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13. ABSTRACT (Maximum 200 Words)

This report presents the activities of the National Academy of Sciences/Institute of Medicine's Committee on Military Nutrition Research (CMNR) for the period June 1, 2001 to May 31, 2003. Activities during this time period which are described in detail in this report include meetings of the CMNR, meetings of other committees working under the auspices of CMNR and reports published by those committees or subcommittees. During the period covered by this report, there were three publications; and one workshop was held. The reports published were: *Caffeine for the Sustainment of Mental Task Performance: Formulations for Military Operations; High-Energy, Nutrient-Dense Emergency Relief Food Product;* and *Pennington Biomedical Research Center: June 2001 Program Review.* The workshop on Biomarkers for Metabolic Monitoring was held in January of 2003, and the agenda and speaker manuscripts are included in the Appendices of this report.

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Committee on Military Nutrition Research

Award Number DAMD17-99-1-9478

Annual Report for Period June 1, 2001 to May 31, 2003

FOREWORD

The purpose of this project is to continue the activities of the Institute of Medicine's Food and Nutrition Board standing Committee on Military Nutrition Research (CMNR). The purpose of the CMNR is to provide review and recommendations to the Commander, U.S. Army Medical Research and Materiel Command (USAMRMC), on research projects, programs, and products as they relate to the nutrition and performance of military personnel. This multidisciplinary standing committee is made up of experts in nutrition, food science, pharmacology, physiology, and behavior, and with knowledge of military procedures. The committee meets at least once per year and oversees the activity of subcommittees that have the necessary in-depth expertise to address each specific task requested by the military. This report covers the activities of the CMNR and its various subcommittees and committees for the period June 1, 2000 to May 31 2003.

INTRODUCTION

The CMNR was established in October 1982 following a request by the Assistant Surgeon General of the Army that the Board on Military Supplies of the National Academy of Sciences set up a special committee to advise the U.S. Department of Defense on the need for and conduct of nutrition research and related issues. This newly formed committee was transferred to the oversight of FNB in 1983. The committee's primary tasks are to identify nutritional factors that may critically influence the physical and mental performance of military personnel under all environmental extremes, to identify knowledge gaps, to recommend research that would remedy these deficiencies as well as approaches for studying the relationship of diet to physical and mental performance, and to review and advise on military feeding standards.

As a standing committee of IOM, the membership of CMNR changes periodically, however the disciplines represented consistently have included human nutrition, nutritional biochemistry, performance physiology, food science, dietetics, psychology, and clinical medicine. For issues that require broader expertise than exists within the committee, CMNR has convened workshops, utilized consultants, or appointed committees with expertise in the desired area to provide additional state-of-the art scientific knowledge and informed opinion to aid in the deliberations.

BODY

Standing Committee on Military Nutrition Research

The standing committee met on October 25-26, 2001 to discuss the public release of its report on *Caffeine for the Sustainment of Mental Task Performance: Formulations for Military Operations,* on October 24, 2001 and to review progress of its various subcommittees. The Committee met on April 2-3, 2002 to review the work of its various subcommittees and to discuss a new activity on metabolic monitoring technologies requested by the sponsor. The Committee also met on January 8-10, 2003 in San Antonio TX in conjunction with its newly-formed Committee on Metabolic Monitoring

Subcommittee on Program Review of the Pennington Biomedical Research Center

In June 2001, a specially appointed subcommittee of the CMNR conducted a program review and site visit of the Pennington Biomedical Research Center (PBRC) to assist the Military Nutrition Division of the U.S. Army Institute of Environmental Medicine in its review of a proposed research program submitted by Pennington as follow-on to Grant No. DAMD-17-97-2-7013. The proposed nutrition research was to be conducted with funds earmarked for military nutrition research activities at Pennington in the 2001 Department of Defense Appropriations bill. The Subcommittee on Program Review of the Pennington Biomedical Research Center met in Baton Rouge June 12-14, 2001. Activities of the Subcommittee during the site visit included 1) initial discussion of the PBRC pre-proposal in closed session, 2) presentations from PBRC staff on details and methodology for each of the new tasks proposed, 3) discussion of the progress of previous grants and the new proposal with the Army sponsors, 4) discussion and evaluation of the proposed research in closed session, and 5) development of a brief report to the Army stating the subcommittee's conclusions and recommendations.

The Subcommittee's report *Pennington Biomedical Research Center: June 2001 Program Review* was released on March 4, 2002.

Subcommittee on Technical Specifications for a High-Energy Emergency Relief Ration

This subcommittee, which was established in March of 2001 and held its first meeting on March 19-21, 2001 at the U.S. Army Research Institute of Environmental Medicine in Natick, MA, held its second meeting in May of 2001 and subsequently completed its writing tasks by email and conference calls. Following completion of the National Academy of Sciences (NAS) external review process, the subcommittee's final report, *High-Energy, Nutrient-Dense Emergency Relief Food Product* was released on February 27, 2002.

Subcommittee on Military Weight Management Programs

This subcommittee has met 4 times since its inception (agenda and abstracts from its workshop were provided in the previous annual report). The chair and vice-chair of the subcommittee have met with committee staff subsequently to complete the report. The report entered the NAS external review process in June, 2002. An extensive response to reviewer comments and revision of the report has been submitted to the NAS Review Coordinator and final approval for public release of this report is pending.

Committee on Metabolic Monitoring for Military Field Applications

This new committee of CMNR was appointed in December, 2002. The committee held its first meeting at Brooks City Air Force Base in San Antonio TX on January 8-10, 2003. The agenda of the workshop is included in the Appendix of this report. The committee held its second meeting on February 28, 2003 to review first drafts of material for its report. The third meeting of the committee was held on April 3, 2003. Significant progress has been made in development of this committee's final report. The committee is scheduled to meet again in conjunction with the CMNR standing committee on July 29-31, 2003.

KEY RESEARCH ACCOMPLISHMENTS

Extensive reviews of the literature have been completed for the two current projects.

1. A thorough review of the scientific literature has been completed on factors known to affect body weight, such as genetics, gender, age, activity, diet and nutrition, and environmental factors. Critical components of effective weight management have also been reviewed and incorporated in the revision of the report on military weight management programs.
2. A thorough review of the scientific literature has also been completed with respect to potential biomarkers for use in monitoring physical, physiological and cognitive function of military personnel during intensive training and combat operations.
3. A workshop was held in January, 2003 that brought together key researches in the identification of biomarkers for fluid and hydration status, energy expenditure, muscle turnover, bone turnover, and cognitive function.

REPORTABLE OUTCOMES and CONCLUSIONS

Three reports have been completed and released.

1. *Caffeine for the Sustainment of Mental Task Performance: Formulations for Military Operations*
2. *High-Energy, Nutrient-Dense Emergency Relief Food Product*
3. *Pennington Biomedical Research Center: June 2001 Program Review*

APPENDIX A

Committee Rosters

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APPENDIX B

Agenda

Workshop on Metabolic Monitoring Technologies

Workshop Agenda

Metabolic Monitoring Technologies for Military Field Applications

Committee on Military Nutrition Research
Food and Nutrition Board. Institute of Medicine, The National Academies

January 8-9, 2003
School of Aerospace Medicine
Brooks Air Force Base, San Antonio, Texas

Wednesday January 8, 2003

8:30 am Registration and check-in

8:45 am Welcome on Behalf of Brooks Air Force Base
Commander, Brooks Air Force Base

8:50 am Welcome on Behalf of the Committee on Military Nutrition Research
Dr. John Vanderveen, Chair, Committee on Military Nutrition Research

9:00 am Overview of Military Interest in Technologies for Metabolic Monitoring
LTC Karl E. Friedl, PhD, U.S. Army Medical Research and Materiel Command, Fort Detrick, Frederick, MD

9:30 am Overview of Field Applications of Physiological Monitoring
Dr. Reed W. Hoyt, U.S. Army Research Institute on Environmental Medicine (USARIEM) Natick, MA

Part I: Biomarkers and Monitoring Technologies for Heat Production and Hydration Status, and Carbohydrate Metabolism
(Moderator: Johanna Dwyer, DSc, RD)

10:00 am Biomarkers of Physiological Strain During Exposure to Cold and Hot Environments
Dr. Andrew J. Young, Chief, Military Nutrition Division, USARIEM

10:30 am Hydration Status Monitoring
Dr. Michael Sawka, Chief, Thermal and Mountain Medicine Division, USARIEM

11:00 am BREAK

11:10 am Technologies for Monitoring Glucose and Lactate
Dr. David Klonoff, Mills-Penninsula Health Services

11:40 am Utility of Insulin-Like Growth Factor-I for Assessing Metabolic Status
Capt. Bradley C. Nindl, Ph.D., USARIEM

12:10 pm Discussion

12:30 pm LUNCH

Part II: Biomarkers and Technologies for Monitoring Physiologic Status and Work Capacity (Moderator: William Morgan, Ph.D.)

1:30 pm The Use of Portable Accelerometers in Predicting Activity Energy Expenditure
Dr. Kong Chen, Vanderbilt University Medical Center

2:00 pm Humans, Hills, and the Metabolic Cost of Locomotion: Simple Explanations from Putting Foot-Ground Contact Times to Work
Dr. Peter Weyand, USARIEM

Part III: Biomarkers and Technologies for Monitoring Muscle Protein Turnover and Metabolism (Moderator: Bruce Bistrian, MD, PhD)

2:30 pm Biomarkers for Changes in Protein Turnover of Muscle and Other Tissues
Dr. Robert R. Wolfe, University of Texas Medical Branch ,Shriners Burn Hospital, Galveston, TX.

3:00 pm BREAK

3:10 pm Potential Real Time Markers Muscle Fatigue or Environmental Stress
Dr. T. Peter Stein, New Jersey School of Osteopathic Medicine, University of Medicine and Dentistry of New Jersey

3:40 pm Muscle Protein Biomarkers to Predict the Occurrence of Physical Stress and Muscle Fatigue or Muscle Inflammatory Responses to Extreme Levels of Physical Activity
Dr. William J. Evans, University of Arkansas Medical School

Part IV: Biomarkers and Technologies for Predicting Bone Turnover (Moderator: Helen Lane, Ph.D., R.D.)

4:10 pm Biomarkers of Bone and Muscle turnover: Effects of Exercise
Dr. Cliff Rosen, Maine Center for Osteoporosis Research and Education, St. Joseph Hospital

4:40 pm Discussion

5:00 pm ADJOURN

6:00 pm Dinner

Thursday January 9, 2003

Part IV: Biomarkers and Technologies for Predicting Bone Turnover (continued)
(Moderator: Helen Lane, Ph.D., R.D.)

9:00 am Biomarkers for Monitoring Bone Turnover and Predicting Bone Stress
Dr. Michael Kleerekoper, School of Medicine, Wayne State University

9:30 am Biomarkers to Predict the Occurrence of Bone Stress and Matrix Abnormalities Due to Sustained and Intensive Physical Activity
Dr. Wendy Kohrt, PhD, University of Colorado Health Sciences Center

10:00 am Discussion

10:15 am BREAK

Part V: Biomarkers and Technologies for Monitoring Cognitive and Physiological Status in **Relation to Stress**

(Moderator: Esther Sternberg, M.D.)

10:30 am Technologies for Monitoring Cognitive Status to Predict the Occurrence of Mental and Physical Stress
Dr. Julian Thayer, National Institutes of Health

11:00 am Use of Sweat Patch Technology to Monitor Neuroendocrine Status
Dr. Giovanni Cizza, National Institutes of Health

11:30 am Discussion

11:45 am LUNCH

Part VI: Biomarkers and Technologies for Monitoring Mental Status, Cognitive Function and Alertness
(Moderator: Dr. Patrick O'Neil)

1:00 pm Biomarkers for Brain Hypometabolism Due to Sleep Deprivation
Dr. Nancy Westensten, Department of Behavioral Biology, Walter Reed Army Institute of Research

1:30 pm Electroencephalographic Indicators of Impaired Aviator Status During Sleep Deprivation
Dr. John Caldwell, Airforce Research Laboratory, Brooks Air Force Base

2:00 pm Circulating Plasma Markers of Cognitive Status
Dr. Harris Lieberman, USARIEM)

2:30 pm Discussion

3:00 pm BREAK

Part VII: Future Possibilities for Monitoring Physiological and Cognitive Function.
(Moderator: Beverly Tepper, PhD)

3:15 pm Odors as Biomarkers to Predict the Occurrence of Mental and Physical Stress
Dr. Gary Beauchamp, Director, Monell Chemical Senses Research Institute

3:45 pm	Use of Brain Imaging Technologies to Monitor Cognitive Status to Predict the Occurrence of Mental and Physical Stress *Dr. Mark George, Center for Advanced Imaging Research, Brain Stimulation Laboratory, Medical University of South Carolina*
4:15 pm	Molecular Markers of Mechanical Activity/Inactivity Induced Anabolic and Catabolic States in Striated Muscle *Dr. Kenneth Baldwin, Dept. Of Physiology and Biophysics, University of California - Irvine*
4:45 pm	Discussion
5:00 pm	Summary of the Workshop *Dr. John Vanderveen, Char, Committee on Military Nutrition Research*
5:30 pm	ADJOURN

APPENDIX C

Speaker Manuscripts

Workshop on Metabolic Monitoring Technologies For Military Field Applications

Molecular Markers of Mechanical Activity/Inactivity Induced Anabolic and Catabolic States in Striated Muscle

K. M. Baldwin, F. Haddad; and G. R. Adams; Department of Physiology and Biophysics; University of California Irvine; Irvine, CA 92697

INTRODUCTION AND BACKGROUND

Striated muscle is highly plastic in that the individual cells or myocytes comprising this complex system have the capacity to change their mass, metabolic capacity, and contractile properties in accordance with the chronic functional demands (or lack thereof) imposed on it (Baldwin and Haddad, 2001). In the last ~30 years, considerable evidence has accumulated to suggest that several key processes involving gene expression are closely linked in the regulation of both the amount and types of protein that are expressed in the muscle cells thereby enabling them to adapt to various environmental stimuli (Adams, 2002; Baldwin and Haddad, 2001; Booth and Baldwin, 1996). Therefore, the goal of this report is to determine if different activity/inactivity paradigms can induce altered expression/activity in certain molecular markers (studied in an acute setting) in order to predict long term adaptations reflecting changes in either the phenotype and/or net protein balance (anabolic and catabolic states) in skeletal muscle.

Fundamental Concepts of Gene Expression

Figure 1 presents a schematic of how the expression of a gene is typically regulated via collective molecular processes in order to produce a specific protein product. Through these processes as depicted for a single gene, it is now recognized that expression of a variety of genes could contribute collectively to the regulation of many fundamental processes occurring in the cell. These are illustrated by, but are not limited to the following processes that are known to undergo dramatic alteration in their functional properties: a) the contraction process (e.g., actin and myosin interaction); b) aerobic and anaerobic energy transformations; c) muscle growth regulation (growth factor expression); d) protein synthetic pathways; and e) protein degradation pathways. Also depicted are key steps in the cascade that interact to control the amount of protein that is expressed, depending on how each step in the cascade is regulated. These steps include: transcription and pretranslational processes, which combine to produce the message substrate (mature mRNA) of the gene for producing the protein. The mRNA is then translated into protein, a process that is commonly referred to as protein synthesis. This process is known to be regulated by several important steps, the chief of which is at the "protein initiation" step. Also operating simultaneously are post-translational events, including the process whereby proteins becomes targeted for subsequent degradation. It should be noted that all proteins within the cell undergo turnover (synthesis and degradation). It is through this process of protein turnover that both the type and amount of protein expression in the muscle can be changed from one functional state to another.

Factors Defining Protein Balance in Muscle

Based on the above, it is apparent that the amount of the protein maintained in a given muscle cell is controlled by the balance of those processes that transcribe/translate a given protein relative to those processes that regulate its degradation. When the muscle is in a stable steady state (e.g., neither growing nor atrophying), the synthetic processes are in balance with the degradation processes. However, when the muscle is exposed to stimuli that induce a net accumulation of protein (referred to herein as an anabolic state) the transcriptional/translational processes of the muscle must be greater than those operating on the degradation side. On the other hand if the conditions are such that the transcriptional/translational processes cannot match that of degradation, then the muscle enters a state of catabolism, which results in net protein loss leading to its atrophy. Thus, it is important to note that all of

the processes operating in the cascade can undergo altered rates of operation to thereby significantly influence the net protein balance in the muscle cell.

The Importance of Protein Isoforms

Coupled to this general scheme of gene/protein regulation is the fact that the genome of mammalian species contains a variety of multi gene families. These consist of groups of very similar genes that encode slight variants of the protein product that have slightly different functional properties. An example of this is the myosin heavy chain (MHC) gene family, which collectively encode several different isoforms or species of myosin. Each isoform has distinct functional properties that ultimately dictate the intrinsic contraction properties (speed of contraction; fatigability) of the cells in which it is expressed. Depending on how this gene family is regulated in a given fiber, it is possible to repress one type of MHC gene and increase expression of another MHC type(s). This plasticity of gene expression enables the muscle to transform its intrinsic contractile properties. Thus, it is possible for the muscle to change both its size and its contraction phenotype, depending on how this complex cascade in Figure 1 is regulated from one functional state to another.

Signaling Pathways in Adaptive Processes

Presented in Figure 2 is a complex array of processes/pathways that collectively operate to modulate those proteins/enzymes that coordinate the functional operation of the cascade depicted in Figure 1. While it is beyond the scope of this short review to describe these signaling molecules and pathways in detail, it is important to emphasize that there are both up stream initiation factors (e.g. growth factors) and down stream effector proteins that regulate the integrated events governing transcription, translation, and degradation processes thereby enabling the muscle cell to remodel its structure and functional properties. Importantly, this simplified scheme in no way reflects all of the signaling molecules and regulatory factors that control adaptive processes in striated muscle.

Activity Paradigms for Studying Adaptations in Skeletal Muscle: Animals Models

In this report we will focus on three different activity/inactivity paradigms: 1) a model of chronic functional overload (FO) in which a smaller target muscle(s) is continually overloaded due to the surgical removal of its larger synergist(s) (Adams et. al., 2002); 2) intermittent resistance overload training (RT), in which the target muscle is trained with a specified contraction regimen spanning one-two training sessions (Haddad and Adams, 2002); and 3) the model of spinal isolation (SI) in which the target muscles are rendered ~completely inactive by mid-thoracic/sacral spinal cord transectioning that is coupled to a dorsal rhizotomy procedure. This procedure eliminates all sensory and higher center input to the motor unit pool of the lower extremity muscles while keeping the muscle-nerve connections intact (Huey et. al., 2001). This latter model, in essence, provides essentially a "ground zero" catabolic reference state to which the anabolic mechanical overload paradigms can be compared.

METHODS AND MATERIALS

All the animal projects involved adult, female rats. Functional overload and resistance training procedures were as described in detail elsewhere (Adams et.al., 2002; Baldwin and Haddad, 2001; Haddad and Adams, 2002). The spinal isolation model involved surgical procedures as described by (Huey et. al., 2001). The biochemical/ molecular analyses of marker protein phosphorylation, RNA concentration, and mRNA levels (via RT-PCR techniques) for specific genes were adapted from procedures described previously (Adams, et. al., 2002; Haddad and Adams, 2002). For comparative purposes, we report some initial findings (unpublished results) on humans that have undergone a combination of limb unloading plus resistance training in an attempt to ameliorate the atrophy that occurs in unloaded human skeletal muscle (Carrithers et. al., 2003).

RESULTS AND DISCUSSION

Early Events Leading to Net Protein Accumulation in Response to Mechanical Loading

Previous studies show that infusing physiological levels of IGF-1 directly into the muscle can induce significant hypertrophy within several days (Adams and McCue, 1998). The question is whether a mechanical stimulus, in and of itself, can induce rapid increases in muscle derived IGF-1 expression in muscle thereby stimulating compensatory growth? If such a response occurs, it would suggest the involvement of an autocrine/paracrine process in the anabolic cascade following mechanical loading. As shown in Figure 3, there is a rapid increase in mRNA expression for both IGF-1, as well as a variant isoform of IGF-1 (Adams et. al, 1999; Adams et. al., 2002), called mechanical growth factor (MGF) in response to functional overload. This response occurs early-on in the adaptive response, and it is seen in both FO and isometric RT paradigms (Adams et. al., 2002; Haddad and Adams, 2002; Huey et. al., 2001) suggesting that growth factors are likely playing a key role in inducing anabolic responses in muscle under conditions that produce high mechanical stress on the muscle.

In addition to the response of growth factors, we also determined if there are rapid adaptive changes in the machinery that translates mature-mRNA into protein (figure 1). Therefore, we examined levels of total RNA in skeletal muscle, since ~85 % of the RNA pool exists as ribosomal RNA. Ribosomal RNA provides the scaffolding to which the mature mRNA is attached providing the template for synthesizing the encoded protein. As shown in Figure 4, there is a rapid increase in the concentration and content of total RNA in response to FO, suggesting that this is an important adaptive response to provide the machinery for producing more protein.

Based on the above observations it is apparent that there are early events occurring to enable the muscle to enter into an anabolic state. Therefore, it was of interest to determine if there are adaptive changes occurring in the pathways that are considered to be rate limiting steps in protein synthesis, e.g., the initiation steps in protein translation. We examined two different but complementary markers of this process. The first involves the phosphorylation of p70S6 kinase (pS6K). When this kinase is phosphorylated, it increases phosphorylation/activity levels of other proteins involved in the translation of mRNAs encoding proteins comprising the ribosomal machinery. As shown in Figure 5A, there was a marked increase in the phosphorylation state of pS6K indicative that this pathway was activated. This observation is also consistent with the increase in total RNA presented above in Figure 4. Also, we examined the phosphorylation state of another marker of protein initiation, e.g., eukaryotic initiation factor 4E binding protein, (4EBP-1). This factor normally functions as a negative regulator of the formation of the 43 kD pre-initiation complex that is essential for protein translation. However, when 4EBP-1 undergoes increased phosphorylation, it dissociates from the protein, eIF4E, a key protein subunit that is necessary for the 43S complex to form so that the initiation process can occur. As presented in Figure 5B, 4EBP-1 also undergoes increased phophorylation at the early stages of mechanical loading, which is also indicative that protein initiation processes are being activated. Thus, we have demonstrated that there are several molecular markers that can serve as early-event signaling molecules in order to predict that the muscle is entering a state of positive protein balance. All of the markers that have been identified above to predict that an anabolic state is occurring in response to functional overload show similar adaptive responses when the mechanical stimulus is intermittent rather than continuous. For example, when isometric resistant training paradigms are imposed on the muscle it responds in a fashion similar to that seen the in functional overload paradigm (Haddad and Adams, 2002).

Early Responses of Molecular Markers During Muscle Atrophy

Do inactivity paradigms that induce marked degrees of muscle atrophy cause the opposite responses of those markers presented above in response to anabolic stimuli? The answer to this question appears to be negative, since some of the markers (IGF-1, p6SK, 4EBP-1) that are highly responsive to mechanical loading, either are maintained at normal levels or show some level of increased expression or increased activity when the target muscles undergo rapid atrophy in response to SI (Haddad et. al.,

submitted manuscript). Instead, there appears to be a different set of molecular markers that are highly sensitive to the unloading state. These include the following. 1) There is, at the onset of muscle unloading, a decrease in the transcriptional activity of key genes that encode important structural/functional proteins that comprise the sarcomere machinery, i.e., the system that produces contraction (e.g. myosin heavy chain and actin). This is depicted in Figure 6, which shows that transcriptional activity of actin as well as the slow type I MHC gene (which predominates in load-sensitive muscle cells), are significantly reduced. 2) There is a reduction in total RNA as well as specific mRNA expression for both actin and total MHC. These responses are indicative of a reduction in both the substrate and the machinery necessary for carrying out translation of key proteins (data not shown). 3) Genes encoding enzymes that are involved in the process of protein ubiquitination are up-regulated (Figure 7). These enzymes define the process whereby specific proteins become targeted for degradation by the proteasome system, which is the major pathway for protein degradation in muscle cells. These collective responses provide a mechanism to rapidly reduce muscle mass by a) decreasing the ability of the muscle to accumulate protein, while b) increasing the processes for decreasing protein pools thereby creating a catabolic state and net protein loss. Since those processes that regulate factors such as IGF-1 and the phosphorylation of pS6K and 4EBP-1 do not appear to be down-regulated, it is apparent that the loss of muscle protein is not necessarily the result of a "shutting down" of those processes that cause muscle cell enlargement. Thus, one must focus on a different set of molecular markers to distinguish a net catabolic state from that which defines a net anabolic state in predicting a protein balance profile of the muscle under different physiological conditions.

Do The Molecular Responses Seen in Animal Models Have Relevance to Adaptation in Human Muscle?

While there is abundant evidence that there are viable human models such as resistance training, bed rest, and the unique model of unilateral limb suspension (ULLS) that can mimick, to a certain extent, the gross responses seen in animal models of hypertrophy and of atrophy, questions arise as to whether acute changes in the mechanical stress imposed on human skeletal muscle induce the same type of responses as reported herein for rodent muscle? In an attempt to addressed this issue, we performed preliminary analyses in conjunction with Dr. Per Tesch at the Karolinska Institute in Stockholm (Carrithers et. al., 2003) on selected molecular markers in biopsy samples obtained from three groups of subjects (n=8 each): 1) a group subjected to ULLS for three weeks (left limb unloaded; right limb ambulatory; 2) a group of subjects subjected to ULLS plus a resistance training paradigm (Carrithers et. al., 2003); and 3) a group of fully ambulatory subjects that received the same resistance training paradigm as seen for ULLS-trained group. The results indicated that the ULLS caused a reduction in strength and muscle mass in the suspended limb. This response was attenuated in the ULLS plus resistance-trained group. The resistance training of the ambulatory subjects did not significantly enhance muscle mass or muscle strength beyond that which was observed for the ULLS plus trained group. Biopsies were obtained on each subject at the beginning of and at the end of the experimental protocol. As presented in figure 8A, there was a deficit in the pre/post change in muscle total RNA concentration for the ULLS versus the two resistance trained groups. Also there were net deficits in both the MHC and actin mRNA responses in the ULLS group versus that seen for the two resistance trained groups (Figure 8B and 8C). Thus, we propose that the same general adaptive processes that operate in the muscles of animal models also are seen in the human subjects when they are exposed to perturbations that alter the homeostasis of the skeletal muscles under different loading states.

SUMMARY AND CONCLUSION

In this report we have demonstrated that skeletal muscle of both animal and human subjects possess a high level of plasticity (ability to change in response to altered environment) of gene expression in response to altered states of loading and/or mechanical stress. This phenomena makes it possible to establish molecular marker profiles based on adaptive responses to acute disruptions in muscle homeostasis that predict impending alterations in catabolic and anabolic states that affect outcomes in the

net protein balance in muscle cells. This information paves the way for the eventual development of technologies with the capability of monitoring the muscle's molecular status for predicting outcomes to paradigms that may have either a positive or negative impact on the structure and function of the skeletal muscle system.

This research was supported by a grant from the National Space Biomedical Research Institute (NCC9-78-70) and NIH grants AR 30346 (KMB) and AR 45594 (GRA).

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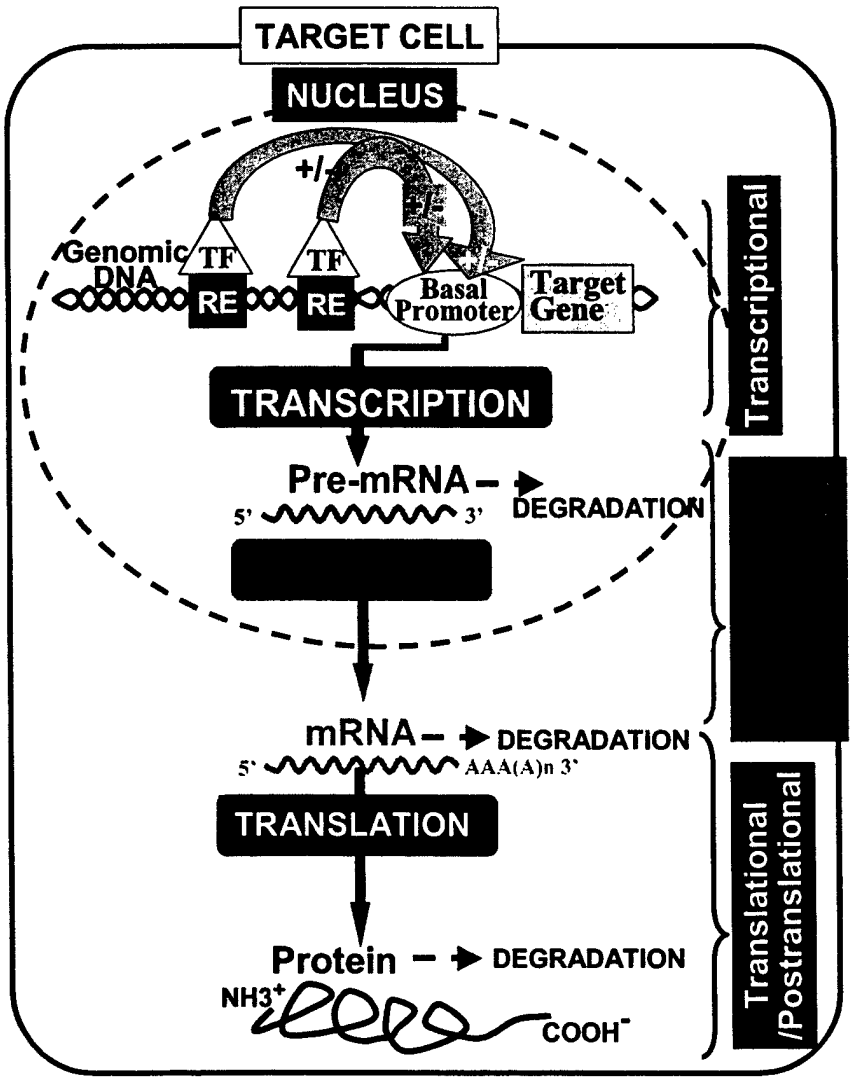


FIGURE 1 Processess regulating gene function.

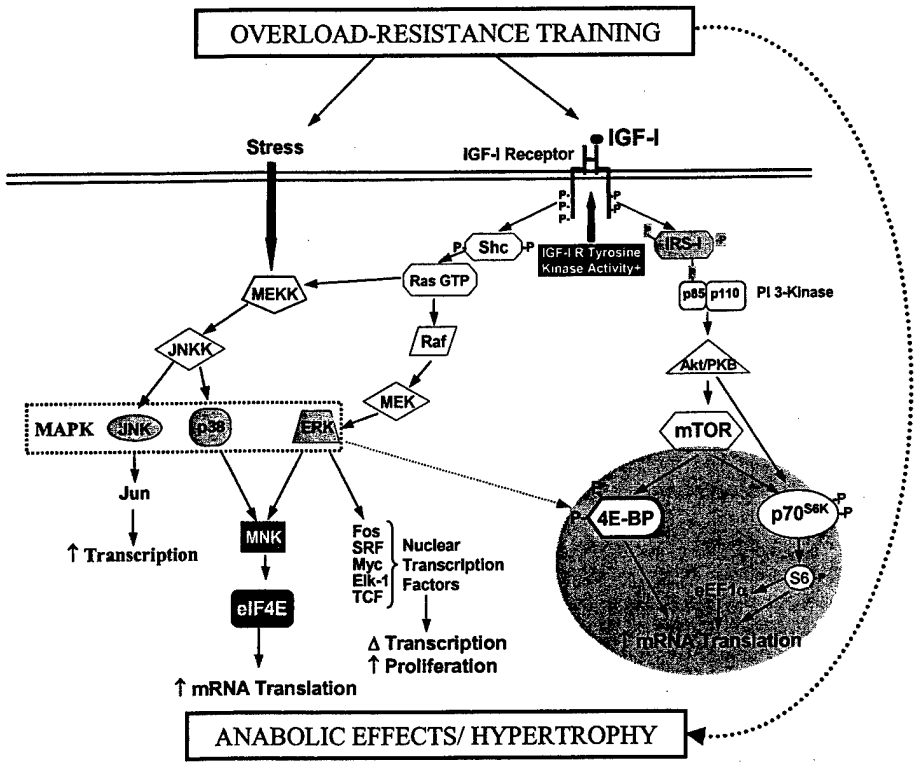


FIGURE 2 Signaling pathways in adaptive processes

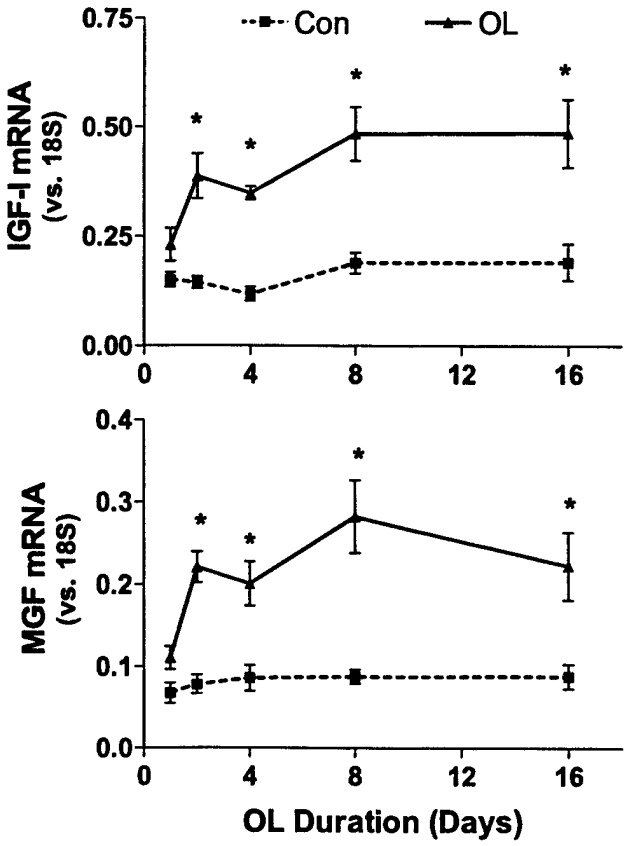


FIGURE 3 Soleus IGF-I and MGF mRNA expression in response to overload.

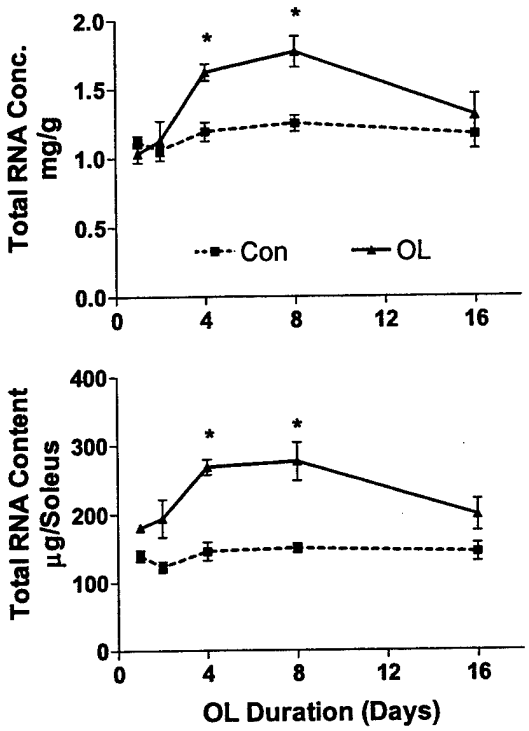


FIGURE 4 Soleus total RNA concentration and content in response to overload.

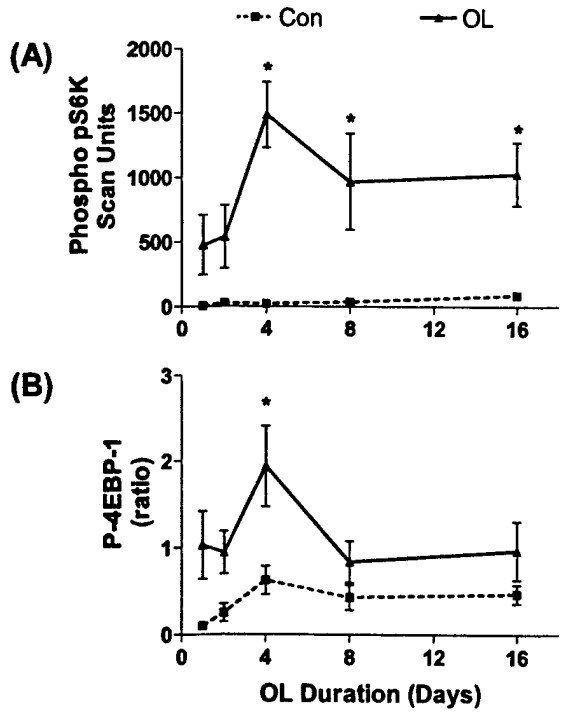


FIGURE 5 Phosphorylation level of pS6K (A) and 4EBP-1 (B) in overloaded soleus.

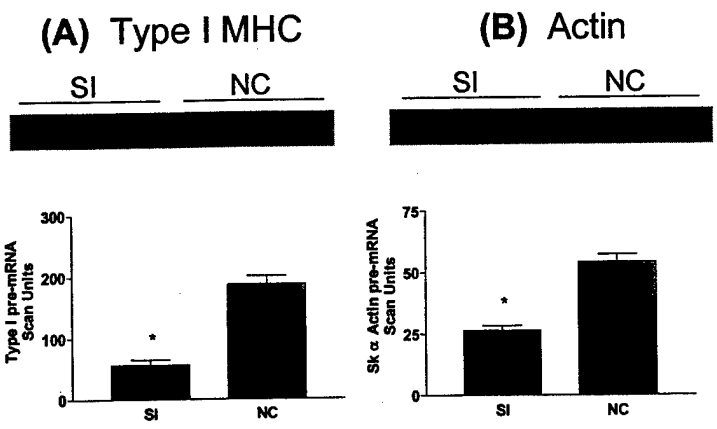


FIGURE 6 Type I MHC (A) and Actin (B) pre-mRNA expression in control and 8 days SI soleus muscle. Pre-mRNA is the nascent transcriptional product and changes in its expression represent changes in gene transcriptional activity.

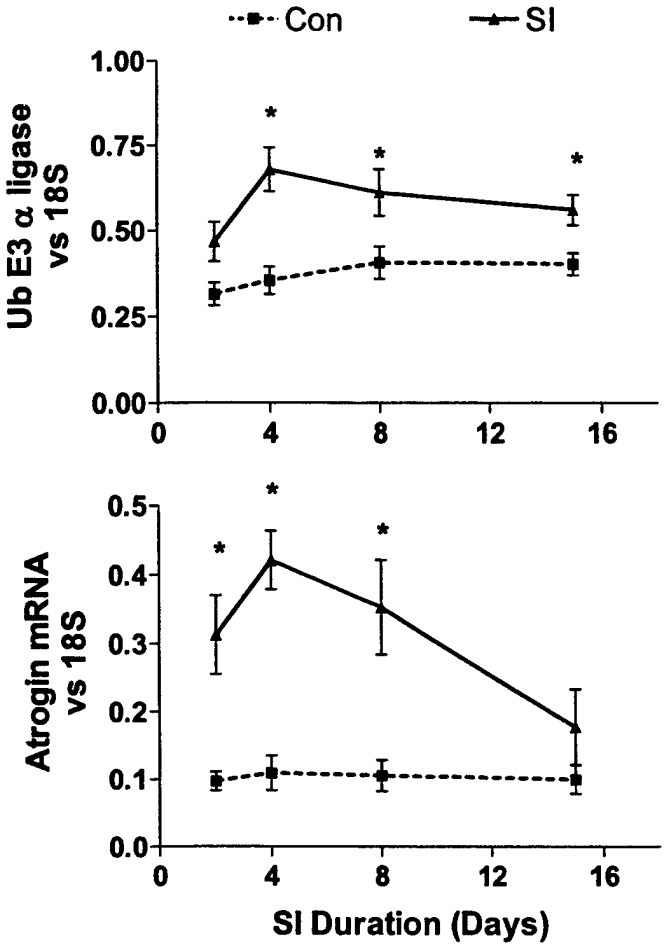


FIGURE 7 Degradation marker mRNAs expression in soleus in response to SI.

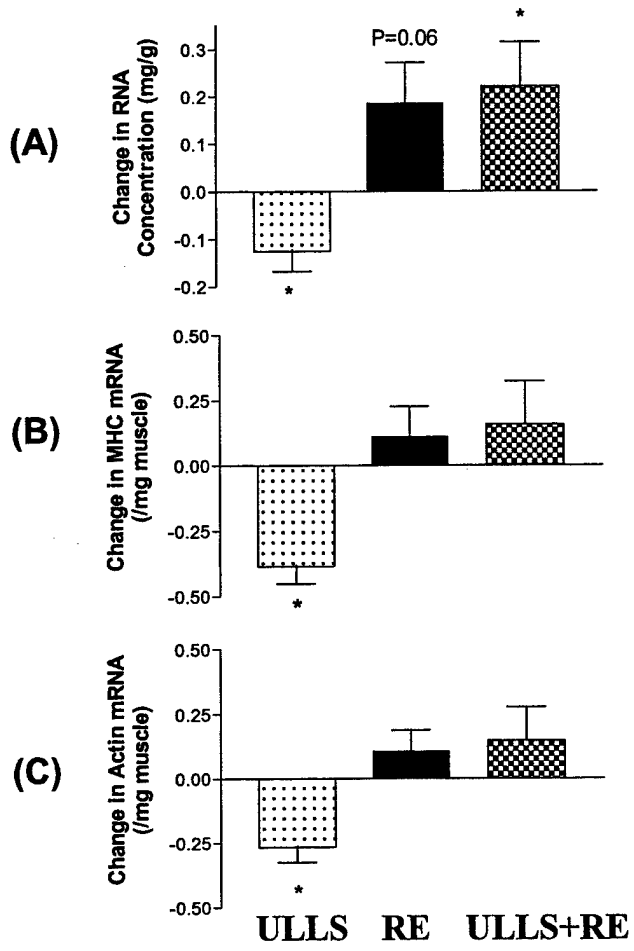


FIGURE 8 Changes in total RNA concentration (A), total MHC (B) and actin (C) mRNA expression in human muscle when subjected to unilateral limb suspension (ULLS), resistance exercise (RE) or ULLS+RE.

Odors as Biomarkers

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INTRODUCTION

Chemical signals (herein termed body odors or just odors) provide information on many characteristics of an organism and are involved in coordination and regulation of all aspects of behavior and physiology. Typically, body odors have been divided into two broad classes, those termed pheromones and all others. In the former category are included chemical signals that have evolved to convey very specific information, elicit specific behavioral and physiological responses, and are in principle rather simple chemically. Examples include odorants that elicit behavioral responses such as sexual attraction and aggression (often termed releaser pheromones) and those that elicit physiological responses such as estrus synchrony and sexual maturation (often termed primer pheromones). The other broad class (sometimes included in the pheromone category but at other times excluded) encompasses odors that signal information such as individual identity, age, emotional status, and health. However, both in practice and in principle, the distinctions between these two categories are often difficult to discern (see Beauchamp et al., 1976; Wysocki and Preti, 1998). In the remainder of this essay this distinction will be ignored.

Body odors have a number of inherent characteristics that should make them particularly useful for those interested in monitoring organic states of individual humans. First, many body odors evolved to communicate messages between individuals. As a consequence, these messages ought to be relatively unambiguous and difficult to falsify. Second, unlike many visual and auditory signals odors often persist in the environment. Indeed, many species make use of this characteristic during territorial scent marking such as dogs urinating on posts. Darwin noted that the odor on a handkerchief that he had rubbed on a scent gland of an animal persisted on the cloth for years in spite of repeated washings. Third, body odors often directly reflect physiological processes. For example, odors associated with stress have been suggested to arise from action of stress hormones on odor-producing body structures. Fourth, odors can be detected from a distance and hence non-invasively. It has often been noted that when a dog follows a scent of an individual person, it does not put its nose directly on the ground but instead holds it above the presumed odor source. Finally, in principle it ought to be relatively straight-forward to develop devices to detect and recognize specific chemical signatures indicative of particular physiological states. In practice, however, this has remained a challenge as will be described below.

In the following brief essay, I will first discuss the kinds of information that exist in body secretions and excretions. Next, the possible use of body odor to identify messages signaling physiological states such as stress will be discussed. Finally, I will speculate on the future use of body volatiles in monitoring physiological status.

MESSAGES IN BODY ODOR

Work with body odors in non-human animals has clearly demonstrated that a variety of messages are transmitted and influence the behavior and physiology of the receiver. The categories of information are illustrated in Fig 1.

Core Messages

Core messages (1 in Figure 1) involve characteristics of the animal that are relatively fixed. Thus animals are able to determine the species of the odor producer and its individual identity. For example, a

large series of studies have demonstrated that the individual identity of a mouse is coded in part by the genes of the Major Histocompatibility Complex, the same genes involved in self—non-self recognition within the context of the immune system (Yamazaki et al., 1999; Penn and Potts, 1998). Presumably an individual mouse's odor (and very likely an individual human's odor as well) is a fixed characteristic of that animal. Based on these ideas we are now attempting to identify the odorous materials in mouse and human emanations with a long-range goal of developing sensors that could recognize individuals by their genetically determined characteristic body odors.

Life-span Messages

At a second level (2 in figure 1) there are a number of messages that are relatively stable yet do vary over the life span of an individual. Two of these are age and odor-expressed gender. Consider first, gender. Although basic biological gender is fixed at conception, many data indicate that body odors reflecting this do change over the course on an individual's life. Most dramatic are odor changes that accompany sexual maturation. Indeed, in human males almost the first easily observable sign of male puberty is a change in body odor—this clearly precedes changes in body hair and voice. These changes clearly reflect changes in amounts of circulating sex hormones. As a practical example, it is well known that castration reduces male body odor in pigs, reducing "boar taint" in male pig meat.

In many species it has been speculated that information on the age of an animal may serve to modulate mate choice. Older males may be preferred mates due to the fact since they survived, they must possess "good" genes that are advantageous for the female to pass on to her offspring. Recently we (Osada et al., In press) have identified some of the volatile chemicals in mouse urine that change with age that may underlie age-related discriminative odors. Several of these are plausibly linked to changes in immune function. This raises the possibility that immune system activity could be determined by sensors that detect body odors (see below).

Varying messages

Finally, there is a series of odor-based messages that are quite variable (3 in figure 1). Included here is information on sexual receptivity or willingness to mate, incidence of disease and emotional state. For example, many animal studies have documented changes in female odor as a function of estrus cycle and there is some evidence that the body odors of women change over the menstrual cycle (independent of the odors associated with menses; e.g. Stern and McClintock, 1998). In non-human animals female odors associated with sexual receptivity are often highly attractive to males.

That body odors can be indicative of disease has a long history in medical practice (see Penn and Potts, 1998). Nevertheless, little systematic study of this topic has been conducted. For example, there are many anecdotes of dogs identifying the presence of cancers prior to formal diagnosis but few experimental studies documenting this in a rigorous fashion.

As a first model system to investigate disease and body odor, we (Yamazaki et al., 2002) have recently reported on a model system - the Mouse Mammary Tumor Virus (MMTV). It is possible in this model to test for changes in odor profiles that arise prior to overt disease. Mouse mammary tumors are notably lacking in cachectic, metastatic and other general systemic effects on the host that might be expected to alter body odor in a non-specific manner. Our studies revealed that mice can be trained to discriminate female or male intact mice or their urine odors as a function of the presence of MMTV, either acquired through infection or genetically. Furthermore, odor distinction based on the presence of virus occurs in the absence of overt disease. We are currently investigating the chemical pattern change that occurs following infection and are attempting to identify biologically relevant odorants. More generally, however, these studies suggest that it may be possible to identify and diagnose certain diseases (e.g. viral diseases such as AIDS and smallpox) before they are otherwise obvious and via the relatively non-invasive route of body odors.

BODY ODORS INDICATIVE OF OTHER PHYSIOLOGICAL STATES

Very little experimental work has been conducted on odors indicative of emotion (e.g. fear, anger, happiness) or fatigue in humans. Nevertheless, there is a widespread belief that an individual's emotional state is reflected in changes in body odor and this belief is reinforced by the results of some animal studies. For example, there are a number of studies that indicate that stressed animals emit a distinctive odor. It has been suggested that these odors may function to warn others of danger; these odor often elicit avoidance.

Anecdotal evidence in humans is consistent with animal studies. It is said that when one is under stress that sweating increases and this in turn leads to increases in body odor. Whether this purported change in odor is qualitative (new odorants being produced specifically indicating stress) or quantitative is not known. There is a plausible mechanism for a change in odor production with stress, however, since it is well known that certain neurotransmitters such as epinephrine stimulate heightened sweat gland activity.

As far as can be determined, there are no studies on changes in body odor with fatigue. Also, there is very little research on body odor changes with other emotional variations with two exceptions. Chen and Haviland-Jones (2000) have reported that arousal of the emotions of happiness and fear by film clips results in production of body odors that can be discriminated by human noses. Similarly, Ackerl et al. (2002) have also reported that women made "fearful" by watching a scary movie produce an axillary odor that can be discriminated (by other women) from axillary odors collected under non-fear conditions. These studies are admittedly tentative but in light of their implications, a number of investigators are following them up.

BODY ODORS AS SIGNALS: FUTURE PROSPECTS

Based on studies with non-human animals and much more limited work with humans, it is safe to say that body odor is a rich potential medium for monitoring physiological states. There are however, a number of problems that make it difficult to put this potential into practice at the present time.

Production and Communication

The first problem is our current lack of definitive studies on what information *human* body odors contain. Such studies are difficult to conduct for a variety of reasons but they are not impossible. It is encouraging that DARPA has recently called for research proposals on the potential to identify individuals based on their body odors. Here it is assumed that there is a genetically based individual odor but whether this can be reliably discriminated in spite of variation in such factors as diet, perfume use, and odors associated with home and work place remains a major question. Apparently dogs can discern the individual signature of a person in spite of these potential distracters indicating that, at least in principle, it should be possible for a device to do this as well.

Similar studies should be encouraged to study further how odors reflect emotional states. The Chen and Haviland-Jones and Ackerl et al. work represent just the very beginning. Both of these investigations asked whether humans can make olfactory discriminations between samples of body odors based on the emotional state of the odor donor. Based on non-human animal work, it is highly likely that human stress induces specific odor changes but this must be rigorously demonstrated before programs to try to identify specific odorants and to develop sensors are instituted. It is also important to recognize that for volatile signals indicative of emotional states to be useful for monitoring emotion, it is not necessary that human noses be able to detect these substances. More discriminative devices, be they other biological ones such as rats or dogs, or specialized non-biological sensors (see below), may be able to detect these volatile signals and thereby serve as monitors even if other people find these discriminations difficult or impossible.

Detection and Discrimination

A second major problem involves techniques to identify and monitor odorants. In nature the olfactory system has evolved to be astoundingly sensitive to small molecules. Recently, much progress has been made in our understanding of this system although many mysteries remain. Briefly, it is now thought that mammals have about 1000 different molecular receptors for odorants (in humans two thirds of these are not functional, however). Each receptor, located on an individual receptor cell that is actually a primary sensory neuron, is responsive to a variety of structurally similar odorants (e.g. Zhang and Firestein, 2002). It is thus the pattern of receptor activity that is monitored and that determines odor quality and intensity. Processing and fine-tuning this pattern occurs beginning at the first synapse in the olfactory bulbs but how further CNS processing occurs remains mostly unknown.

One strategy is to develop devices that mimic or even use biological principles to detect specific body odors. Particularly attractive is the idea that one might be able to express olfactory receptors in a device that monitors their activity using, for example, fluorescence to express overall patterned activity. This is a promising approach but it clearly is quite far in the future.

A very active research area involves using a variety of kinds of artificial sensors to develop so-called e-noses or artificial odor sensing devices. Although success of these devices has been mixed (initial claims turned out to be highly exaggerated), there is no doubt that progress is being made in sensors and sensor-interpretation interfaces. It seems likely that for highly accurate sensors that a knowledge of the specific odorants of interest will be needed. Hence, detector device development must go hand in hand with studies on the biology and chemistry of odors of interest.

CONCLUSIONS AND PROSPECTUS

Non-human animal studies confirm that body odors are a rich source of information about an organism. Human studies are few; nevertheless it is highly likely that our odors serve communicative functions. Because these odors presumably evolved to communicate, the messages should be much more readily useful for monitoring physiological states than, for example, hormones or metabolites from body fluids. In this latter case, multiple extraneous factors can obviously interfere with what is measured since there have been no evolutionary constraints to insure a high signal-to-noise ratio. For an evolved signal like an odor, in contrast, the signal to noise ratio should be high and the information content buffered against disruption from environmental and physiological variables. Consequently, additional work aimed at investigating odors for monitoring various physiological states is a very promising line of inquiry. Future work should reveal what information is available in body odor and the chemical identity of the odorants. In parallel, devices to accurately and reliably monitor these odors will be developed.

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Hierarchical organization of body odor messages

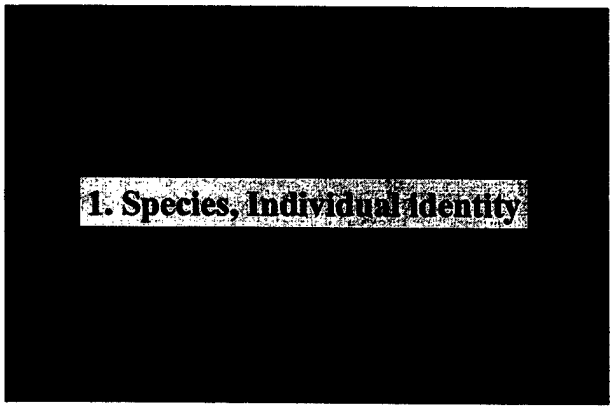


FIGURE 1 Three levels of chemical signals derived from body odors illustrated by the mouse system. 1. Core messages largely innately determined and with little variation across the life span. 2. Messages that are relatively fixed but do vary in expression across the life span of the organism. 3. Messages that vary from time to time and may reflect short term physiological fluctuations.

Electroencephalographic Indicators Of Impaired Aviator Status During Sleep Deprivation

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INTRODUCTION

Monitoring the brain activity of aviators for indications of degraded cognitive/performance capacity is desirable for enhancing flight safety. Research has shown that degraded pilot status has caused serious mishaps. For instance, McCann and Schulze (1963) reported that a substantial number of fatal aviation accidents have resulted from pilot incapacitation due to hypoxia, hyperventilation, or blackout; and Yacavone (1993) found that serious flight mishaps have been associated with inadequate crew coordination or decrements in the physical or mental status of pilots. Pilot fatigue is now recognized as a serious threat to aviation safety especially in operations that involve sleep loss from circadian disruptions, extended duty periods without sleep, and episodes of night duty during which alertness is typically impaired due to circadian factors (Akerstedt, 1995). Aviator fatigue degrades response accuracy and speed, impairs the capacity to integrate information, and narrows attention (Perry, 1974). Fatigued pilots tend to decrease their physical activity, withdraw from social interactions, and lose the ability to effectively divide mental resources among different tasks. These effects are compounded by the fact that increased sleepiness in the cockpit is associated with less consistent performance and deteriorations in vigilance (Dinges, 1990).

Kirsh (1996) estimates that fatigue may be involved in 4-7% of civil aviation mishaps, and data from the U.S. Army suggest fatigue is involved in 4% of Army accidents (Caldwell and Gilreath, 2001). Furthermore 25% of the Air Force's night tactical fighter Class A accidents were attributed to fatigue between 1974 and 1992, and 12.2% of the Navy's total Class A mishaps were thought to be the result of aircrew fatigue from 1977 to 1990 (Ramsey and McGlohn,1997). Especially noteworthy mishaps in the commercial aviation sector include the crash of Korean Air flight 801 in which 228 people died (NTSB, 1999); the near crash of China Airlines flight 006 in which two people were severely injured while numerous other passengers were traumatized (Kolstad, 1989); and the accident involving American Airlines 1420 in which 11 people died (Krause, 1999). In each of these cases, crew fatigue from insufficient sleep and/or circadian factors was implicated.

It is regrettable that a suitable metric has not been developed to determine the point at which aviator fatigue becomes a hazard to safe flight. In fact, neither the military nor the civilian aviation sector has identified a better fatigue countermeasure than the age-old strategy of flight-time or duty-time limitations. Unfortunately, this approach fails to account for the known effects of fatigue-inducing factors such as 1) the quality of sleep prior to reporting for duty, 2) the deleterious impact of chronically restricted sleep periods, and 3) the hour-by-hour fluctuations in physiological alertness that stems from circadian rhythms. What is needed is a validated, objective measurement of aviator status that ultimately can be monitored continuously and in real time.

Since the electroencephalogram (EEG) is the most direct indication of central nervous system functioning (which presumably underlies all cognition and performance) this measure holds great promise for objectively and accurately monitoring the fatigue state of operators. The fact that EEG activity can be collected without interfering with the primary task of flying the aircraft (Caldwell, Hall, and Erickson, 2002) supports the feasibility of continuous, real-time monitoring. In addition, numerous ground-based studies have established the sensitivity of EEG activity to work-related stressors such as sleep deprivation. Comperatore et al. (1993), Caldwell et al. (1996), Lorenzo et al. (1995), Pigeau et al. (1987) and others all have shown that slow-wave EEG activity (i.e., delta and/or theta) is significantly elevated

by even moderate sleep loss. Recently, Caldwell and Hall (2001) reported that both delta and theta are reliably accentuated after 23-26 hours of continuous wakefulness, approximately the same time that both mood and performance are adversely affected.

Although studies relating in-flight EEG data to the readiness level of aviators are virtually nonexistent, a few investigators have suggested a link. Sterman et al. (1987) demonstrated changes in EEG theta and alpha as a function of increased flying demands as well as increased EEG asymmetries as a function of increased workload, and Wilson and Hankins (1994) found differences in EEG theta activity attributable to alterations in attention and cognitive demands in flight. With regard to the appearance of EEG indications of in-flight fatigue, Samel et al. (1997), Cabon et al. (1993), Rosekind et al. (1994), and Wright and McGown (2001) all have reported EEG microsleeps (bursts of slow-wave EEG) in aircrews during trips ranging from 8 to 15 hours in duration. Since such events signal an impaired ability to respond to incoming stimuli (Belyavin and Wright, 1987; Ogilvie et al. 1989, 1991), these findings are relevant to aviation safety.

In this study, EEG data were systematically collected from sleep-deprived subjects in a specially-instrumented rotary-wing aircraft to determine whether the typical increases in theta and reductions in alpha (recorded under controlled conditions in the laboratory) would occur in the in-flight environment, particularly while pilots were at the controls of the aircraft. The magnitude of differences at selected points during 29 hours of continuous wakefulness was examined. In addition, the extent to which EEG changes were associated with fatigue was assessed by collecting cognitive and mood data between flight times.

MATERIALS AND METHODS

Ten UH-60 current and qualified aviators served as subjects. The average age of the participants was 31.2 years (with a range of 26 to 46). Resting (eyes-open/eyes-closed) EEG evaluations were completed both in the laboratory and in the aircraft (while the safety pilot was on the controls). In addition, EEG evaluations were performed while the pilot was flying the aircraft. Performance and mood evaluations were conducted between flights in the laboratory.

In-flight EEG evaluations were conducted using a Cadwell Laboratory Airborne Spectrum 32 which transmitted data to a standard ground-based Cadwell Spectrum 32 for review and analysis. Laboratory EEG evaluations were made with a standard Cadwell Spectrum 32. The low filters were set at 0.53 Hz, the high filters were set at 100 Hz, and the 60 Hz notch filters were used. Grass E5SH electrodes were used to detect EEG.

Subjective mood evaluations were made (in the lab.) using the Profile of Mood States (POMS) (McNair, Lorr, & Droppleman, 1981), a 65-item test which measures 1) tension-anxiety, 2) depression-dejection, 3) anger-hostility, 4) vigor-activity, 5) fatigue-inertia, and 6) confusion-bewilderment. Subjective sleepiness/alertness was measured via the Visual Analog Scale (VAS). Several items were included such as sleepy, alert, energetic, talkative, etc.

Basic cognitive abilities were examined (in the lab.) with the Multi-Attribute Task Battery (MATB), a test that requires subjects to track a target and tune a communications radio while monitoring fuel levels and warning lights and dials.

The test schedule included three training sessions on the first day of participation. These were followed by three testing sessions which began on the second day of participation, continued during the night, and ended on the morning of the third day. On the training day, subjects arrived at the Laboratory at approximately 1000 and were released by approximately 2200. On the following (testing) day, subjects awakened at 0700, reported to the laboratory at 1700, and remained in the laboratory (except for the flights) until approximately 1200 the next day (no sleep was permitted).

On the testing day, EEG electrodes were attached, and the subject proceeded to the first EEG test in the laboratory. The subject was instructed to sit quietly for 5 minutes with eyes open, followed by 5 minutes with eyes closed. Following EEG testing, the subject completed one VAS, one POMS, and performed the MATB for 30 minutes. Afterwards, he completed another resting EEG, VAS, and POMS. Once laboratory testing for the session was complete, the subject was driven to an airfield for the first flight at 2300. After reaching altitude, with the safety pilot at the controls, the subject completed an eyes-open/eyes-closed EEG while the safety pilot was in control of the aircraft. Afterwards, the subject flew several standard flight maneuvers. At the conclusion of the flight, the subject was driven back to the laboratory for the next test session (EEG, VAS, POMS, MATB, EEG, VAS, and POMS) at 0200. Afterwards, the subject departed for the second flight (at approximately 0400). Following this flight, there was one final laboratory test session at 0700 and one final flight at 0900.

RESULTS

A variety of detailed analyses were conducted in this study. For the sake of brevity these will be summarized here, but a detailed account is available in Caldwell, Hall, and Erickson (2002).

EEG Laboratory Data

The ANOVA on *delta activity* included two factors: session (2045, 2140, 0145, 0240, 0645, and 0740) and eyes (eyes open and eyes closed). There were session main effects at Fz , Cz , and Pz; eyes main effects at Fz, Cz, and Pz; and session-by-eyes interactions at Cz and Pz ($p<.05$). Delta power increased from 2045 to 0740 and was greater under eyes closed than eyes open. The session-by-eyes interaction at Cz was due to the fact that there was a small increase in delta from eyes-open to eyes-closed early in the deprivation period (at 2045), followed by a much larger increase later in the deprivation period (at 0645). A similar pattern occurred at Pz. The analysis of *theta activity* revealed session main effects at Fz, Cz, and Pz primarily because of linear increases from the first to the last sessions of the deprivation cycle. Eyes main effects at all three electrodes were due to less theta at eyes-open than at eyes-closed. Session-by-eyes interactions at Fz, Cz, and Pz were all because of more theta under eyes-closed than eyes-open at various points in the deprivation cycle (particularly at 2045, 0145, 0645 and 0740), with the differences being more noticeable at certain times than at others. The ANOVA on *alpha activity* indicated session main effects and eyes main effects at Fz, Cz, and Pz. There were session-by-eyes interactions at Fz and Cz ($p<.05$). A decrease in alpha activity occurred from the first to the last part of the deprivation period at Fz and Cz, and an increase in alpha occurred under the eyes-closed versus the eyes-open condition at all three electrodes. The session-by-eyes interactions were the result of large differences between the eyes-open and eyes-closed conditions at 2045, 2140, 0145, and 0740, with smaller or more variable differences at 0240 and particularly at 0645. *Beta activity* revealed a session difference only at Pz ($p<.05$) which was the result of higher amounts of beta during the first part of the deprivation period (from 2045 to 0145) than at 0645, after which there was a rebound at 0740. Eyes main effects occurred at all three sites ($p<.05$) because of greater amounts of beta under eyes-closed than eyes-open. There were no significant interactions.

EEG In-flight Data

In the in-flight (aircraft) setting, EEG data were collected under a resting eyes-open condition (with the safety pilot on the controls) at the beginning of each flight and subsequently during each of the 15 maneuvers (with the participant on the controls). Only time-related effects will be reported here.

The ANOVA on *delta activity* for flight (2300, 0400, and 0900) and segment (resting, maneuver 1, maneuver 2, maneuver 3, . . . maneuver 15) indicated there was a flight- (or session) related difference only at Pz ($p<.05$). This was due to increased delta from the first two flights to the third. *Theta power* at Fz, Cz, and Pz increased from the first to the last flight, and theta at Pz showed a particularly striking increase by the time of the third flight. *Alpha power* at Fz, Cz, and Pz increased from the 2300 flight to the 0900 flight as well, but EEG *beta activity* did not change as a function of flight time.

POMS

One-way ANOVAs of the scales from the POMS given at 2100, 2155, 0200, 0255, 0700, and 0755 revealed main effects on *tension-anxiety*, *vigor-activity*, *fatigue-inertia*, and *confusion-bewilderment* ($p<.05$). These occurred because mood deteriorated as the hours of continuous wakefulness increased.

VAS

The one-way ANOVAs on the VAS given after the POMS (at 2100, 2155, 0200, 0255, 0700, and 0755) indicated significant session differences on six of the eight subscales: *alertness*, *energy*, *confidence*, *irritability*, *sleepiness*, and *talkativeness*. ($p<.05$). Once again, these were due to linear deteriorations in mood from the first to the last test sessions. Alertness, energy, confidence, and talkativeness declined generally from the beginning to the end of the deprivation period; whereas irritability and sleepiness increased.

MATB

There were statistically significant effects on the *reaction times to warning lights and dials*, the *standard deviation of reaction times to the dials*, and the *root-mean-square (RMS) errors* in the tracking task, due to a linear deterioration in performance from the 2105 session to the 0705 session in all four cases ($p<.05$). In addition, there were quadratic trends in the reaction times to lights, the standard deviation of reaction times to dials, and the tracking RMS errors due to more pronounced decrements towards the end of the deprivation period than the beginning.

DISCUSSION

There were EEG effects in both the laboratory and the in-flight testing situations, and theta activity was affected consistently across the two settings. Theta activity (3.0-8.0 Hz) progressively increased from the beginning to the end of the deprivation period, suggesting that fatigue from sleep deprivation was exerting a negative impact on the physiological alertness of the pilots. In addition, lower-frequency delta (1.5-3.0 Hz) activity also was accentuated as a function of sleep deprivation in both testing situations, but the effect was localized to Pz in the aircraft, whereas it was seen at all three recording sites in the laboratory. Increases in delta activity are primarily associated with sleep in normal adult subjects (Ray, 1990). Differences in alpha activity were not consistent from the laboratory to the aircraft, possibly because of environmental effects (the laboratory environment is more soporific than the noisier and less comfortable in-flight environment). However, the uniform effects in both delta and theta strongly suggest: 1) that participants were becoming more fatigued as the deprivation period progressed, and 2) that this increase in fatigue was detectable via EEG recordings both in the more traditional laboratory setting and in the less-well-researched aircraft setting.

These EEG findings agree with the subjective mood data (from the POMS and the VAS) which indicated that the pilots were adversely affected by sleep deprivation. Ratings of fatigue, sleepiness, irritability, tension, and confusion all increased significantly as a function of prolonged wakefulness, whereas ratings of vigor, alertness, energy, confidence, and talkativeness decreased. These decrements no doubt contributed to the deterioration in basic cognitive abilities observed on the MATB. Although less than half of the MATB outcome measures apparently were sensitive to the effects of sleep loss and fatigue, the ones that did degrade seem particularly pertinent to aviator performance. Degradations in the reaction time to warning lights and out-of-bounds dial indications, along with more variable performance

and increased tracking errors, became more pronounced as the amount of sleep deprivation progressed. Thus, not only were self-perceptions of alertness declining with increased hours awake, but objective measures of performance were deteriorating as well.

Overall the findings from this study suggest it is feasible to monitor increases in the fatigue levels of pilots via the real time acquisition of EEG activity from the in-flight environment. Thus, it is possible to gain insight into the functional status of aviators without disrupting performance on the primary task of flying the aircraft. However, future studies are needed to establish whether there are significant correlations between in-flight physiological changes and in-flight performance changes.

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The use of portable accelerometers in predicting activity energy expenditure

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INTRODUCTION

"*A soldier's level of physical fitness has a direct impact on his combat readiness*" (US Army, 1998). The balance of energy intake (EI) and energy expenditure (EE) can significantly affect soldiers' physical fitness, conditioning, and overall health. The predominant contributor to the variations of EE is physical activity. Unlike most civilian populations, soldiers are often subjected to negative energy balance (EE significantly exceeds EI) (Fridel et al., 1997). For optimum designs of food rations and physical training, accurate and detailed measurements of EE are crucial. However, our current techniques in assessing physical activity are limited, such that possible associations between physical activity and the related EE (EE_{ACT}) with respect to the health and performance in military personnel have not been well determined.

Daily EE can be categorized into three major components: basal or resting EE (also called basal metabolic rate, or BMR), thermic effect of food (or food-induced thermogenesis), and EE_{ACT}. *Resting EE* is the rate of EE measured in postabsorptive, well rested, and thermoneutral conditions. In sedentary subjects, resting EE is the major component of EE (Flatt, 1978). Inter-individual variations in resting EE of normal humans can be explained by differences in fat-free mass (the primary contributor), age, sex, familial traits, and fat mass (Ravussin et al., 1986, 1987). *Thermic effect of food* represents the increase in EE following meal ingestion for absorbing, processing, and storing the nutrients. There are two recognized subcomponents, obligatory and facultative thermogenesis, which combine to represent a small component to total EE (<8-10%) (Jéquier et al.,1988; Wells et al., 1981) under normal conditions. $\underline{EE_{ACT}}$

is the largest variability to total EE in humans. Moderate walking can increase EE by 3 times, while a more vigorous activity such as running can elevate EE by 10 times. Compared to civilians who generally have more sedentary lifestyles, EE_{ACT} is particularly important in soldiers' nutritional and physiological state, affecting performance and overall health (Burstein et al., 1996; DeLany et al., 1989).

MATERIAL AND METHODS

Measuring energy expenditure

Doubly Labeled Water (DLW) **is considered as the "gold standard" for measuring EE in the field or free-living conditions. It determines the net disappearance of hydrogen (through water) and oxygen (through water and carbon dioxide) by stable isotope labeling, i.e., $^2H_2{}^{18}O$ (Schoeller et al., 1982, 1996). The major advantage of the DLW is its non-invasiveness and non-intrusiveness. It has been used to assess EE of soldiers in the field and the impact of different rations (DeLany, 1989), climates (Burstein, 1996), and other training conditions (Forbes-Ewan et al., 1989). However, the main limitation of DLW is that it measures total EE during a period of 7-14 days, without being able to detect the type, duration, and intensity of physical activity, or to trace variations in physical activity and related EE within certain periods. Furthermore, the high cost and relative limited availability of ^{18}O make this method difficult to apply.**

Indirect calorimetry is the "gold standard" method of EE measurement under laboratory environments. It uses a facemask, a ventilated hood, or a respiratory chamber (Sun et al., 1994), to measure oxygen consumption and carbon dioxide production continuously and non-invasively. Major advantages of indirect calorimetry are the immediate and detailed measurements of the rates of EE during different activities and the macronutrient oxidations. The major disadvantage is the limited application under free-living conditions.

Methods of assessing physical activity

Studying the relationship between physical activity and health in humans is complicated by the highly variable nature of physical activity. A particularly challenging area has been the development and application of accurate, valid, and cost-effective techniques to quantify physical activity under field conditions (Paffenbarger et al., 1993; Washburn et al., 1986; Wilson et al., 1986). Numerous methods have been utilized to measure EE during physical activities. They vary greatly in their usefulness in different study populations and designs (Shultz et al., 2001). They can generally be categorized as subjective and objective methods.

Subjective methods

Subjective methods include the use of *direct observations, physical activity records, survey,* and *recall questionnaires*. These techniques are used for various time periods and settings. Although inexpensive and easy to implement, their accuracies are greatly limited by the recording, recall, interviewer, and other biases. Predictions of EE_{ACT} using these methods could be further flawed by interpretation and translation errors. Results from most subjective monitoring methods are thus difficult to quantify and to compare inter-individually.

Objective Methods

Objective methods for current measurements of physical activity mainly consist of mechanical/electronic devices. Since walking and running are the most common types of physical activities, *step counters* are often used estimate overall activity levels. Several types of step counters exist, including pedometers using a mechanical movement counter (Bassey et al., 1987; Washburn et al., 1980), mercury switches (Cauley et al., 1987), and electronic load transducers and foot contact monitors inserted into the heels of shoes sensing loads held, lifted, or carried, and walking activity (Barber et al., 1973; Dion et al., 1982; Hoyt et al., 1994; Weyand et al., 2001). These are generally simple, small, and relatively inexpensive devices that are based on the principle that EE_{ACT} is correlated with individual step frequency and foot contact times (Kram et al., 1990). The main limitation is that the sensitivity and accuracy of step counting may vary significantly among activity types, inter- and intra-individually. Furthermore, stride lengths, a crucial element of the velocity and distance traveled, can only be estimated.

Researchers have recently focused on an array of new activity monitors based on *accelerometers*, which directly measure body movements in terms of acceleration. The most currently used are the piezoelectric sensors that detect accelerations in one (typically vertical direction) or in three orthogonal planes (anterior-posterior, lateral, and vertical). Results can be recorded in a microcomputer. Most current marketed monitors are usually placed on the hip or waist (for its closeness to the center of body mass), although ankle or wrist monitors are also used. Caltrac, Tritrac-R3D (both by Hemokinetics, Madison WI), RT3 (Stayhealthy, Monrovia CA), Computer Science and Application (CSA, Shalimar FL), Tracmor (Maastricht, The Netherlands), and ActiWatch (Minimitter, Sunriver OR) are just a few examples of marketed systems. In several validation studies using these monitors, correlation values ranged from 0.65 to 0.92 between EE measured by indirect calorimetry and accelerometer readings during various activities (Bouten et al., 1994; Bray et al., 1994; Chen et al., 1997; Freedson et al., 1998), where level walking showed the highest correlation with the hip-worn triaxial accelerometers. The advantages of the accelerometry devices include their small size, non-invasiveness, and minimally intrusive to normal subject movements during daily activities. Additionally, they are easy to use for subjects and testers, sensitive to relative intensity, frequency, and duration detections, and have extended measuring periods (minute-by-minute data for up to 28 days), thus making free-living monitoring more feasible. The major limitations include their inability to detect activity types, for which the associations between measured acceleration and EE_{ACT} are dependent upon, single site monitoring that is unable to detect movements from various body segments, limited prediction algorithms to estimate EE_{ACT} across a wide range, and inability to differentiate EE due to postural changes and other low intensity physical activities (Chen, 1997). To compensate for these errors, a combination of using accelerometry devices and inclinometer(s) or mercury switches was used to detect postural changes and motions were reported (Levine et al., 2001; Walker et al., 1997). Recently, several research labs have tested the feasibility of using accelerometer arrays that were positioned at different body segments, mainly the chest, trunks, and thighs, to monitor the types of activities by postural identifications (Bussman et al., 2001; Fahrenberg et al., 1997; Foerster et al., 2000; Zhang et al., 2003). However, EE_{ACT} predictions from these monitors have yet to be carefully validated.

Works from the Vanderbilt Energy Balance Lab

Equipped with the state-of-the-art *whole-room indirect calorimeter* at Vanderbilt, we are in a unique and ideal environment to develop and validate portable activity monitors for EE_{ACT} predictions. The room calorimeter is a small, airtight environmental room (2.6x3.3x2.3 m^3, 19,500 liters in net volume), equipped with a desk, chair, outside window, toilet, sink, telephone, TV/VCR, audio system/alarm clock, and fold-down mattress to simulate free-living conditions (Figure 1). Oxygen consumption and carbon dioxide production are calculated by measuring the changes of oxygen and carbon dioxide concentrations of the air inside the calorimeter and the flow rate of the purged air in an open-circuit design. A special multi-channel air sampling system was designed to ensure an even sampling of the gas expired by the subject. Temperature, barometric pressure, and humidity of the room

are precisely controlled and monitored. With the optimized controls and precision measurements, the minute-by-minute EE is calculated with the highest precision reported (>90% with each minute and >99% over 24 hours) (Sun, 1994).

RESULTS

In a previous study (Chen, 1997), we used a hip-worn triaxial accelerometer monitor, the Tritrac-R3D Research Ergometer (Hemokinetics, Inc. Madison WI), to detect body motion during physical activities. A heterogeneous group of healthy adult volunteers (85 women and 40 men) each spent two separate 24-hr periods (one day with non-intensive walking and stepping exercises and the other day without, respectively denoted the Exercise and Normal Days) in our room calorimeter, where each subject's minute-by-minute EE and body movements were measured simultaneously. The Tritrac-R3D's simple linear prediction model, using the combined signal from all three axes, significantly underestimated EE_{ACT} (by 33% and 49%) and total EE (by 17% and 26%) for normal and exercise days, respectively (Figure 2, parts A and B). Using the EE and acceleration data measured during the Exercise Day, body acceleration components *(A)* measured by the Tritrac-R3D were fitted into a non-linear two-parameter model:

$$EE_{ACT} = a \times A_{horizontal}^{p1} + b \times A_{vertical}^{p2},$$

where coefficients *a, b, p1, and p2* were determined by optimization with the least sum-squared error for each individual. Results showed significant improvements (all $P<0.001$) in modeling total EE (Figure 2, part C), standard error estimation parameters, and correlation coefficients. We then cross-validated these models by applying them to the acceleration recorded during the second 24-hr period (Normal Day) and demonstrated that the predicted total EE was now comparable to the measured values (Figure 2, part D). Furthermore, we showed that a generalized model, using subject's gender, weight, height, and age to replace the individualized coefficients (*a, b, p1, and p2* from the equation above, shown in Figure 2, parts C and D), was also significantly more accurate compared to the one-parameter-linear model by Tritrac-R3D.

However, this model underestimated the EE_{ACT} during low intensities, potentially due to inadequate movement detections of the upper body motion. In a recent study (unpublished), we used a similar study design and measured EE during a 24-hr period in the room calorimeter in 60 healthy volunteers. Body movements were simultaneously measured using the same Tritrac-R3D triaxial accelerometer (worn at the hip). We added a wrist accelerometer (ActiWatch64, Minimitter, Sunriver OR) on the dominant arm for upper body movement measurements. The non-linear power-fitting model was then expanded to include the arm accelerations:

$$EE_{ACT} = a \times A_{hip,\,horizontal}^{p1} + b \times A_{hip,\,vertical}^{p2} + c \times A_{arm}^{p3},$$

We found the Tritrac-R3D and the ActiWatch combined model accurately estimated EE_{ACT} in all intensity categories compared to measured EE_{ACT} by the calorimeter (Figure 3). The particular improvements were in the measurement of lower intensity physical activities, in which sedentary individuals tend to spend most of their time. A second 24-hour study was repeated in a subgroup of 12 volunteers and showed accurate EE_{ACT} prediction compare to measured values (Figure 4).

DISCUSSION AND CONCLUSIONS

In view of the number of current field techniques for measuring detailed physical activity, accelerometers have been shown to be valid and useful. However, the applications of portable monitors to accurately predict energy demands in military personnel during training and field operations are unique. Compared to the more sedentary civilian populations, for whom the most current activity monitors are

designed, soldiers participate in routine training regiments that are often subject to increased physical demands. Marching and running with significant added loads (>10kg), crawling, jumping, climbing, and many other lifting or pulling activities are just a few of the activity types that will present challenges to existing technologies. Furthermore, many trainings and operations are conducted in extreme external environmental conditions, such as hot or cold climates (Burstein, 1996), dry desert or humid jungles (Forbes-Ewan, 1989), and high altitude (Hoyt et al,, 1994), while the internal stress from the imbalance of high total energy demands vs. low energy intake, sleep deprivation, fatigue, and psychological stress (Nindl et al., 2002; Troumbley et al., 1990) may further exacerbate the complexity of the physical activity and EE_{ACT} estimations. Thus, we need to develop and optimize more specific portable methods for the measurements of the various activity types, intensities, durations, and frequencies, and extend to the associated energy demands in military personnel during sustained field operations. Two general areas of improvement are: sensor designs and model development.

Current marketed accelerometry activity monitors primarily use the piezoresistive sensors, either stand-alone or built-in (surface-mounted and integrated) chips. Although mostly unpublished, the ranges of acceleration are generally 0.05-1.0 g, with resolution of 0.02 or worse, and sampling rates of 32 Hz or lower. Although this may be sufficient for monitoring majority of the physical movements of the center of mass (e.g., for the hip-worn monits), movements of upper extremities contain higher frequency components in short bursts which may exceed the maximum range. These limitations would introduce inaccuracies in measurements. Most current activity monitors only use the dynamic component (or the AC component) of the raw signals from the sensors, partially to minimize the drifts from the baseline (or the static or DC component) due to temperature and directional changes. However, if the sensor(s) are positioned at proper locations, such as the chest, it may be useful to access such baseline change with respect to sensor direction for assessing body postures, which may indicate the type of the activities. The dynamic signal from the sensor is generally filtered (corrected for baseline drifts), digitized, full-wave rectified (turn the negative values to positive), and integrated to 15-second epoch or longer to yield the output of activity counts. Although most of the current accelerometry monitors are packaged for easy operations for field researchers, almost all current marketed monitors do not allow user to change key parameters such as sampling rate or to allow raw signal collections, which are crucial to enable fundamental improvements in sensor designs.

Since current available sensors have limited ability to detect wide ranges of physical activity types and intensities, the modeling of the acceleration output to predict EE_{ACT} is an area that needs much more development. We have demonstrated that the acceleration components recorded in the separate directions can be weighed differently to enhance EE_{ACT} prediction, due to body movements in the vertical axis normally demand more energy due to the increased work against gravity, such as in the cases of weight-bearing activities walking, running, and stepping (Haymes et al., 1993; Wong et al., 1981). Furthermore, the linear relationship between the acceleration and EE_{ACT} may not be the pertinent model for all activity types and intensities. Thus, we have systematically developed and cross-validated a relatively simple multi-component power prediction model that significantly improved the EE_{ACT} estimation.

The placement of the monitor is also important. Previous studies have confirmed that the center of mass is the ideal site for monitoring, particularly for weight-bearing activities that contribute to the largest dynamic changes in energy cost. From our unpublished data, we have also seen that minute-to-minute EE during a 24-hr period correlated significantly better with raw measurements of physical activity by a hip-worn triaxial accelerometer ($R=0.825\pm0.046$) than with a wrist-worn uniaxial accelerometer (0.646 ± 0.093, $P<0.001$, $N=60$). However, previous studies also illustrated that a single hip-worn monitor would be inadequate in measuring various physical activity types and intensities. Therefore, combination models that combine signals from multiple body segments need to be explored for improved accuracy in predicting EE_{ACT}.

In addition, other assessment techniques involve physiological measurements may also be incorporated with simultaneous accelerometry monitoring to further improve EE_{ACT} modeling. An example is the use of heart rate monitors, a simple and objective method for the estimation of EE during certain levels of physical activity and exercise (Spurr et al., 1986). Moreover, heart rate

monitoring may facilitate the measurements on fatigue, state of hydration, body temperature changes, and emotional state (stress) that could affect the energy metabolism (Nielsen et al., 1993; Yoshida et al., 1994). Other physiological parameters, such as core body temperature (Gass et al., 1998; van Marken et al., 2001), galvanic skin conductance (estimating heat loss through sweating), and surface electromyography (measures of muscular activity), may also be explored to reveal their potentials to facilitate the prediction of EE_{ACT}.

In summary, to enhance our abilities to assess the energy demands in soldiers in the field, future research in technologies should focus on small and wireless sensors that can be positioned non-invasively and non-intrusively to measure body movements as well as physiological responses. Accelerometers are suitable for many aspects of the physical activity monitoring; however, much can be improved to increase their sensitivity and further reduce their size. The complex characteristics of the human physical activity, large inter- and intra-individual differences in energetic efficiencies, and inherent limitations of the sensors, dictate that the development of advanced models to accurately predict EE_{ACT} should integrate more unique features of the signals from the sensors, rather than simple averaged signal outputs. This requires that we collect the raw signals from sensors while measure EE_{ACT} simultaneously. Moreover, advanced pattern recognition and automated classification modeling techniques, such as artificial neural networks that can incorporate multiple input parameters and output feedbacks for non-linear and adaptive modeling, need to be explored. The ideal development processes of such portable activity monitors should include the use of a respiratory chamber for sensor and model explorations under laboratory conditions, portable indirect calorimetry units for short-term field evaluations, and DLW for overall validations. Furthermore, we should optimize such monitoring systems to the specific applications through modeling, such as weather conditions and external loads, while broadening the general applications to benefit civilian medical research.

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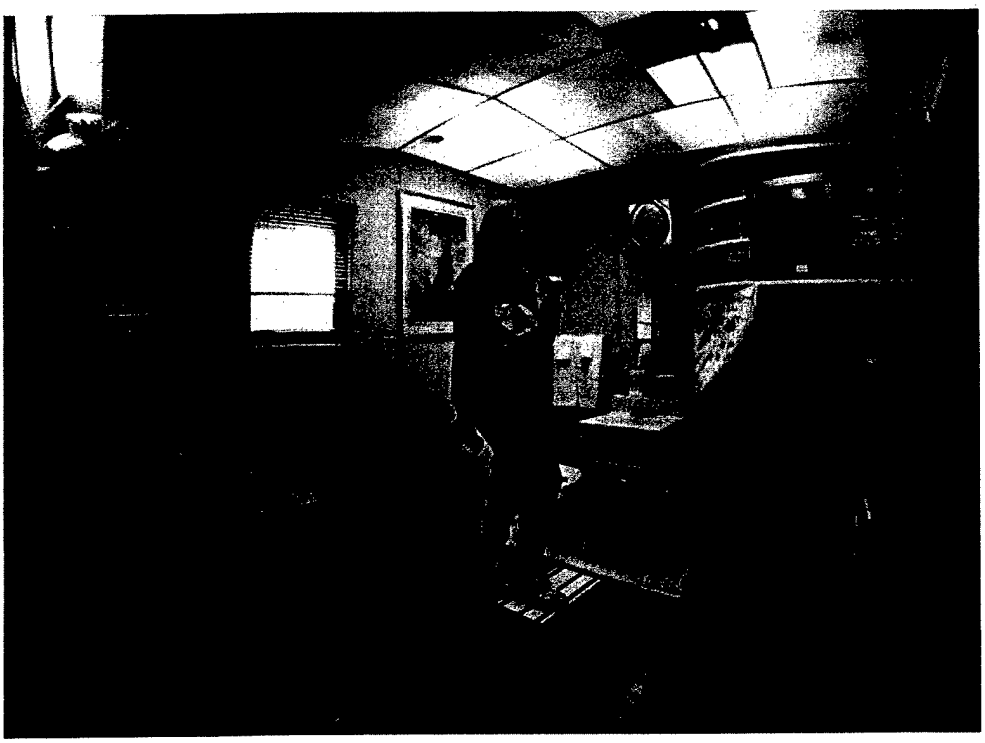


FIGURE 1 **The whole-room indirect calorimeter at Vanderbilt University.**

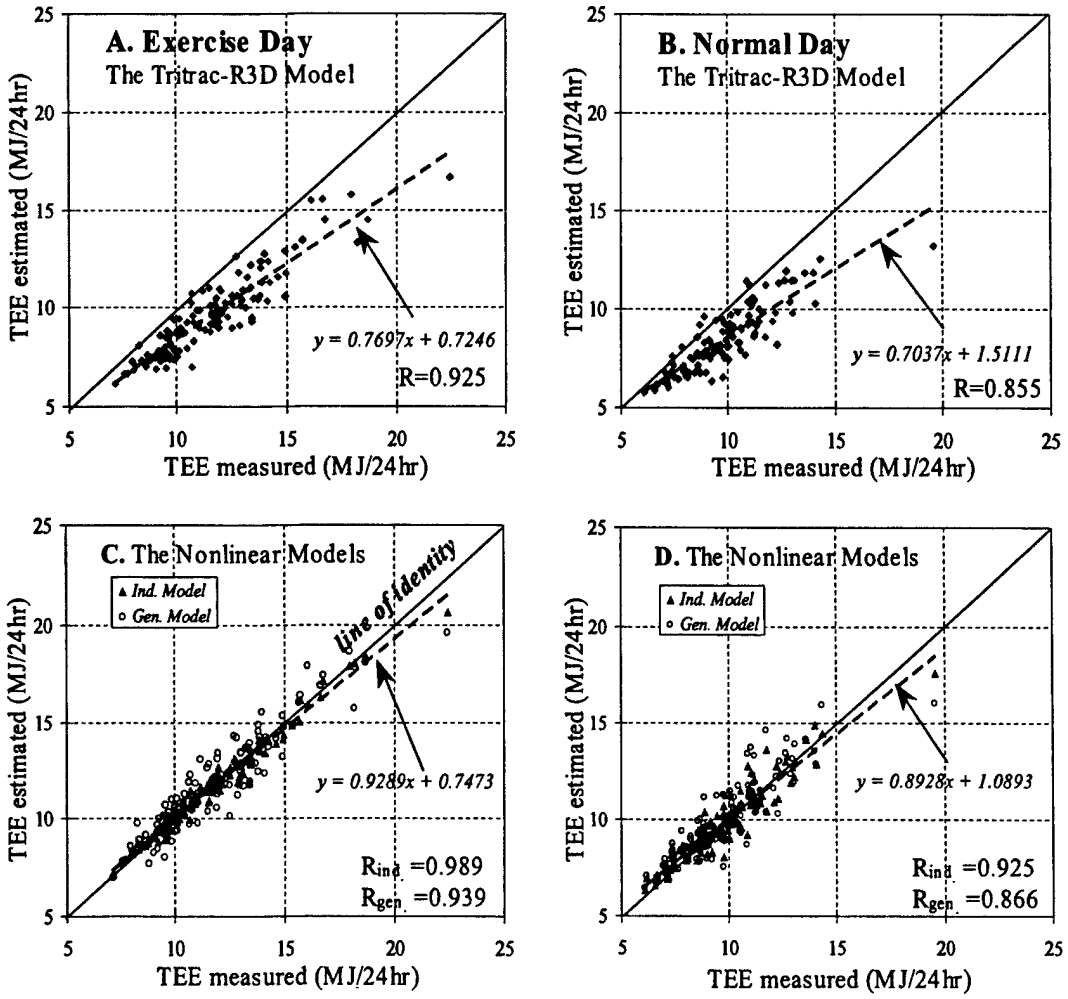


FIGURE 2 **Total daily energy expenditure (TEE) estimated by the Tritrac-R3D model (A. Exercise Day, and B. Normal Day) in 85 healthy women and 40 men, and by the two-component nonlinear models (C. Exercise Day, and D. Normal Day) versus TEE measured by the calorimeter. The line of identity signifies a perfect match between the estimated and the measured values in the room calorimeter. In C and D, individual (Ind.) model represents the parameters fitted for each volunteer, and general (Gen.) model represents the model using only the subject's gender, weight, height, and age to replace the individualized coefficients.**

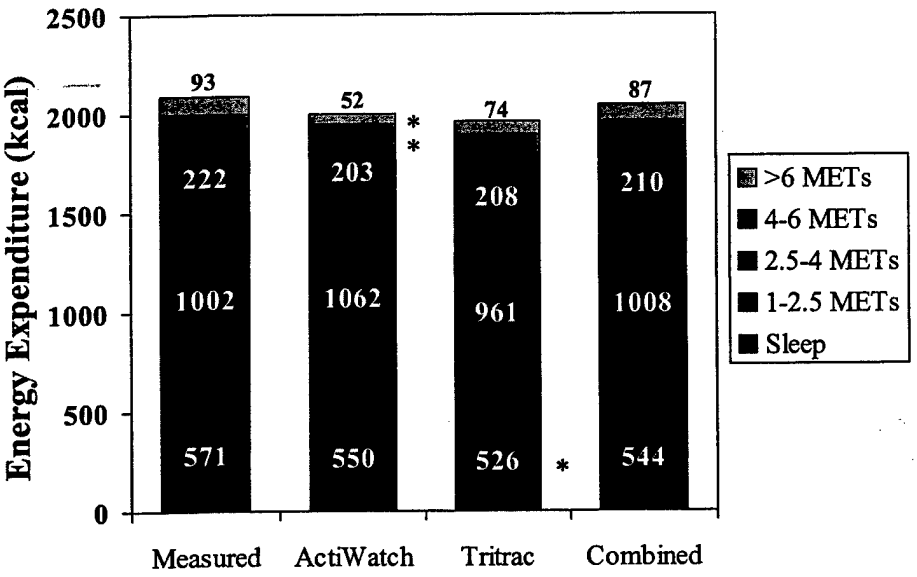


FIGURE 3 **Averaged energy expenditure (EE) in separate intensity categories of one 24-hour period in 60 healthy sedentary women (age 35.4±9.0 years and BMI 30.0±5.9 kg/m^2). Comparison between EE measured in the whole-room indirect calorimeter, estimated by the ActiWatch, the Tritrac-R3D, and the ActiWatch and Tritrac-R3D monitors combined. METs: metabolic equivalents, calculated as ratio of individual energy expenditure and resting energy expenditure. (* P<0.05 compared to the measured values).**

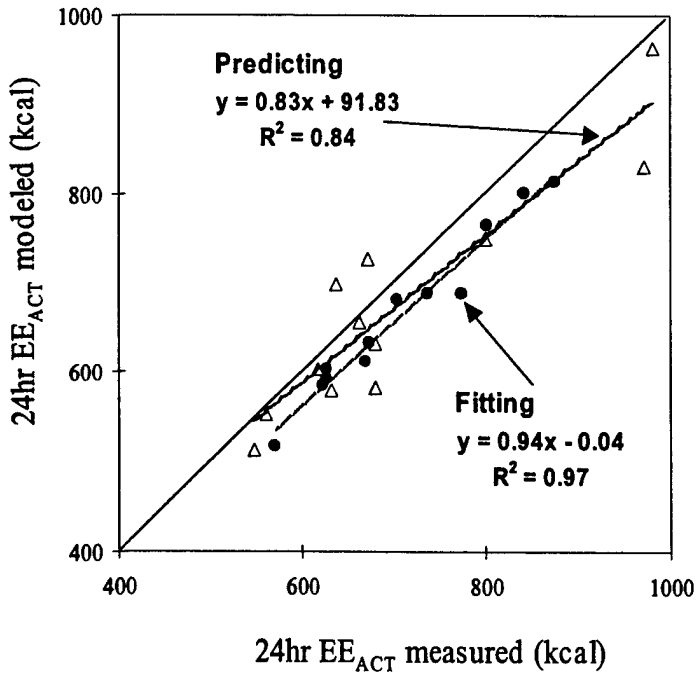


FIGURE 4 **Total energy expenditure of physical activity (EE_{ACT}) in 12 healthy women during two 24-hr periods (identical protocol) measured in the room calorimeter, compared to the estimated from the activity monitors. One day was randomly selected for fitting with combinations of ActiWatch on the wrist of the dominant hand and Tritrac-R3D at the waist, and the second day used as prediction validation.**

SWEAT PATCH AS A NOVEL APPROACH TO MONITOR THE LEVEL OF ACTIVITY OF THE STRESS SYSTEM: POTENTIAL APPLICATION FOR STUDIES CONDUCTED IN THE FIELD

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INTRODUCTION

The Stress System: General Characteristics

The stress system has evolved to maintain homeostasis in response to disruptive internal or external stimuli (Crousos et al., 1995). A highly conserved system, the stress system is represented in lower species and has a central as well as a peripheral component. The central component involves several areas in the central nervous system including the paraventricular nucleus of the hypothalamus, the main source of corticotrophin-releasing-hormone (CRH), the locus coeruleus, the main source of catecholamines, and the pre-frontal cortex, the cortical coordinating center. The afferent limbs of the stress system include the pituitary-adrenal axis with its main effector molecule, cortisol, and the sympathoadrenal system with its two main effectors, the catecholamines norepinephrine and epinephrine, produced by the sympathetic terminal nerves and by the adrenal medullary gland, respectively.

These effectors of the stress system regulate a highly coordinated response aimed at mobilizing energy, increasing arousal, and restoration of homeostasis in the face of threatening stimuli. Other endocrine systems, including the GH/IGF-1, the reproductive and the thyroid hormone axes are inhibited by the stress. During acute and chronic stress, complex alterations of the immune system also take place which result in a switching from a cellular immune pattern of response to a humoral response (TH1 to TH2) (Chrousos et al., 2000). Such a coordinated series of responses is essential to survival. A complex network of inhibitory feed-back loops within and among the above components of the stress system has evolved to ensure that the stress response is effective but contained.

The Stress Response: Specific or Non-Specific?

Initially the stress response was thought to be homogeneous in response and independent of the nature of the perturbatory challenge, a concept originally formulated by Hans Selye and known as the doctrine of the non-specificity of the stress response. More recently it is being recognized that different stressful stimuli may elicit different patterns of stress responses. In addition, there is growing evidence suggesting that genetic variability as well as differences in experiences during the first years of life, or even during intrauterine life, may "imprint" the stress responsivity of any given individual in a stable fashion. A

corollary of the specificity of the stress response is that different stressful stimuli, if protracted and/or severe enough, are associated with different diseases.

The Stress System: Theoretical and Practical Challenges in Monitoring its Activity in Vivo

There are several challenges to measuring the levels of activity of the stress system (Eskandari et al., 2002): a) the complexity of the system and the multiplicity of the effector molecules to be measured; b) the methodology used to measure the activity of the stress system must not perturb the system; c) the intrinsic variability of several hormones due to their circadian rhythmicity; d) the importance of measuring stress reactivity as well as baseline stress response measures; e) finally, for studies conducted in the field, the feasibility of collecting integrated measures without using large volumes of blood or other biological samples.

The approaches currently used to measure the activity of the stress system in field studies originally evolved from the approaches used to measure the activity of the HPA axis in selected categories of patients, including patients with hyperactivity of the stress system such as patients with Cushing's disease, patients with major depression or patients with a rare tumor of the adrenal gland, the pheochromocytoma (Cizza et al., 1997). The clinical methods used in these cases involve measurements of the relevant circulating hormones during both basal and stimulated conditions, and often, given the diurnal rhythmicity of these hormones, around the clock measurements. Biological fluids collected in clinical settings include blood and sometimes cerebrospinal fluid or hypophysial portal blood for specific research purposes; more frequently in an outpatient setting urine or saliva are collected. However in the field would be necessary to collect biological fluids in a non invasive manner, with a non-bulky collection apparatus, minimal discomfort and cooperation from the subject. The purpose of this chapter is to provide support for the sweat patch method in which sweat collected by means of a commercially available cutaneously applied patch may represent a viable option for monitoring indices of stress system activity in the field.

Biology of the Stress Response and Bone Mass

An example of a serious medical consequence resulting from chronic stress is osteoporosis. Bone loss and fractures are often observed as a consequence of hypercortisolism resulting from endogenous Cushing's syndrome or the chronic use of steroids (Cizza et al., 1996). It is becoming more evident that subjects suffering from major depression also exhibit bone loss likely due to hypercortisolism (Figure 1) (Cizza et al., 2001). CRH hypersecretion and hypercortisolism in turn lead to the inhibition of the reproductive axis and hypogonadism. The latter is an established risk factor for bone loss in both genders. CRH hypersecretion and hypercortisolism also decrease the activity of the GH– IGF-1 axis, an important enhancer of bone formation. In depression, a dysregulation of several inflammatory mediators including interleukin-6 has also been reported. Interleukin-6, a major mediator of bone resorption, is elevated in depressed subjects, especially at an older age. Increased sympathetic activity, often observed in depressed subjects also is associated with increased interleukin-6 secretion. This cytokine may be implicated in some of the other medical consequences of major depression, such as cardiovascular disease and insulin resistance.

Stress fractures are often observed in young military recruits of both genders during intense military training or operations (Imeida et al., 1999). In addition to the obvious physical component of mechanical overload associated with marching for a long period time with heavy loads, it is reasonable to hypothesize that some of the endocrine responses associated with the psychological stress may accelerate bone resorption and decrease bone formation. Specifically, an increase in cortisol and a decrease in IGF-1, an important contributor to skeletal integrity, may synergistically with mechanical overload, decrease bone mass at specific skeletal sites below the threshold for fractures (Munoz-Torres et al., 2001). Stress fractures are a common problem in young people who engage in vigorous physical activity especially endurance training. For example, stress fractures during basic training occur in approximately 7% of male and 14% of female recruits. It is therefore important to identify subjects at greater risk; known risk

factors include low level of physical fitness, current or past history of smoking, more than 10 alcoholic beverages/week, use of corticosteroids, and low body weight. In women additional factors include amenorrhea, delayed menarche and use of depo-medroxyprogesterone acetate. In young men, low levels of testosterone, a hormone with an anabolic effect on bone, in a hypogonadal range are reported during intense training. To the best of our knowledge, there are no studies addressing the potential association between patterns of the individual stress response and subsequent risk of stress fractures, most likely because of the lack of a feasable method to measure the stress response in the field in an integrated fashion.

Sweat: Background and Physiology

Summarized below is information supporting the notion that sweat may represent a biological fluid from which it is feasible to measure endogenous substances released during stress in ambulatory or field situations (Guyton et al., 2000). In humans, three types of sweat glands are present. *Apocrine* sweat glands are largely confined to the axillary and perineal regions and their ducts open directly into hair follicles. The *apoeccrine* sweat glands are present in adult axillae. They develop from eccrine-like precursor glands and their ducts open directly onto the skin surface. The *eccrine* sweat glands are distributed over the entire body. Generalized eccrine sweating is the physiologic response to an increased body temperature. This is the most effective means by which humans regulate their body temperature through evaporative heat loss.

The eccrine sweat glands develop from the epidermal ridge as a cord of epithelial cells growing downward. These glands are stimulated by the cholinergic sympathetic nervous system. The preoptic hypothalamic area plays an essential role in regulating body temperature. Efferent nerve fibers originating from the hypothalamic preoptic sweat center descend through the ipsilateral brain stem and synapse in the intermediolateral cell columns of the spinal cord without crossing. The myelinated axons rising from the intermediolateral horn of the spinal cord (preganglionic fibers) pass through the anterior roots to reach the sympathetic chain and synapse. Unmyelinated postganglionic sympathetic class C fibers arising from sympathetic ganglions join the major peripheral nerves and end around the sweat glands. The major neurotransmitter released from the periglandular nerve endings is acetylcholine. In addition, ATP, catecholamine, vasoactive intestinal peptide (VIP), natriuretic peptide (ANP), calcitonin gene-related peptide (CGRP) and galanin have been localized in the periglandular nerves.

The eccrine sweat gland consists of two segments, a secretory coil and a duct. The secretory coil secretes an ultrafiltrate of plasma-like fluid called the primary secretion. The concentration of sodium is about 142 mmol/L and chloride about 104 mmol/L, with much smaller concentrations of the other solutes of plasma. In addition, sweat glands excrete heavy metals, organic compounds and macromolecules. As this precursor solution flows through the duct portion of the gland, it is modified by reabsorption of most of the sodium and chloride ions. This reduces the osmotic pressure of the sweat fluid to such a low level that most of the water is then also reabsorbed. The degree of this reabsorption depends on the rate of sweating. When the sweat glands are stimulated only slightly, the primary secretion passes through the duct slowly and essentially all the sodium and chloride ions are reabsorbed. The concentration of each of these falls to as low as 5 mmol/L, followed by reabsorption of water, which concentrates most of the other constituents. Conversely, when the sweat glands are strongly stimulated by the sympathetic nervous system large amounts of primary secretions are formed and the concentrations of the sodium and chloride ions are then at a maximum of about 50 to 60 mmol/L and little of the water is reabsorbed.

Content of Human Sweat in Hormones or Cytokines

Several studies have been reported using skin biopsy specimens or sweat specimens collected over an oil barrier on a plastic film or in a polypropylene sack. Traditionally, sweat is collected after exercise or exposure to intense heat. Interleukin (IL)-1 and IL-1β, IL-6, IL-8 and tumor necrosis factor have been identified in human sweat. Interestingly, to the best of our knowledge there are no published reports on the presence of cortisol or catecholamines in sweat in humans.

Why the Sweat Patch?

We propose to use a cutaneous patch as a convenient and noninvasive technique that may overcome several of the limitations intrinsic to blood and urine collection. Such a technique would have the advantage of being non-invasive, easily applied at any time of the day and of being worn for an extended period of time with minimal discomfort. A series of biochemical markers of bone turn-over, cytokines and neurohormones may be measured in microliters of specimen, using state-of-the art technologies such as recycling immunoaffinity chromatography and cytokine chip technology. These techniques require a minimum amount of biological sample thus overcoming the need for collection of large volumes of blood. Once validated, the cutaneous patch in conjunction with ultramicro analytical immunochemistry method should substantially expand our ability to examine and understand the interactions between the endocrine, immune and nervous systems and its role in stressfull conditions in the field.

*Current Clinical Application of the Cutaneous Patch in Diagnostic Test*ing

A cutaneous patch is approved by FDA for qualitative detection of a variety of drugs and their metabolites, including opioids, benzodiazepines and metamphetamines. It can also be used to measure methadone, caffeine and nicotine. This device is commercially available under the trade name of Osteopatch® and has been used for determinations of free pyridinoline cross-links in sweat. It has been validated in healthy subjects and in subjects affected by metabolic bone disease, including postmenopausal osteoporosis, and hyperparathyroidism. The Osteopatch has been used in subjects treated with estrogen replacement therapy, and treated with alendronate. Sweat determinations of pyridinoline reflected true changes in bone resorption due to metabolic disease and antiresorptive treatments indicating that these measurements were valid and accurate (Sarno et al., 1999, 2001). In addition, as pyridinoline in sweat arises from plasma, measurements of pyridinoline in this biological fluid reflect true bone resorption more closely than urine measurements.

To correct for sweat volume, determinations of potassium are performed. Potassium is consistently recovered from the patch and its secretion is reasonably well correlated with sweat volume. In contrast to sweat sodium and chloride, potassium is relatively insensitive to subject age, diet and methods of fluid replacement (i.e. intake of water only as compared to glucose-electrolyte solution) in situations of extreme heat. The latter characteristics make this test potentially valuable for field studies.

In order to validate the reliability and sensitivity of use of the Osteopatch for collection of stress, neuroendocrine, and immune biomarkers in sweat, it is necessary to: 1) determine the range of stress, neuroendocrine, and immune biomarkers that can be measured in sweat and the stability of these biomarkers under various collection conditions 2) determine the degree to which these sweat biomarkers reflect their concentrations in other biological fluids including blood, urine, and saliva.

METHODS

Description of the Device The Sweat Patch: Advantages and Pitfalls

The transdermal diagnostic skin patch is a device that provides easy, non-invasive, reliable and relatively non variable access to body sweat. The patch is a non-occlusive sweat collection device. It consists of an adhesive layer on a thin transparent film of surgical dressing with a cellulose absorbent pad attached. The patch passively collects and concentrates non-aqueous components of sweat. The outside surface of the patch forms a barrier for substances in the environment. The potential disadvantage of the non-occlusive design is that the volume of the secreted sweat is not measurable and thus the concentrations of analytes cannot be normalized to sweat volume. This limitation may be overcome by normalizing against potassium measurements. The patch can be worn over an extended period of time (usually a few days) and is reported as being well tolerated by the manufacturer. However we propose to limit its application to a period of time not longer than 24 hours. In our limited experience so far we have observed no adverse reaction with the exception of one subject, a 36 year old female normal volunteer

who, six months after the application of a patch, had an area of discoloration on the abdomen in the area in which the adhesive part of the patch had been applied.

Analytical Procedures

Two major challenges are encountered from an analytical perspective when measuring biological analytes in sweat: 1) the available assays are not sufficiently sensitive to detect some analytes and, 2) there may not be sufficient volume to perform all the measurements needed. The application of newer technologies, including Recycling Immunoaffinity Chromatography and the cytokine chip technology, described below, should address both challenges (Brown et al., 2000; Phillips et al., 1997).

Recycling Immunoaffinity chromatography (RIC)

Specimens can be analyzed for cytokines, hormones, biochemical markers of bone turn-over or any other substance of interest, using a 25-μL sample injected into a modified liquid chromatography system, equipped with a panel of 25-30 immunoaffinity columns packed with antibody-coated glass beads. The specimen passes through the columns in a serpentine fashion, each column extracting a single analyte, while allowing the non-reactive materials to pass to the next column. The bound analytes are measured by sequential acid elution of each column followed by laser-induced fluorescence detection. Concentrations of each recovered analyte is calculated by comparing them to standard curves constructed by running known amounts of pure analyte through identical conditions.

Cytokine Chip Technology

Glass chips are constructed by covalently immobilizing 200 nanoliter (nL) spots of avidin to the glass surface via a robotics system. The chips are heat annealed, washed in 0.01M phosphate buffer, pH 7.4, and blocked with 0.1% bovine albumin. The chips are then re-washed in phosphate buffer, dried and stored under nitrogen at -70°C. Biotinylated antibodies, directed against the analytes of interest are spotted in appropriate patterns onto re-hydrated chips and incubated in a moist chamber for 60 minutes at 37°C. The chips are then incubated with fluorescent-labeled specimens for 60 minutes at 37°C, washed in phosphate buffer and read in a laboratory-built laser-induced fluorescence reader (Instrument Development Resource, DBEPS, ORS, NIH). The concentration of each analyte is calculated from calibration curves constructed by subjecting known standards to the same analytical procedure.

RESULTS

Table 1 lists analytes which can be detected either under baseline conditons or after a brief bout of exercise in sweat collected by the means of the patch. The panel of substances which can be measured in sweat includes inflammatory cytokines such as IL-1, TNF-alpha, IL-6, and IL-8; neuropeptides stimulated by pain such as substance P; hormones increased during the stress response, such as cortisol or prolactin; IGF-1 an important factor for bone regeneration, as well as several chemokines. In addition this device is already marketed for the measurements of several markers of bone turn-over.

SUMMARY

In summary, we have collected sweat at baseline conditions and after a short course of exercise from which we measured several biomarkers, including stress hormones, neuropeptides, and cytokines, by applying ultrasensitive techniques requiring minute amounts of biological samples. Many of the analytes that we have detected in sweat have never been described in this biological fluid. As the endocrine and immune responses to stress are highly interconnected, the ability to measure many of the molecules involved in these responses in the same sample greatly enhances the ability to more precisely define those complex interactions at an individual level. Since the Osteopatch is designed and approved for measurements of biomarkers of bone turn-over, the integration of these bone measures in sweat, together

with stress hormone and immune cytokine measures which contribute to bone loss, should provide a sensitive method for detection of predictive conditions leading to deleterious effects.

In conclusion, the sweat patch provides the opportunity to conduct naturalistic studies outside of the laboratory on a very large number of subjects. Once validated in a reference population, this technique would allow for the early identification of subjects who, because of their individual physiological responses to stress, may be at greater risk during intense training of stress fractures, acute infections, or other stress-related accidents.

Conflict of Interest: None of the authors has any commercial interest in the development of the device described in the current chapter or any other similar device

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TABLE 1 Substances Detected in Sweat Collected by the Osteopatch

Type	Substance
Cytokines	TNFα, IL-1α*, IL-1 β, IL-6, IL-8
Hormones	Cortisol, Substance P, Calcitonin Gene Related Peptide, NPY, βendorphin, Prolactin*, VIP, Angiotensin, IGF-1*, GH
Miscellaneous Compounds	PCAP*, NT-3*, TGFß, LIF*, IP-10*, EGF, CNTF, NGF, ßFGF*, MIP-α*, NGF

*substance that can be detected in sweat only after a 12 min walking-running test

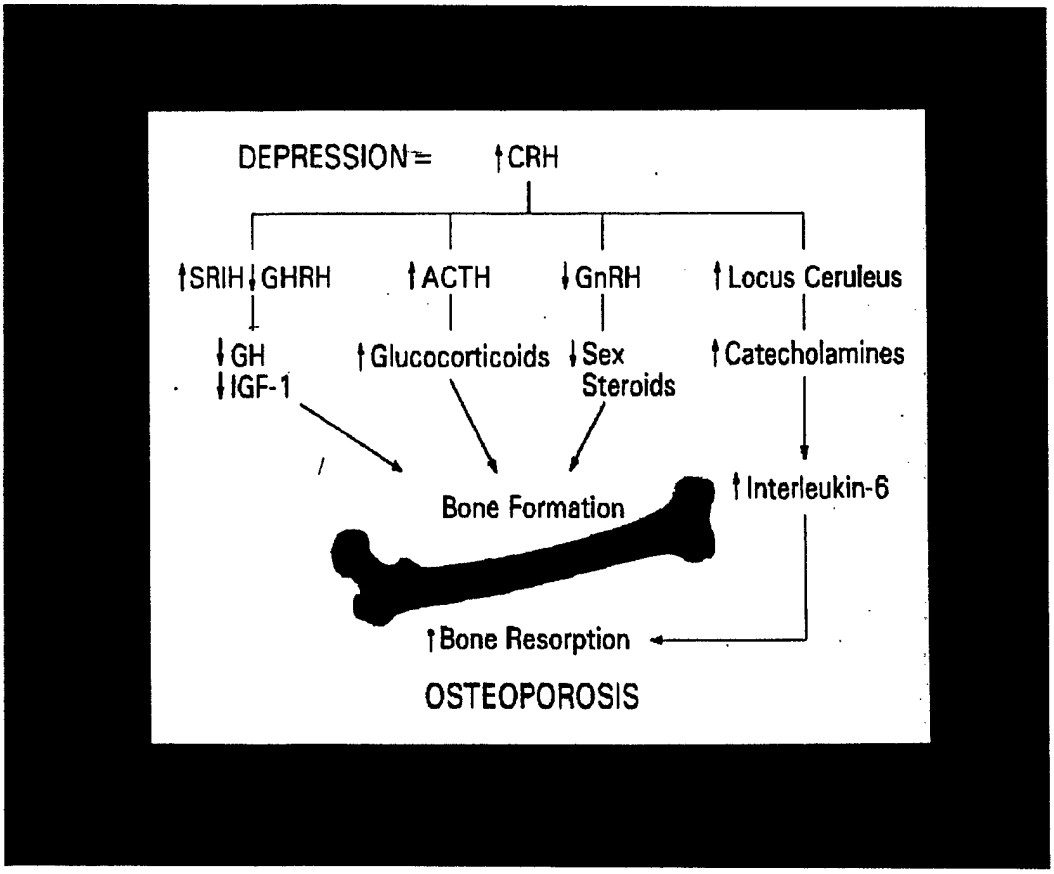

DEPRESSION
↑CRH
↑SRIH ↓GHRH
↑ACTH
↓GnRH
↑Locus Ceruleus
↓GH
↓IGF-1
↑Glucocorticoids
↓Sex Steroids
↑Catecholamines
Bone Formation
↑Interleukin-6
↑Bone Resorption
OSTEOPOROSIS

FIGURE 1

Predicting and Protecting Performance using Metabolic Monitoring Strategies: It's all wet stuff anyway, isn't it?

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INTRODUCTION

The ultimate reductionistic view of the Military Operational Medicine Research Program (MOMRP) centers on metabolism as the answer to all questions. For every problem we are trying to solve in the MOMRP program, we will someday complete the connection to a metabolic basis. This includes Soldier performance problems that range from extended physical stamina to sustaining optimal mood and behavior. While this first principles approach is not likely to provide many near term solutions to MOMRP problems, we can exploit the emerging physiology to develop monitoring technologies. Insight into this metabolic activity should help predict individual status and physiological reserve. This is based on the premise that these metabolic processes are the basis of the responses that allow organisms to survive in the face of environmental challenges and are the earliest indicators of a change in physiological status. This calls for a thoughtful review of currently known regulatory mechanisms that suggest promising predictive markers of status and impending failure of adaptive response capabilities. We should also consider applications of the most promising monitoring technologies that are currently available. The focus of this workshop is to address: what are the best metabolic targets for monitoring and what are the most promising monitoring technologies?

This is needed for predictions about the readiness status of individuals in training and operational settings where human performance is important. We have formidable monitoring capabilities on military systems but lack real time information on the status of our own troops. This serves U.S. Defense priorities to "assure readiness of the Armed Forces" and to "transform the Department of Defense" (including experimenting with new approaches to warfare).

RESEARCH REQUIREMENTS FOR PHYSIOLOGICAL MONITORING

Monitoring Soldier status has become increasingly important because of new lethal and complex technologies that require high reliability of the human operator, and new tactics that reduce line-of-sight contact with team members and increase geographical distance and isolation. No longer is Soldier monitoring just a nice-to-have technological replacement for common sense or for good leadership that includes understanding the signs of Soldier limits. Soldiers may not know they are reaching dangerous levels of overheating and dehydration and, if they are fully encapsulated in protective suits and operating in a remote site, their team leaders also may not know they are heading for trouble. An alert to the individual on their future helmet visor display and/or an automatic "911" message to their squad leader can provoke a prompt intervention and save a mission.

The Navy is designing ships with substantially reduced crew sizes, calling for greater reliance on each individual. Monitoring the status of these sailors becomes especially important if they are incapacitated

in an isolated crew compartment especially during high risk damage control operations fighting fires or flooding. The concept of the Reduced Ships-Crew by Virtual Presence (RSVP) is for smart ships to continuously receive data on the status of the ship, as well as the crew within the ship (Street et al., 2002).

Today's high performance aircraft can easily exceed the limits of human physiological tolerances and one concept for physiological monitoring includes monitoring approaching loss of consciousness to trigger an automatic take over of the controls (Forster et al., 1994). This calls for a highly responsive and rapid system that identifies a major lapse in the pilot capabilities with high reliability.

Monitoring in training is at least as important as in operational environments. It may be most useful for leaders to use physiological monitoring to learn the limits of their own soldiers during training operations and then during an actual operational mission only rely on specific warnings about real time status. Other aspects of metabolic monitoring may not require a wearable system but simply periodic testing to determine, for example, if individuals have reached a high state of bone and muscle remodeling during their training and can reduce a high probability of injury by resting the next day. This kind of feedback will be broadly useful to learning limits of individuals and units.

Physiological monitoring is also being explored for a wide variety of other military applications, including the forensic "black box" flight recorder-type of analysis of a pilot's mental state after a class A accident, in order to prevent future accidents (Forster, 2002). There is also a need for overall "whole body" health markers for easy assessment of global indices of service member health at regular intervals throughout their career. This could eventually represent some combination of psychological and physiological health, monitoring brain metabolites through MRS scans, whole body oxidative stress load assessments, mitochondrial redox potential of critical brain cells, etc. as common final pathways of health status.

RECENT EVOLUTION OF MONITORING RESEARCH

Physiological monitoring concepts are not new but the measurement technologies have advanced more rapidly than our understanding of what the measurements mean to health and performance. Fifty years ago, the Office of Naval Research and the Army Surgeon General cooperatively studied infantrymen in combat to identify metabolic predictors of mental status (Davis et al., 1952). Using neuropsychological testing (including visual flicker fusion and auditory flutter fusion tests), and blood and urine testing, they assessed hydration status, adrenal stress markers, and corresponding changes in cognitive functioning. Studies by the Air Force explored the use of EEG to monitor pilot performance as early as the 1950s (Sem-Jacobsen, 1959). Current studies are examining many of the same factors and relationships that were tested in the studies 50 years ago. Although these empirical studies have some technological advantages, most notably electronic computing power, the studies have largely relied on available technologies instead of exploration of the most suitable measurement targets and development of specifically needed monitoring technology. Many of the available technologies are simply telemetered applications of clinical monitoring systems, limiting advances to spin offs from standards of medical care. We have spent too much time trying to find uses for new measurement technologies, instead of pushing the development of technology to systematically test what we understand about physiology and predict outcomes of greatest importance.

The greatest barrier to advances in performance monitoring has been the lack of suitably defined performance outcome measures. Until recently, aviator performance has been the most extensively studied model for physiological monitoring. Military aviators have been a logical focus because of the need (i.e. the high costs associated with catastrophic performance failures) and because of the experimental advantages. Performance outcome measures are better defined for aviator tasks, especially the ultimate outcome of successful landing versus disaster. The cockpit also provides a friendly setting for clunky prototype monitoring systems that are power hungry and tethered to heavy equipment. Aviator studies can provide early proof of concept for systems that are later reduced in size, weight, power, invasiveness for untethered applications in Soldiers, marines, and sailors. Nevertheless, the aviator monitoring studies are not generalizable without the further development of performance assessment methods and metrics.

Without suitable performance measures, results from lab-based studies cannot be translated into militarily-relevant outcomes. These measures are also needed for field studies which are otherwise forced to rely on simple dichotomies of "no bad outcome" or catastrophic failure (e.g., heat stroke, serious injury, or mission failure). The MOMRP has invested heavily in the development and standardization of practical neuropsychological tests (e.g., the Automated Neuropsychological Assessment Metric, ANAM)(Kane and Kay, 1992) and current field studies are attempting to link these test results with military performance. For example, simple reaction time remained impaired following sports concussions in military cadets even after they were cleared for return to duty by clinical criteria; the significance of this finding to other performance measures is being further investigated. Cold water immersion reliably affected the matching-to-sample test; what this means to Navy diver performance capabilities is being further investigated. One eventual monitoring application would be to embed informative tests into common military tasks that could be monitored to obtain unobtrusive periodic assessments of an individuals performance status. We are currently sponsoring a DoD review of methods and metrics for performance assessment that synthesizes the current state of the knowledge on militarily-relevant performance assessments and models (Ness et al., in preparation). We have also launched a new research initiative on the development of military performance assessment methods based on measures of neurological function such as voice stress analysis and eye saccades ("Science Technology Evaluation Package 3.C").

Physiological monitoring moved from a research sidelight to a central objective in the Army research program under the guidance of Dr. Fred Hegge in 1996. The goal of the Warfighter Physiological Status Monitoring (WPSM) initiative is to make real time performance predictions that leaders can use to assess the readiness status of their forces. The concept is to develop a Soldier-acceptable, minimally invasive (SAMI) sensor set with on-the-soldier analysis. The output (which can be queried for further information) will be a simple "green" (within normal limits), "amber" (physiological challenges are present), or "red" (systems have failed and the Soldier is a casualty). This relies on the vast trove of environmental physiology and psychological data collected and modeled in DoD research programs for many years. A key feature of the approach is that these systems must also learn the usual range of responses for its soldier, accounting for individual variability. Currently, WPSM is a research "tool kit" to learn more about normal and abnormal physiological signals encountered in real soldier environments; these include a range of responses that routinely exceed those that can be obtained in an ethically developed experimental laboratory setting. WPSM will ultimately be reduced to the minimal sensor set needed for highly reliable and important predictions. Reed Hoyt currently leads this program with development of experimental signal acquisition and data handling systems, and data collection studies with Marines and Soldiers in challenging training environments (Hoyt et al., 1997; Hoyt et al., 2001). The immediate requirements for WPSM are to provide status for thermal strain, live-dead

detection, sleep history, and energy expenditure for the Land Warrior system. In later iterations of this system (e.g., the Objective Force Warrior), more sophisticated monitoring capabilities and performance predictions are planned that will also include early casualty triage capabilities.

EXAMPLES OF CURRENT RESEARCH EFFORTS, CONSIDERATIONS, AND LEVERAGING FROM RELATED PROGRAMS

We have chosen several critical areas for review: hydration and heat production; substrate utilization and energy metabolism; muscle and bone remodeling; and brain function. These traditionally separate research areas are interrelated through metabolic processes. For example, exertional rhabdomyolysis has elements of hydration and heat exposure, energy flux, and muscle remodeling, with early effects on mental status (Gardner and Kark, 1994). The topics are also closely interrelated through common measures that might signal changes in one or more of these physiological categories. For example, shivering may indicate a variety of threats that when combined with one or two other measurements can unambiguously distinguish impending hypothermia risk, exposure to a neurotoxic chemical, or intense psychological fear. Brain function reflected in cognitive, mood, or psychomotor measures (e.g., speed of mental processing, irritability, and marksmanship) may be a common and sensitive marker of deficits of all the other stressors and functional deficits of interest. These may include each of the topics in this workshop, including carbohydrate metabolism in physical exhaustion (Frier, 2001), dehydration or significant fluid shifts such as those observed in the brain with acute mountain sickness (Singh et al., 1990), and perhaps even cytokine-mediated changes in brain function following intense muscular exertion (Febbraio and Pedersen, 2002). Brain function is both an early indicator of many stressors of concern and a direct reflection of specific performance capabilities.

Early changes to defend critical functions are likely to be more promising prognostic indicators than awaiting change in the critical function itself (e.g., blood glucose, serum osmolality, core body temperature). The critical function may be so well defended, such as serum osmolality and sodium concentration, that when a significant change is detected homeostatic mechanisms have failed and the individual is already a casualty. Earlier changes in interstitial fluid or osmoregulatory hormones may signal a heroic defense against a threat to intravascular volume. There are also conditions under which the critical function measurement, such as body temperature, may have a wider range of measurements at performance extremes in healthy individuals that may even be appropriate compensation to sustain peak performance, defying definitive classification of an impending performance failure until regulatory mechanisms fail. For example, core body temperature may be as low as 35 C at the circadian nadir in Ranger students who have lost most of their insulative fat and have metabolically adjusted to a reduced energy intake (Hoyt et al., 1997), and it may be sustained at 40 C for several hours in marathoners during

their race (Maron et al., 1977). Monitoring the signs of compensation (e.g., changes in heat flux, activation of sweating or shivering mechanisms, cardiac responses, and mental functioning) may predict a trajectory to danger ("amber") before detection of unambiguous changes in core body temperature ("red").

Bone and muscle turnover studies are important to the military to solve near term problems of high rates of injury during physical training, most importantly during the rapid train up phase of an 8–12 initial entry training course in every Service (half of all female Soldiers incur musculoskeletal injury during basic training). A peak incidence of stress fractures by about the third week of training was hypothesized to be associated with high rates of bone remodeling stimulated by the training. This led to a major Army study that examined the benefits of a physical training rest period in the third week of training (Popovich et al., 2000). Unfortunately, this did not modify the injury profile, suggesting a more complicated pathogenesis, including individual variability. The development of specific markers of susceptibility and impending injury in individuals is still urgently needed.

Table 1 suggests some of the outcomes that might be logical targets for monitoring within the next decade, and some of the technologies that exist or could be developed for such monitoring. The boundary between current and near-term approaches is slightly blurred by the overlap of current technologies that require far more validation and projected near-term technologies that are just beginning to demonstrate promise. For example, fitness for duty based on various peripheral indicators of brain function is an important but elusive goal. In the past, there was a hope that performance could be predicted from recent sleep history measured by wrist-worn actigraphy (Redmond and Hegge, 1985); the current status of fatigue-performance models is too immature and individual responses to this single measure are too variable to make this useful by itself. Potentially noninvasive measurement methods that could be mounted in a helmet, such as pupillometry and saccadic eye movements, are being explored but have so far not held up well compared to lab measures such as the psychomotor vigilance task. A method developed by NASA that follows slow eye closure ("droopy' eyelids) shows great promise but will have to be proven in a helmet-type platform that keeps the monitor in line with the subject's eyes (Dinges et al.,

1998). Voice analysis is specifically affected by emotional load in Soldiers, returning to normal with psychological adaptation even while general activation (e.g., accelerated heart rate) continues (Wittels et al., 2002); however, this measure has not yet been demonstrated to correspond to specific performance decrements. EEG analyses in fatigued subjects or during sustained vigilance tasks have been studied in at least three military laboratories and show promise but remain to be demonstrated as strong predictors of impending deficits (Caldwell et al., 2002).

Far future technologies are concepts that might be achievable but have not been seriously explored and remain "marks on the wall." Mitochondrial redox state in specific brain tissues has been suggested as the key marker of brain function status, based on the importance of neural cell bioenergetics. Perhaps the far future final common pathway to monitor would be something like this and everyone will submit to a minor transsphenoidal surgical procedure for a rice grain-sized monitor of brain status! Intracerebral monitoring of energy-related metabolites is being done with neurosurgical patients now to follow acute conditions involving hypoxia and ischemia. As we learn more about what we need to measure, the technologists may be able to develop the noninvasive monitoring devices to our emerging specifications. For example, with the higher powered magnets, researchers are now detecting glutamate peaks in MRS brain pixels. An elevated frontal lobe glutamate might signify a range of acute metabolic insults that would be very important to detect and countermand. We now have magnet systems that operate with very low power; why not a technology for brain spectroscopy built into a helmet in the future?

The current military research programs are leveraged with special Congressional appropriations that accelerate basic metabolic research in specific topic areas. The Bone Health and Military Medical Readiness (BHMMR) research program (supported by the National Osteoporosis and Related Bone Disorders Coalition) is focused on the improved understanding of bone remodeling processes, and includes projects exploring markers of impending stress fracture injury. The Technologies for Metabolic Monitoring (TMM) research program (supported by the Juvenile Diabetes Research Foundation) is testing novel approaches to measure functional outcomes related to biochemical status and energy metabolism, notably glucose regulation but including development of lactate sensors and exploration of physiological indicators of metabolic status. Projects supported by the Force Health Protection (FHP) research program examine methods to monitor global health status in soldiers including the use of breath condensates to measure cytokines and other markers of lung function following blast or toxic inhalation exposures. Two large projects are assessing the association of brain magnetic resonance spectroscopy measures and symptom reporting in chronic multisymptom illnesses, to determine objective markers of well-being. Another program is dedicated to investigation of eye saccades and pupil responses as indices of fatigue

and fitness for duty, as described in a recent review by MG(ret.) Gary Rapmund (2002). The Neurotoxin Exposure Treatment Research Program (NETRP) (sponsored by the Parkinson's Action Network) includes exploration of voice analysis and neuropsychological testing methods for early detection of neurological changes.

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TABLE 1 Technology forecast for practical metabolic assessment measures (measured endpoints & *conceivable te*

	Present	Near Future	Far Future
Past			
Energy balance and fuel availability • blood & urine biochemistry • ratings of perceived exertion *home test glucose monitors; lab tests*	• "gluco-watch" • activity-based predictions *reverse iontophoresis; actigraphy*	• subdermal continuous glucose, lactate, pH, FFA *semi-invasive implantable sensors and "tattoos"*	• functional nerve con thermal flu *noninvasive p built into clothi*
Brain metabolic function • paper and pencil tests	• computerized neuropsychological testing • EEG spectral analysis *palm-top test; dry electrodes in a hat band*	• saccades & pupil responses • voice analysis • task embedded psych tests *doppler etc in soldier helmet/spectacles*	• sweat/exha • volatile cm • brain blooc *chemical nose; personal intra-systems*
Hydration and water balance • urine specific gravity	• balance based on intake & predicted losses • whole body water estimates *Instrumented canteen/camelbak; bioelectrical resistance*	• intercellular fluid assessment • whole body water changes *subdermal wicks; boot-sensor body weight tracking with electrolyte and BIA sensors*	• changes in • endocrine · water volu *skin mechanic semi-invasive osmoregulator*
Bone and muscle turnover • loss of strength & delayed onset muscle soreness • "hot spots" *thermography*	• specific blood and urinary markers (e.g., telopeptides; myoglobin, CPK, IGF-1) *lab tests*	• sweat markers of calcium and protein metabolism • altered biomechanics *practical field test systems*	• changes in • regional n changes *deep muscle bi*

CURRENT STATUS OF FIELD APPLICATIONS OF PHYSIOLOGICAL MONITORING FOR THE DISMOUNTED SOLDIER

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INTRODUCTION

The Dismounted Warfighter's workplace is fairly unique within the variety of occupational challenges encountered by the American population. Modern foot Soldiers commonly engage in intense, mentally and physically demanding three-to-10 day missions, often in rugged terrain or complex urban settings. These warriors carry heavy loads (35 to 65 kg) and are often food and sleep-restricted. Environmental conditions–ambient temperature, humidity, wind speed, solar load, and barometric pressure-can vary widely. Consider as recent examples of the operational environment the desert heat conditions of the Persian Gulf, cold wet weather in Bosnia, and cold and high altitude challenges in the mountains of Afghanistan.

Warfighter Physiological Status Monitoring (WPSM) Concept

Why is physiological monitoring in the field needed? Wearable metabolic and physiological status monitoring can play important roles in (a) sustaining physical and mental performance, (b) reducing the likelihood of non-battle injuries such as heat stroke, frostbite, and acute mountain sickness, and (c) improving casualty management in remote situations.

Ambulatory WPSM technologies are being developed to provide useful performance and health status indicators for warfighters, medics, commanders, and logisticians. The goal is to maximize the operational effectiveness of Soldiers, reduce the occurrence of non-battle casualties, and improve remote casualty management. Currently, the WPSM program is using a novel research "tool kit" to collect ambulatory physiologic data from Soldiers operating in stressful field environments. Analysis of these data sets is providing a better understanding of the physiological strains associated with operations in a multi-stressor environment. The data is also guiding the development a Soldier-acceptable WSPM system for advanced combat systems for dismounted warfighters including Land Warrior and the Objective Force Warrior.

Sensor hardware first comes to mind when thinking about ambulatory metabolic/physiologic monitoring. In practice, however, sensor development is one of a series of steps needed to reliably generate a useful flow of health state information in a harsh and highly constrained wearable environment. These steps include reliable sensor data collection, data

cleaning, data reduction and interpretation, and the communication, synthesis, interpretation, and presentation of the data. Key technologies that support this process, including *post hoc* time series data management and the medical Personal Area Network (PAN), are reviewed elsewhere (Hoyt et al., 2002).

Power, weight, and volume constraints, and the need for truly "wear-and-forget" comfort, limit the functionality of wearable sensors. What can be sensed may be unconventional, for example, estimating sleep by monitoring activity is practical, but it is not currently practical to do so by electroencephalogram. Furthermore, wearable sensors are usually less reliable than their laboratory counterparts due to factors such as motion artifact and environmental effects (water, temperature, pressure). An intelligent sensor network that reliably generates useful information from a number of disparate sources is needed to provide a holistic rather than a "keyhole" view of the physiological status of the individual.

CURRENT COMPONENTS OF PHYSIOLOGICAL STATUS

A prototype WPSM user interface (display) for the Medic or field Commander (Fig. 1) illustrates relevant types of contextual and physiological information.This heuristic display shows (1) thermal/work strain as Physiological Strain Index (PSI)(Moran et al., 1998), (2) hydration state or water balance, (3) metabolic rate, (4) environmental conditions, (5) cognitive/sleep status (hours of sleep, etc.), and (6) clinical status and location information.This knowledge display requires data from multiple sources, including baseline characterization of the individual, real time Soldier and environmental sensor input, and historical and group mean data.

Warfighter Characteristics

Warfighter characteristics, along with clothing, diet, load, geolocation, and meteorological conditions (air temperature, solar load, wind speed, humidity), are important determinants of the individual's physiological and pathophysiological responses to environmental stresses and trauma. Relevant warfighter characteristics include job type (military occupational specialty or MOS), gender, ethnicity, age, height, body weight, percent body fat, thermal and altitude acclimation history, and aerobic fitness. These factors change slowly, if at all, and can be recorded well before any training or combat mission. Body fat percent can be estimated simply from waist circumference (Wright and Wilmore, 1974). Simple field techniques for characterizing thermal and altitude acclimation states are currently not well defined. Aerobic fitness can be estimated from the Army Physical Fitness Test two mile run for time score (Mello et al., 1988), or from foot-ground contact time and heart rate using the method of Weyand et al. (2001).

Heat Strain

Understanding why hot weather injuries occur, and developing ways to prevent heat injuries, are important concerns given the approximately 120 heat stroke/sun stroke injuries occur per year and the associated $10M/y cost (Sawka et al., 1996; http://amsa.army.mil). The graphical display shows core temperature measured by ingested thermometer pill (O'Brien et al., 1998) and heart rate, typically derived from the electrocardiogram. The Physiological Stain Index (PSI), a lumped core temperature/heart rate index that reflects thermal/work strain on a scale of 1 to 10 (Moran et al., 1988), is currently used to generate green/amber/red alerts as thresholds are passed. The PSI values may prove useful in assessing acclimation status, guiding heat acclimation routines, and setting the timing and duration of work/rest cycles. A first principles thermal strain model, called Scenario, estimates core temperature from work rate, clothing characteristics, and ambient meteorological conditions (Kraning and Gonzalez, 1997). This and other surrogate measures of core temperature may be appropriate when risk of hypo- or hyperthermia is moderate

and more precise core temperature measurements, such as those provided by ingested radio thermometer pill, are not needed. The core temperature requirement is likely to be replaced by improvements in heat flux modeling from measures of cutaneous responses and temperatures; combined with other sensor measurements this may provide strong inferences not only about thermal status but also about shock and hemorrhage.

Cold Strain

Cold injuries, that is, hypothermia and peripheral cold injuries, are also a major concern for Soldiers (King and Lum, 2002). Temperature pills can be used to monitor for hypothermia (O'Brien et al., 1998). Peripheral temperature and heat flux sensors can be used to assess the risk of peripheral cold injury, and to guide improvements in clothing, boots and gloves. The Cold Strain Index (CSI)(Moran et al., 1999) uses core and peripheral temperatures to track cold strain. However, this algorithm needs to be modified to account for altered thermoregulation during underfeeding and sleep. See Toner and McArdle (1988) for a discussion of physiological adjustment of humans to the cold.

Hydration

Under- or over-hydration can lead to decrements in physical and cognitive performance, increased risk of heat injury, hyponatremia, or death (Montain et al., 2001; Pandolf et al., 1988). Mission water requirements, which are largely driven by basal water needs and sweat losses, can be predicted based on the anticipated weather, clothing, load weight, and metabolic rate during the mission (Kraning and Gonzalez, 1997). Technologies to monitor water intake from bladder-type canteens, the "drink-o-meter" concept, can help ensure adequate water intake (water discipline). However, practical field methods to assess overall body tissue hydration, or to monitor hydration through urine output, have yet to be developed. Tests of the use of body resistance measurements have consistently failed to demonstrate accurate tracking of water changes, perhaps in part because of inability to control for variability in electrolyte concentrations during various types of dehydration (Berneis and Keller, 2000; Koulmann et al., 2000). It may be possible to improve electrical resistance derived estimates of hydration with minimally invasive subdermal electrolyte sensors in the future. Alternatively, future automatic monitoring of urinary excretion rates and solute concentrations may provide valuable insight into hydration status and other aspects of acute Soldier health.

Metabolic Status/Energy Reserve - Modeling The Metabolic Fuel Requirements Of Soldiers

Field rations may not always meet the nutritional needs of Soldiers (Friedl and Hoyt, 1997). Negative fat balance, commonly associated with under-eating in the field, can usually be managed with little consequence by drawing on substantial body fat reserves. Body fat energy reserves can be calculated from percent body fat, estimated from waist circumference, less the five percent absolute minimum body fat levels attainable in underfed healthy male Soldiers (Friedl et al., 1994). However, negative carbohydrate balance, which is common in the field and associated with decreased endurance capacity and loss of lean mass, is more difficult to manage due to the body's limited carbohydrate reserves. Can monitoring technologies help ensure that field rations meet the fuel requirements of physically active Soldiers?

Carbohydrate requirements of Soldiers can be estimated from aerobic fitness, daily activity patterns, and the metabolic cost of locomotion (Hoyt et al., 1997). Maximum aerobic capacity can be derived from the Army's Annual Physical Fitness Test two mile run for time results (Mello et al., 1988). Daily activity patterns can be derived from heart rate or actigraphy (Redmond and Hegge, 1985). The metabolic cost of locomotion can be derived from total weight

and foot-ground contact times (pedometry)(Hoyt and Weyand, 1996; Kram and Taylor, 1990), or from the Pandolf equation and body weight, load weight, and geolocation (including velocity of movement, grade, and footing)(Pandolf et al., 1977). Knowing metabolic rate and the maximum aerobic capacity for each individual, an exercise intensity profile can be generated (that is, % of maximum aerobic capacity over time). Oxygen consumption can be partitioned into carbohydrate and fat combustion by assuming a given relationship between resting or exercise intensity and nonprotein respiratory exchange ratio (RER = carbon dioxide production/oxygen consumption), and using standard conversion factors. The exercise intensity-RER relationship chosen might be more fat-predominant than that of fully fed individuals (Åstrand and Rodahl, 1986) due to practical limits on the amount of food Soldiers can carry.

Remote Trauma Triage

Warfighters are expected to be widely dispersed on the battlefield and minimal medical care will be available to combat casualties.To help improve remote casualty management, a remote trauma triage system is being developed. This remote triage system, part of the WPSM system, will contain sensors and algorithms that allow medics to remotely detect ballistic wounding events, and to determine casualty life signs and the need for a major surgical life saving intervention (Holcomb et al., 2003). Parameters important in life sign detection after wounding include responsiveness to radio contact, motion, body position, cardiac activity, and systolic blood pressure. Distilled health state information will help the medic use medical resources (time, equipment, supplies) effectively.

Altitude Acclimatization

Soldiers deploying to elevations above 2800 m (~8000 ft) may experience Acute Mountain Sickness (AMS) (Pandolf et al., 1988). AMS is characterized by headache, nausea, fatigue, decreased appetite and poor sleep, often with signs of poor balance, and mild swelling of the face, hands, and feet. Without special preparation, a large proportion of a military unit rapidly inserted at high altitude is likely to develop acutely debilitating symptoms. Normally, AMS is either absent or resolves within three to four days following ascent. However, maladaptation can lead to life-threatening high altitude pulmonary or cerebral edema. Individual acclimatization state can be assessed by comparing blood-oxygen saturation for a given ascent profile (that is, SaO_2 for the reported or measured exposure to hypobaric hypoxia), with that expected with normal acclimatization. An ability to monitor and model acclimatization status will make it easier to plan high altitude missions and minimize altitude illnesses.

AN EXAMPLE APPLICATION - CHARACTERISTICS OF A HEAT CASUALTY

Heat strain provides a demonstration of nascent capabilities for physiological monitoring. Reliable predictions of soldier mental status and performance capabilities are not yet available while the assessment of frank casualties has been possible for some time through the use of clinical monitoring technologies. Progressive heat strain moves on a continuum from impaired cognitive function to frank casualty and presents one of the first opportunities to provide commanders with useful predictions of failing performance before a Soldier becomes an environmental stress casualty. Collection of field data that includes clear medical outcomes makes it possible to backtrack to earlier indicators of the impending health risk and develop more precise predictive thresholds of individual risk.

A pair of Soldiers engaged in similar training activities during a hot weather field exercise at the Joint Readiness Training Center, Ft. Polk, Louisiana. Although the two Soldiers performed similar activities from about 1130 to 1400 h (ambient temperature = 32 to 34°C;

relative humidity = 46 to 55 %; solar load = 800 to 875 W/m^2; wind speed = 1 to 2 ms^{-1}), and both were fed and hydrated, only one became a heat casualty. Soldier characteristics, including maximal aerobic capacity determined using the method of Weyand et al. (2001) are shown in Table 1. Geolocation data (not shown) was collected using GPS and Dead Reckoning Module (Model DRM III, Point Research Corp., Fountain Valley, CA). Ambulatory heart rate data, from electrocardiography, and core temperature data, via ingested temperature radio telemetry pill (Human Technologies Inc., St. Petersberg, Florida), were also collected. Physiological strain index (PSI) was calculated (Moran et al., 1998). *Post hoc* data analysis showed that the difference in response to heat stress was due to a number of factors. The heat casualty had a higher body fat percent, carried a heavier load, was less physically fit, and was not heat acclimated (by interview), as compared to his unaffected cohort. These results illustrate the importance of integrating multiple data streams in the process of understanding multi-stressor physiologic events.

In conclusion, physiological and metabolic monitoring offers a number of potential benefits for dismounted warfighters. However, achieving these benefits is scientifically and technically challenging.

Acknowledgements

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TABLE 1 Age, physical characteristics, total load carried, and maximal aerobic capacity of two Soldiers – a heat exhaustion casualty (Cadet), and the unaffected 509th Soldier from the 1/509th Infantry Brigade (Airborne). These Soldiers were engaged in similar hot weather training activities at the Joint Readiness Training Center, Fort Polk, Louisiana. During a road march, the non-heat acclimated, less lean, more burdened, less physically fit Cadet became a heat casualty, while the heat acclimated, leaner, less-burdened, more-fit Soldier from the 1/509th Infantry Brigade (Airborne) tolerated the thermal/work stress.

	Age (y)	Height (cm)	Weight (Kg)	Body fat (%)	Load (kg)	VO_{2Max} ($ml\ O_2/kg^{-1} \cdot min^{-1}$)
Cadet	21	175	79.3	18	45.3	47
509th Soldier	22	170	68	13.3	35.3	53

FIGURE 1 Prototype WPSM user interface (display) for the Medic or field Commander (Fig. 1) illustrating context information. This heuristic display shows (1) thermal/work strain as Physiological Strain Index (PSI), (2) hydration state metabolic rate, (4) environmental conditions, (5) cognitive/sleep status (hours of sleep, etc.), and (6) clinical status and locati

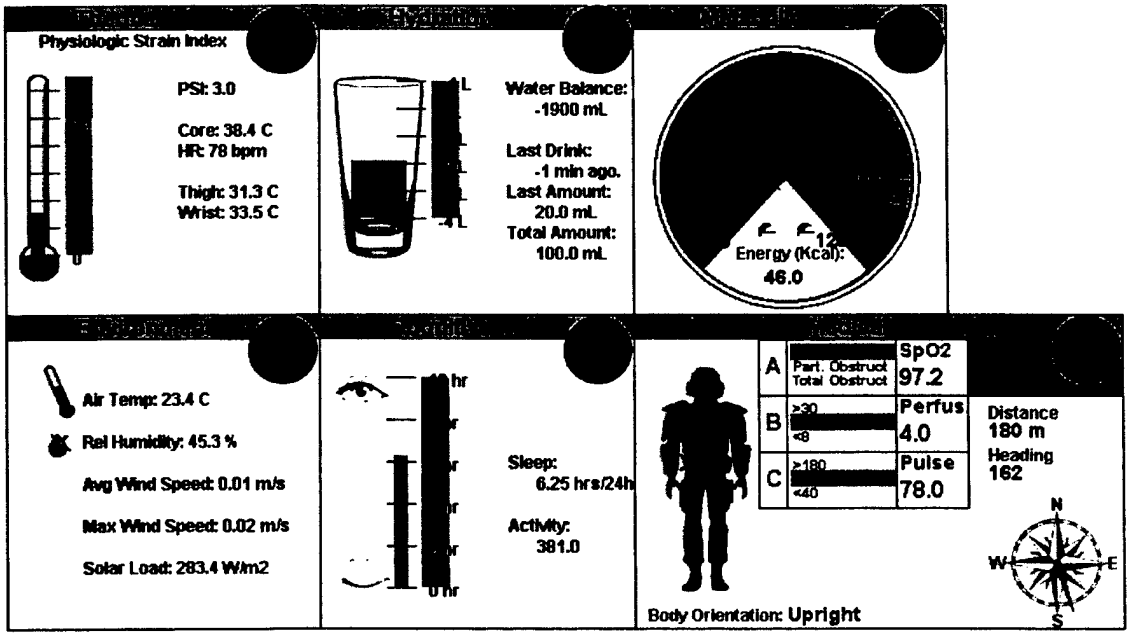
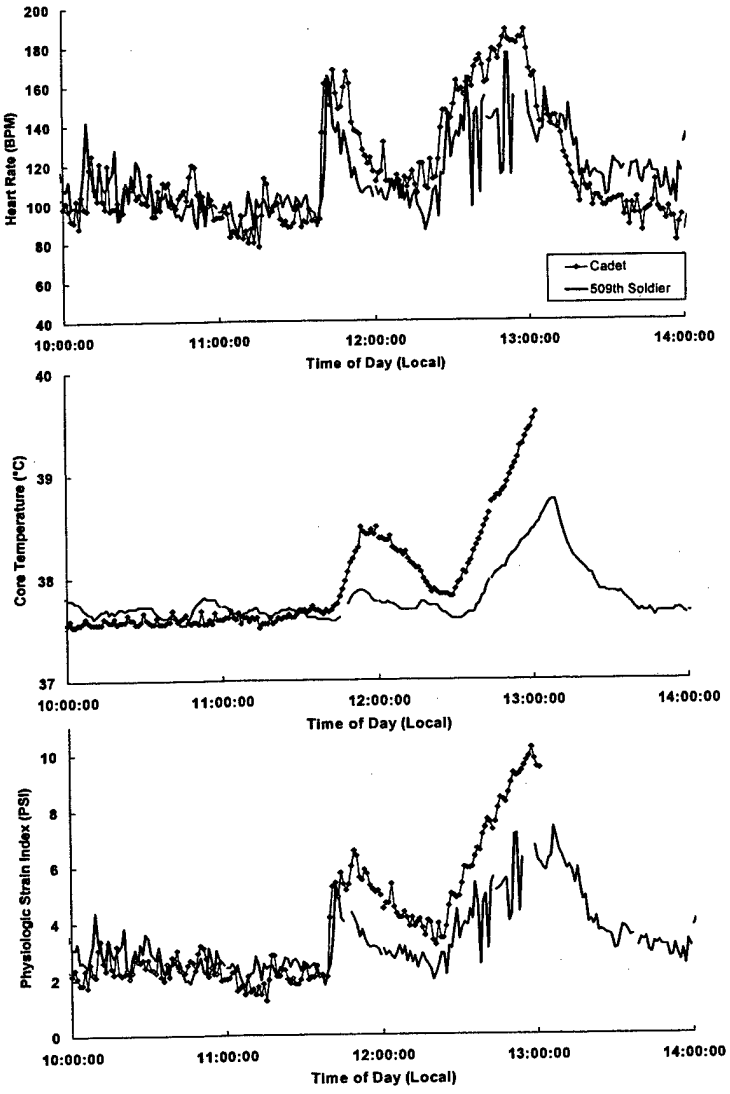


FIGURE 2. Heart rate, core temperature, and physiological strain index (PSI)(Moran et al., 1988) in two Soldiers engaged in similar training activities during a hot weather field exercise at the Joint Readiness Training Center, Ft. Polk, Louisiana. The thermal/work strain levels associated with two bouts of marching (1145-1200 h and 1230-1300 h) were more pronounced in the heat exhaustion casualty (Cadet) than in the less-affected 509th Soldier. The heat casualty had a higher body fat percent, carried a heavier load, was less physically fit, and was not heat acclimated, as compared to his 509th cohort.



Biomarkers for monitoring bone turnover and predicting bone stress

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BONE TURNOVER (REMODELING)

Bone is a structural tissue that, in common with all structural materials, is subject to fatigue damage and fracture if left unprepared. As living tissue one has the unique potential for self-repair of fatigue damage via a process termed bone turnover or bone remodeling. This process is continuous throughout life and is governed by a variety of systemic hormonal and nutritional factors, local factors (cytokines), and local mechanical stress. During intra-uterine and early post-natal life remodeling is very rapid as the cartilaginous "scaffolding" is removed and replacement with early bone elements. This rapid phase in early infancy results in positive skeletal balance as bone is modeled into its adult shape. During childhood the process slows dramatically but remains in slight positive balance, only to accelerate again coincident with the pubertal growth spurt, again maintaining positive balance. Once peak adult bone mass and maturity is reached in the third or fourth decades the balance between removal of older, "damaged" bone (resorption) and replacement with new bone at the same site (formation) is in equilibrium with no net gain or loss of bone. Beginning late in the fifth decade or early in the sixth decade this balance is upset such that, for unknown reasons, resorption exceeds formation with net negative skeletal balance such that bone loss is a universal phenomenon in human aging. The natural menopause in women results in a decline in estrogen levels to a point that local cytokine production is stimulated and bone remodeling rate increases again but to nowhere near the levels seen during early skeletal development. This process is reversed by replacement of estrogen, by administration of antibodies to the local cytokines, or by administration of pharmacologic agents that inhibit bone resorption. The rapid bone loss of the early menopause is short-lived (5-7 years), again via unknown mechanisms since estrogen levels remain low in the untreated state, but age related bone loss continues as seen in Figure 1.

The cells involved in bone remodeling are osteoclasts (responsible for bone resorption), osteoblasts (responsible for bone formation), and osteocytes (responsible for bone nutrition, channels for transport of nutrients and chemicals, and possibly also act as local stretch or stress receptors). A number of metabolic diseases and pharmacologic agents have direct effects on the bone remodeling process resulting in accelerated negative skeletal bone balance. Menopausal and age-related negative skeletal balance results in the disease osteoporosis.

BIOCHEMICAL MARKERS OF BONE TURNOVER

As osteoclasts breakdown the skeleton to begin the process of turnover there is removal of bone mineral (mainly calcium) and bone matrix (mainly type I collagen) and these breakdown products enter the circulation and are subsequently excreted in the urine, largely unchanged. The rise in serum calcium resulting from bone resorption is imperceptible in most circumstances because of rapid renal clearance. While urine calcium increases it is a very non-specific marker of the bone turnover process. The breakdown of type I collagen begins with cleavage of pyridinium cross-links between adjacent molecules collagen. Collagen is a triple helix consisting of two $\alpha 1$ chains and one $\alpha 2$ chain. At each end of the helix is a straight portion known as telopeptides with one at the amino terminal (NTX) and one at the carboxy terminal (CTX). The cross-links are between the telopeptide of one collagen molecule and the helical portion of the adjacent molecule. There are two main cross-links, pyridinoline (PYD) which is the more abundant moiety but less specific for type I collagen and deoxypyridinoline (DPD) which is less abundant but more specific for type I collagen. These breakdown products of bone resorption may be excreted as free moieties or bone to the telopeptides. Thus NTX, CTX, PYD, and DPD constitute the main biomarkers of bone resorption. The cross linking and

breakdown products are depictd in Figure 2. Tartrate-resistant acid Phosphatase (TRAP) particularly the 5b epitope (TRAP 5b) are specific gene products of the osteoclast and can also be measured as an assessment of bone resorption.

Type I collagen is a secretory product of the osteoblast. It leaves the cell as a larger procollagen molecule from which an amino terminal and a carboxy terminal propeptide are cleaved before incorporation into the bone matrix. These extension peptides remain in the circulation where they can be measured (P1NP and P1CP) as markers of osteoblastic activity. Alkaline Phosphatase (AP) is an enzyme secreted by the osteoblast and is involved in bone mineralization. There are many iso-enzymes of AP which differ in post-translational glycosylation. While total AP is a useful marker when levels are quite elevated, the bone-specific iso-enzyme (BSAP) has better sensitivity and specificity. Osteocalcin (OCN) is also a secretory product of the osteoblast and is incorporated into the bone matrix as a non-collagenous protein. While this could qualify OCN as a marker of formation, as part of the bone matrix it is also released during bone resorption so is really a marker of "turnover", with some resultant loss of sensitivity. A summary of the biochemical markers of bone turnover is seen in Table 1.

CLINICAL UTILITY OF BONE MARKERS

In those conditions where a specific disease process directly alters bone turnover (e.g. Paget's disease of bone, osteomalacia, rickets) the level of biochemical markers is usually quite elevated and changes in these levels can be used to monitor progression or regression of the disease in individual patients. In contrast, in diseases that result from a primary abnormality in the remodeling balance, most notably osteoporosis, the markers have lesser sensitivity and specificity for monitoring progression or regression of disease in individual patients. Population studies do suggest that the higher the turnover, the greater the anticipated rate of bone loss but there are only weak correlations between baseline levels of markers and prospectively measured changes in bone mineral density (BMD). Similarly population based studies in the elderly have demonstrated that high levels of markers can predict hip fracture risk, almost as well as can hip BMD, but here too that is of limited sensitivity and specificity in individual patients. Patients with osteoporosis who are treated with drugs that inhibit bone resorption generally have low levels of markers while compliant with therapy. A high level of marker on therapy in a compliant patient suggests that other metabolic bone disease might have supervened on the osteoporosis. With the recent introduction of teriparatide (synthetic amino-terminal parathyroid hormone) as a therapy to directly stimulate bone formation there are likely to be expanded roles for markers in selecting patients for specific therapies and in monitoring the therapeutic response. (Watts, 1999)

BONE TURNOVER MARKERS AND FRACTURES

An acute fracture is a potent stimulus to bone repair and there are resultant changes in markers of bone turnover. However this has been surprisingly little studied and those few studies that have been reported have yielded disappointing results. This is not really a surprise as fracture repair is a local process and the markers of bone turnover reflect global skeletal remodeling.

The most extensive work has come from two back to back articles by Ingle et al. in 1999 (Ingle et al., 1999a; Ingle et al., 1999b). The first followed serial changes after distal forearm fracture and the second after ankle fracture (see Figure 3). The forearm fracture study followed 20 women, mean age 63, for 52 weeks following the fracture. In individual subjects there were marked changes in some of the markers studied but not in any consistent pattern. Overall there was minimal serial change in any of the studied markers.

BONE TURNOVER AND STRESS FRACTURES

Only one group has studied changes in bone markers before and after the development of stress fractures in athletes (Bennell et al., 1998). There were no differences in baseline levels of markers between those

who did or did not sustain a stress fracture. The serial data demonstrated no change in markers from before to after fracture during a total of 12 months of follow-up.

SUMMARY AND CONCLUSIONS

Bone turnover is an efficient mechanism for ongoing repair of micro-damage to the skeleton. Stress fractures occur when the rate of accumulation and propagation of micro-damage exceeds the capacity of the repair process. Several biochemical markers are available to monitor the global rate of bone turnover and have proven useful in monitoring progression or regression of systemic metabolic diseases resulting in abnormal turnover or resulting from abnormalities in the remodeling cycle. Changes in these markers undoubtedly occur during the repair phases following acute traumatic fracture but the extent of skeleton involved is too small to be reflected in these markers of global skeletal activity. It is likely that changes in markers occur during the development of stress fractures and during repair of stress fractures. However here too the extent of skeleton involved is too small to be reflected in these markers of global skeletal activity.

THE FUTURE?

It is extremely unlikely that a marker of bone turnover with sufficient sensitivity to detect change when only small area of the skeleton is damaged will be developed in the foreseeable future. Functional imaging studies (MRI, PET, regional bone scintigraphy) are far more likely to detect changes in local skeletal remodeling that precede stress fractures, even in the asymptomatic state. Whether it will ever be economically feasible to apply these technologies to asymptomatic recruits in the hopes of predicting stress fracture before it occurs will require extensive and expensive prospective studies. Whether such early pre-fracture detection will decrease the "down-time" for recruits recovering from stress fractures is questionable.

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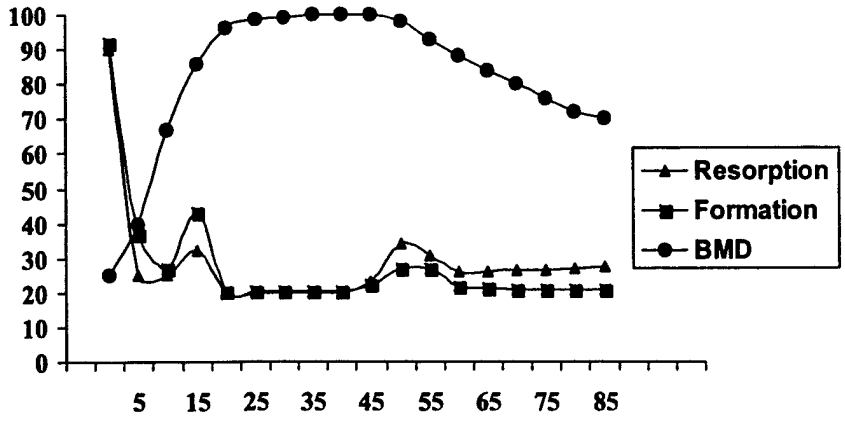


FIGURE 1 Relative changes in bone mineral density (BMD), bone resorption, and bone formation as a function of age.

Stage	Location	Biochemical Marker
Resorption		
	Serum	
		NTX
		CTX
		TRAP
		TRAP 5b
		1CTP
	Urine	
		NTX
		CTX
		DPD
		PYD
Formation		
	Serum	
		BSAP
		P1CP
		P1NP
Turnover		
	Serum	
		OCN
		OPG
		BSP

TABLE 1 Biochemical markers of bone turnover. NTX–the amino-terminal telopeptide of collagen cross-links; CTX–the carboxy-terminal telopeptide of collagen cross-links; TRAP–Tartrate –resistant acid Phosphatase; 1CTP–carboxy-terminal telopeptide of type I collagen; DPD–deoxypyridinoline; PYD–pyridinilone; BSAP–bone specific alkaline Phosphatase; P1CP–Carboxy-terminal fragment of type 1 procollagen; P1NP–Amino-terminal fragment of type I procollagen; OCN–osteocalcin; OPG–osteoprotegerin; BSP–bone sialoprotein.

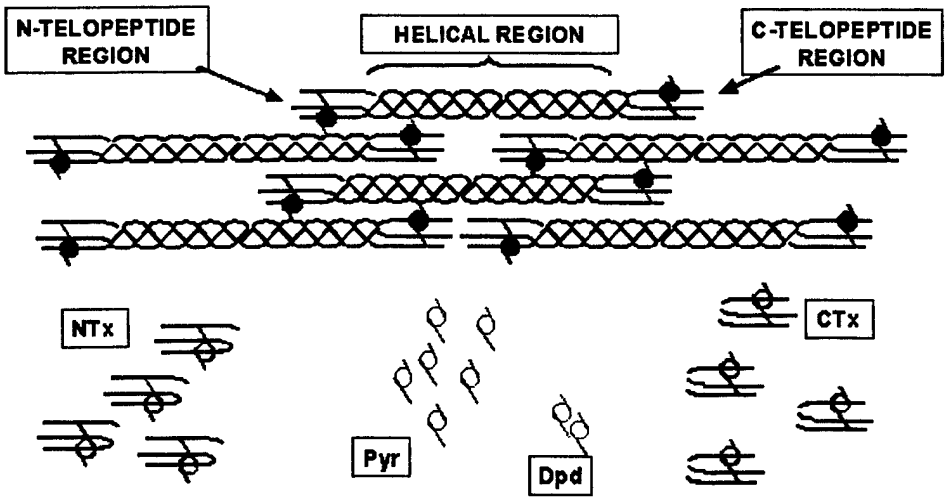


FIGURE 2 A cartoon depicting the cross-linking between molecules of type I collagen and the breakdown products. Reproduced from Watts, 1999 with permission.

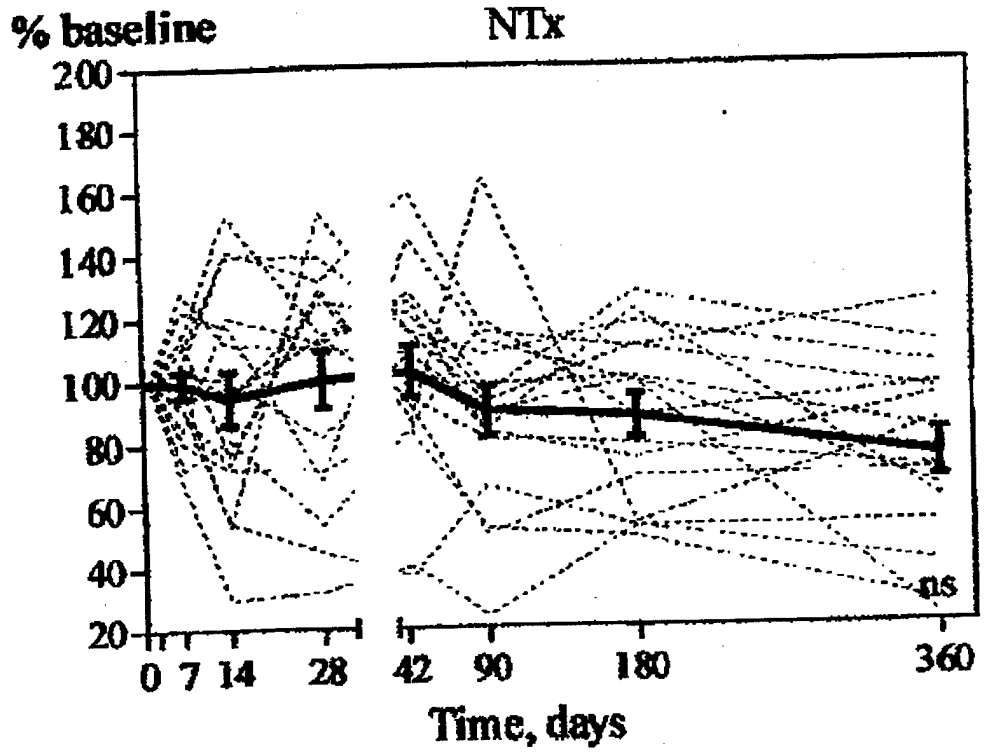


FIGURE 3 Serial changes in urine NTX following ankle fracture. Reproduced from Ingle et al., 1999a with permission.

Technology for Measurement of Blood Lactate

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Glucose is metabolized by cells to produce energy. Glucose metabolism involves progressive oxidation + breakage of Carbon bonds. The oxidation process causes C-H and C-C bonds to be stripped of electrons (oxidized) which are then used to build ATP

The initial steps in breakdown of glucose involve conversion of one 6-carbon molecule of glucose to two 3-carbon molecules of pyruvate. This process is known as glycolysis. Next in the presence of Oxygen, the carbon atoms in pyruvate are converted into three molecules of carbon dioxide in a process known as aerobic metabolism. When Oxygen is available to serve as the final acceptor of electrons, then pyruvate is able to transfer electrons to (or reduce) by way of a series of steps known as the Krebs Cycle or tricarboxycylic acid cycle to the final acceptor, Oxygen. When Oxygen is totally reduced, then it becomes H_2O or water. Meanwhile, the Carbon bonds of pyruvate all become oxidized to CO_2.

Conversely, in the absence of Oxygen, all the electron acceptors "downstream" from pyruvate are reduced and unable to offload electrons to mediators that will carry them towards Oxygen. The carbon bonds are progressively oxidized in the Krebs Cycle and the electrons' energy is drawn off in steps through a process known as oxidative phosphorylation. The process is analogous to water falling down a dam and turning turbines while falling and at the same time the turbines transfer energy to generators which produce electric power. The Krebs Cycle is the dam and the oxidative phosphorylation is the generator. Anaerobic metabolism is a state of water being backed up downstream so that there is no flow of water across the dam. Without Oxygen, the backup of reduced substances reaches pyruvate, which cannot transfer its electrons into any chemicals within the Krebs Cycle. Pyruvate itself then becomes reduced to lactate and broken down no further. The metabolic process that begins with glucose and ends with lactate is known as anaerobic metabolism. Lactate does not accumulate when Oxygen is available.

Aerobic metabolism is preferable to anaerobic metabolism. More energy (defined as the number of ATP molecules generated per glucose molecule broken down) is derived from aerobic metabolism than from anaerobic metabolism. The combination of glucose ignition by way of glycolysis, Krebs Cycle, and oxidative phosphorylation in aerobic metabolism generates 36 ATP molecules per glucose molecule compared to only 2 ATP molecules per glucose molecule that are generated in anaerobic metabolism by glycolysis alone. When exercise is continues past the point of adequate Oxygen delivery (such as during excessive training beyond the ability of cardiac output to supply adequate blood), then glucose breakdown switches from aerobic to anaerobic metabolism. Lactic acid builds up and the acid dissociates to lactate plus free hydrogen ions which lower the pH of the blood. The acid load can damage muscles, including the heart muscle, or even kill.

Currently, technology exists for portable monitoring of lactate to monitor people who are exercising heavily, such as athletes or soldiers in training. The technology is exclusively invasive and intermittent. Unlike the situation with portable monitoring of blood glucose, in which new monitors with advanced

features are regularly introduced, there are only two portable lactate monitors on the market. No portable lactate monitors currently exist or are close to existing which are minimally invasive or noninvasive (only invasive), implanted (only external), continuous (only intermittent), or optical (only chemical).

The molecular weight of lactic acid is 90, compared to that of glucose, which is 180. Resting blood lactic acid concentrations normally range from 0.5–2.0 mmol/L, which are approximately one fourth those of blood glucose. During anaerobic exercise, lactic acid levels may increase 5–10 fold up to 12 mmol/L.

Blood lactate levels can be used to determine the optimal workload for an athlete in training. Below the optimal workload, glucose metabolism is aerobic. At some point when the workload increases, the body's ability to supply increasing amounts of Oxygen to working muscles becomes limited. This is the lactate threshold, or the workload whereby lactate levels no longer rise slowly with increasing exertion, but instead rise rapidly with increasing exertion. At the lactate threshold, glucose metabolism begins to be anaerobic as well as aerobic. Lactic acid builds up quickly if exercise continues to increase beyond this threshold. Below the threshold, the body is able to efficiently break down lactate such that the lactate levels only rise slowly with increasing work, but above the lactate threshold, lactate levels climb sharply. At the inflection point of the curve in which lactate concentration is plotted against workload, it is best for the athlete to decrease their amount of exertion to get back to or just below the lactate threshold. For a given individual, over a short term, heart rate is proportionate to workload, and heart rate is much easier to measure than workload. To identify the optimal workload at which lactate is cleared approximately as fast as it is produced (without accumulation), an athlete's lactate level can be measured and plotted against varying heart rates.

With improved cardiovascular function (i.e. increased fitness), the heart can deliver sufficient Oxygen to maintain aerobic metabolism for progressively greater workloads. Conversely, with deconditioning, at progressively lower workloads, the lactate threshold is met. Therefore, for an athlete in training, determination of the lactate threshold (expressed as a workload level or a heart rate) indicates; 1) the state of fitness (proportionate to the lactate threshold workload); and 2) the optimal work load at which to exercise whereby the workload is challenging, but potentially dangerous lactic acidosis can be avoided. Knowledge of the optimal workload is useful for an athlete in training, such as a soldier, to optimize the exercise regimen.

The lactate threshold can be calculated by performing a series of workouts at varying workloads which can be estimated by the heart rates associated with these workloads. The strategy involves initially exercising well beyond the lactate threshold in order to build up the blood lactate level, then decreasing the exercise to allow the lactate level to fall, and finally increasing the workload slightly to a point where the lactate level starts to rise once again. That point where lactate generation exceeds lactate clearance is the lactate threshold. The specific steps of how to calculate the lactate threshold are as follows. First is the lactate buildup phase, consisting of three 6-minute workouts (easy, medium, and hard) followed by a blood lactate measurement. Second is the lactate clearance phase, consisting initially of a 5-minute workout at a heart rate of 40 beats per minute below that of the workout rate followed by a blood lactate measurement. The 5-minute workout should be repeated at a greater workload defined as a heart rate of 10 beats per minute higher and the blood lactate should be rechecked. Then the workout and lactate measurement should be repeated each time with a heart rate of 5 beats per minute more. Initially the blood lactate will fall from that of the heavy exercise peak value, but with increasing workloads, the blood lactate level will begun to rise. The point, where lactate production comes to exceed clearance, is the lactate threshold.

Lactate monitors can be classified by size. There are three types of lactate monitors. First are portable handheld monitors that are good for monitoring athletes and workers in the field. These include the Accusport/Accutrend (two different names for the same monitor) manufactured by Roche Diagnostics of Germany, and the Lactate Pro manufactured by Ankray of Japan. Second there are small benchtop monitors that can run on batteries and are only slightly mobile. These include the Little Champion monitor by Analox and the YSI 1500 Sport Lactate Analyzer. These devices are somewhat cumbersome to use in the field, but can be so used if the instrument is fairly stationary. There are several benchtop lactate monitors that are used for hospital and research purposes. These devices are not suitable for studying athletes outdoors, but can be used within an indoor training facility. They include the Analox Champion Lactate Analyzer, the YSI 2300 and 2700 Glucose plus Lactate Analyzers, the Kodak Ektachem DT60, and the Eppendorf Biosen 5130.

The portable lactate monitors resemble the blood glucose monitors of the late 1980's in their ease of use. The Accusport/Accutrend requires 20 mcl blood and 60 seconds measuring time. The Lactate Pro requires 5 mcl blood and 50 seconds measuring time. Neither monitor is approved for alternate site testing, and no portable lactate monitor has been developed for minimally invasive or noninvasive lactate testing and none has been developed for implantable, continuous, or optical lactate sensing.

If the need for faster and more convenient lactate measurement of soldiers, athletes, or other workers in the field is evident, then there is room for development of faster, more convenient lactate monitors using smaller volumes of blood. Because lactate has a similar structure as glucose, a goal for manufacturers of lactate monitors could be to produce lactate monitors as user-friendly as portable glucose monitors currently are. There is an untapped potential for measuring lactate in more groups of exercising people and a need for better instruments to perform the monitoring.

Biomarkers to Predict the Occurrence of Bone Stress and Matrix Abnormalities Due to Sustained and Intensive Physical Activity

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INTRODUCTION

There are two major competing hypotheses for the pathogenesis of stress fractures that occur as a result of high-intensity repetitive mechanical loading, such as in basic training for the military. The first hypothesis is that it is mechanical stress, per se, that causes bone to fail. The second hypothesis is that mechanical loading triggers an increase in bone remodeling activity that causes a transient reduction in bone mass, thereby increasing the vulnerability of bone to damage if mechanical loading continues. This brief review will focus on the concept that the initiation of vigorous exercise training could trigger an increase in bone resorption through 3 general pathways: 1) a normal mechanical stress-induced increase in bone remodeling; 2) an increase in bone resorption to repair microdamage caused by mechanical stress; and 3) the effects of exercise training on other physiologic factors that influence bone resorption or formation (see Figure). Finally, biomarkers thought to be potential predictors of risk for stress fracture will be identified.

EPIDEMIOLOGY OF STRESS FRACTURES

Stress fractures are nontraumatic fractures that are due to repeated loading of the skeleton (Burr, 1997). They typically occur in physically active individuals, including soldiers, runners, and dancers. The most common fracture sites are the tibia (soldiers, runners), metatarsals (dancers), and calcaneus. In military recruits, incidence rates of stress fracture are elevated within 2–3 weeks of the onset of training, and peak rates occur after 5–8 weeks (Burr, 1997).

In a recent study of 3758 females U.S. military recruits, the incidence of stress fracture was 8.5 percent during 8 weeks of basic training (Lappe et al., 2001). Women who fractured, compared with those who did not, were: older, more likely to use depo-medroxyprogesterone acetate, had a lower adult body weight, and were more likely to report current or past smoking, alcohol consumption of more than 10 drinks per week, and use of corticosteroids. A history of regular exercise was protective. Such findings suggest that risk for stress fracture is influenced by a number of physiological and behavioral factors.

DEVELOPMENT OF STRESS FRACTURES – MECHANICAL LOADING FACTORS

There is a wealth of evidence from a variety of animal models that repetitive mechanical loading results in bone damage (Burr, 1997; Burr et al., 1997). Furthermore, the rate of development of lesions is consistent with the observation in humans that stress fractures develop in a matter of weeks in response to an abrupt increase in mechanical stress (Burr et al., 1990). Based on theoretical modeling and empirical data from studies of animals, very high levels of bone strain (e.g., 8000 microstrain) will cause bone to fail after

only 10^3 to 10^4 loading cycles. However, in humans peak shear strains of the tibia measured during walking and running under a variety of conditions (e.g., uphill, downhill, zig-zag, while carrying extra weight) are typically less than 2000 microstrain. At this level of strain, it has been estimated that bone can withstand at least 10^6 loading cycles, or roughly 1100 miles of running (Burr, 1997).

Although these observations suggest that mechanical stress, *per se*, is not likely to be the sole cause of stress fractures that occur after only a few weeks of basic training, definitive evidence to rule this out is lacking. It is possible that bone strain is increased under certain conditions, such as when muscles are fatigued (Christina et al., 2001), or that brief periods of mechanical stress in excess of 2000 microstrain induce damage. Although higher degrees of strain may not occur during planned activities (Milgrom et al., 2000), they may occur during unplanned movements. For example, in patients with hip prostheses outfitted with telemetrically monitored force sensors, the highest forces were recorded during unexpected movements, such as stumbling (Bergmann et al., 1993; Bergmann et al., 1995). However, even when all these factors are considered, it seems unlikely that the development of stress fractures after only a few weeks of intensive physical activity is attributable solely to mechanical stress. In an animal model, more than 30,000 loading cycles applied to a limb over a 3-week period resulted in bone damage, whereas applying the same number of loading cycles in 1 day did not (Burr, 1993). The temporal factor suggests that physiological responses to the mechanical stress contribute to the propensity for fracture.

DEVELOPMENT OF STRESS FRACTURES – BONE REMODELING FACTORS

Mechanical stress is thought to trigger an increase in bone remodeling activity that begins with an increase in bone resorption, leading to a transient decrease in bone mass, followed by an increase in bone formation. The transient reduction in bone mass would increase the vulnerability of bone to damage if mechanical loading continues during this period, because forces of the same magnitude would now represent a greater relative stress. This hypothesis is consistent with theoretical models and empirical data on the time course of remodeling and of the development of stress fractures (Burr, 1997). The recruitment of osteoclasts, the bone resorbing cells, typically occurs in a few days. The period of bone resorption lasts about 3 to 4 weeks, with subsequent activation of bone formation activity.

If the induction of bone resorption and consequent decrease in bone mass does, indeed, increase the susceptibility of bone to stress fracture, it could be postulated that use of an anti-resorptive agent would diminish this risk. However, it is likely that mechanical loading results in microdamage to bone, and that an increase in remodeling activity is an obligatory step in the repair of microdamage. In this scenario, use of anti-resorptive agents could result in an accumulation of microdamage and an increase, rather than decrease, risk of stress fracture.

The temporal nature of the response of bone to severe mechanical stress was evaluated by Bentolila and colleagues (Bentolila et al., 1998). In their experiment, the right ulnae of rats was loaded to fatigue on day 1 and the left ulnae underwent the same fatigue loading on day 10; both bones were harvested immediately after the second loading session. Microcrack density was significantly higher in the acutely loaded ulnae than in the ulnae that had been stressed 10 days earlier, suggesting that some healing had occurred in the intervening period. However, bone resorption activity was evident only in the bones that had been stressed 10 days earlier and tended to be concentrated in regions of microcracks. Three-quarters of all microcracks were associated with resorption spaces, but resorption spaces were also visualized in regions of bone in which there was no detectable matrix damage. This suggests that resorptive activity was initiated as part of the normal remodeling response to mechanical stress (in undamaged regions) and to repair areas of microdamage. Thus, it seems plausible that the degree of activation of bone resorption, and the extent of transient bone loss, depends on the severity of the mechanical stress and the extent of damage that it causes.

The notion that inhibiting bone remodeling could lead to an accumulation of microdamage and increase bone fragility has been studied in animals using bisphosphonate therapy (Hirano et al., 2000; Mashiba et al., 2001a; Mahiba et al., 2001b). One year of etidronate therapy at a dose 100-times higher than the recommended clinical dose in humans resulted in increased osteoid volume and a high incidence of spontaneous fractures. At a dose 10-times the clinical dose, there was evidence of microdamage accumulation but no significant increase in spontaneous fractures. The relevance of these findings to the

concept of using anti-resorptive therapy to prevent stress fractures remains uncertain. It will be important to determine the effects of lower doses of bisphosphonates and other anti-resorptive agents, and to specifically evaluate the effects on bone microdamage and fragility under conditions of increased mechanical stress.

DEVELOPMENT OF STRESS FRACTURES – OTHER PHYSIOLOGIC FACTORS

The fact that stress fractures develop in only a few weeks in response to an increase in mechanical loading is temporally consistent with the hypothesis that an increase in bone resorption triggers a decrease in bone mass that transiently increases the vulnerability of bone to fracture. In this context, other physiologic factors that may further exaggerate bone resorption or impair the coupling with subsequent bone formation activity should be considered. The following discussion is not meant to be an exhaustive list of possible factors, but rather an overview of a few factors that can be influenced by vigorous exercise training and are known to affect bone metabolism.

Sex hormones

Both estradiol and testosterone have potent effects on bone metabolism (Riggs et al., 2002), and levels of these sex hormones have been reported to be decreased in highly trained athletes (Laughlin et al., 1998; Roberts et al., 1993). There is emerging evidence that it is low energy availability during vigorous training, rather than the exercise, *per se*, that causes this hormonal dysregulation, at least in women (Loucks, 2001; Loucks and Thuma, 2003). Whatever the cause, if estradiol levels decrease during vigorous exercise training, this would be expected to stimulate an increase in bone resorption, because even normal fluctuations in estradiol across the menstrual cycle are inversely associated with bone resorption rate (Chiu et al., 1999). There have been few studies of bone metabolism and testosterone levels in young male athletes, but the suppression of androgens in men results in a dramatic increase in the rate of bone resorption (Stoch et al., 2001). Because estradiol appears to play a more important role than testosterone in maintaining bone mass in men, it is important to note that reductions in serum testosterone in men will be accompanied by reductions in estradiol because the primary source of estradiol in men is the aromatization of testosterone (Riggs et al., 2002).

Sex hormones have independent effects on bone metabolism, but may also influence risk for stress fracture through other mechanisms. Recent studies have found an increase in apoptosis of rat (Tomkinson et al., 1998) and human (Tomkinson et al., 1997) osteocytes in response to estrogen withdrawal. The investigators suggested that, because the capacity of bone to repair microdamage and to modulate the effects of mechanical strain may be dependent on osteocyte viability, this could be a mechanism by which estrogen deficiency leads to bone fragility. It has also been demonstrated that estrogen receptor alpha is involved in generating the bone response to mechanical stress (Cheng et al., 2002; Damien et al., 2000) and that the combined effects of estradiol and mechanical stress on bone formation activity are additive or synergistic (Cheng et al., 1997; Kohrt et al., 1995). Thus, the effectiveness of mechanical loading to favorably affect bone metabolism may be diminished in the estrogen-deficient state.

Glucocorticoids

The physical and psychological stresses of basic training and survival training can increase the secretion of stress hormones, including cortisol (Helhammer et al., 1997; Morgan et al., 2002), which has a potent, negative effect on bone. With respect to direct actions on bone metabolism, cortisol both increases bone resorption, by stimulating osteoclastogenesis, and inhibits bone formation, by inhibiting osteoblastic cell replication and differentiation and increasing apoptosis of mature osteoblasts (Canalis and Delany, 2002). Cortisol may also influence bone metabolism through indirect actions (Manelli and Giustina, 2000). Glucocorticoids have been found to decrease calcium absorption, modify vitamin D metabolism, and inhibit activity of both the gonadotropic and the somatotropic axis.

Growth Hormone, IGF-1

Growth hormone and growth factors such as IGF-1 have potent and complex effects on bone metabolism (Rosen and Donahue, 1998). The effects of physical stress to suppress the somatotropic axis and the potential adverse consequences on bone metabolism were presented by other participants in the workshop (BC Nindl and CJ Rosen, respectively) and are reviewed elsewhere in this publication.

NSAID Use

The use of non-steroidal anti-inflammatory drugs (NSAIDs) is known to impair fracture healing (Simon et al., 2002). In animal models, NSAIDs also impair the bone formation response to mechanical loading (Cheng et al., 1997; Chow and Chambers, 1994). The likely mechanism of this action is the inhibition by NSAIDs of cyclooxygenase activity, which catalyzes the conversion of arachidonate to prostanoids. Prostaglandin E_2 has been identified as an important signaling factor in mechanotransduction in bone (Chow, 2000) and has been found to increase in response to mechanical loading in humans (Thorsen et al., 1996). Currently, there are no controlled studies of the potential adverse effects of NSAIDs on the bone formation response to mechanical stress in humans. However, the compelling findings from animal studies suggest that attention should be directed to this issue, particularly since NSAID use is likely to be increased during periods of vigorous physical activity.

POTENTIAL BIOMARKERS FOR THE DEVELOPMENT OF STRESS FRACTURES

Based on the discussion above, potential biomarkers to predict the development of stress fractures could fall within 3 general categories: mechanical stress, bone metabolism, and physiologic factors.

Biomarkers of Mechanical Stress

Although it is unlikely that stress fractures develop solely as a result of mechanical loading, the extent of mechanical stress may influence other predictors of stress fracture. The number of loading cycles is thought to be of less importance than the stress magnitude, which could potentially be monitored by load sensors in the shoes. However, of even greater importance would be the ability to monitor the bone response to mechanical stress, that is, strain. Strain gauges positioned on regions of bone prone to stress fracture (e.g., anterior tibia) could potentially measure strain magnitude and strain rate, since both are important determinants of the bone remodeling response. Because bone is weaker in shear than in compression, it may be particularly important to monitor shear strain. A more futuristic goal would be the early detection of microdamage in bone. The development of such methodologies as vibration analysis, ultrasound, or peripheral quantitative computed tomography for this purpose should be considered.

Biomarkers of Bone Metabolism

If the concept put forth above is correct, that an exaggerated increase in bone resorption in response to multiple stimuli increases the vulnerability of bone to fracture, it will be very important to monitor markers of bone resorption and formation. However, the methodologies currently available measure these markers in serum or urine, which reflect whole body bone metabolism, and are not likely to be useful in identifying targeted changes in bone remodeling that occur in response

to localized strain and microdamage. Methodologies to measure local changes in markers of resorption and formation are not currently available.

Other Physiologic Factors as Biomarkers

The identification of appropriate physiologic factors that predict the development of stress fractures will depend on extending the current state of knowledge of the mechanisms for the pathogenesis of stress fractures in humans. Candidate markers that are likely to be important include those that change in response to vigorous physical training and have potent effects on bone metabolism, such as estradiol and cortisol.

Currently, the signaling pathway by which mechanical stress generates the appropriate cellular responses in bone remains poorly defined. It will be important to promote research directed toward furthering our understanding of the cellular mechanisms of mechanotransduction. However, it will be equally important to support clinical research that evaluates these mechanisms in humans using applied, integrative approaches to determine the relative importance in maintaining bone health. For example, prostaglandin E_2 has been found to play a critical role in the bone formation response to mechanical stress in both isolated cell and *in vivo* animal models, and the response is abrogated by NSAIDs. Despite the widespread use of NSAIDs by humans, particularly in conjunction with exercise training, there is no knowledge of the potential adverse effects on bone metabolism. Because it is likely that risk for stress fractures is multi-factorial, involving both localized parameters of strain and bone metabolism and systemic hormonal modulators of bone metabolism, understanding the pathophysiology of stress fracture development in humans will likely require collaborative research that considers these factors in an integrative fashion.

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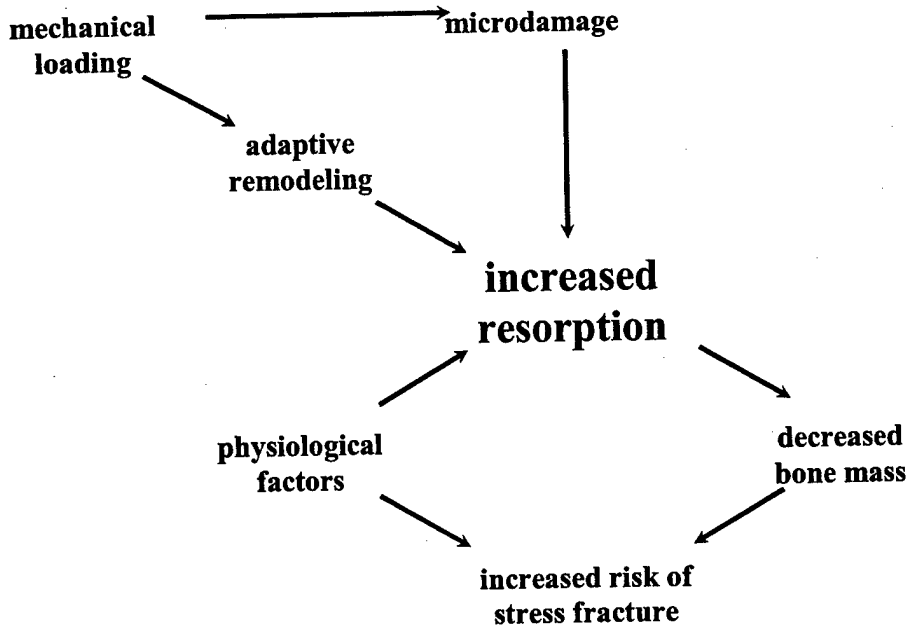


FIGURE 1 Theoretical model for an increase in bone resorption in response to a) loading-induced remodeling activity, b) microdamage that occurs as a result of mechanical loading, and c) exercise-induced changes in physiological factors that increase bone resorption. The 'hyper-resorptive' state would result in a transient reduction in bone mass, increasing the vulnerability of bone to stress fracture. Physiologic factors may also exacerbate risk of stress fracture through other mechanisms (e.g., reduced calcium absorption consequent to increased cortisol excretion).

Circulating Plasma Markers of Cognitive Status

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BACKGROUND AND INTRODUCTION

Basic scientists and clinicians have been searching for biochemical markers of cognitive state for many years. Unfortunately, little progress has been made with regard to identification of markers that, in normal individuals, relate metabolic status to cognitive function or assess general cognitive state. It would be a significant breakthrough for basic science and clinical practice to have reliable plasma markers of cognitive function. Many devastating diseases are either cognitive in nature or produce secondary cognitive deficits. Biochemical tests for the cognitive deficits associated with Alzheimer's disease, depression or Attention Deficit Hyperactivity Disorder (ADHD) would be of extraordinary value to society. In addition, it would be very useful for understanding the biological basis of human behavior to have objective plasma markers of cognitive state. On the battlefield such markers could also be of significant value. They could potentially be employed to optimize warfighter cognitive function and to prevent errors associated with the stress of combat and illnesses associated with combat, such as Post-Traumatic Stress Disorder (PTSD) or Gulf War Syndrome-like diseases.

Current State of the Field

Many peripheral metabolic diseases such as diabetes, hyperthyroid syndromes and Cushing's disease (elevated cortisol) are associated with impaired cognitive function. Frequently, the metabolic markers of the disease are biochemical markers of cognitive state, and sometimes these indicators can provide information about cognitive status in healthy humans. For example, elevated plasma cortisol is an indicator of acute stress, and is negatively correlated with various aspects of cognitive function. The adverse effects of elevated cortisol on cognitive function can be observed in various disease states, and also when exogenous cortisol is administered to normal humans (for a review see Jameison & Dinan, 2001). Unfortunately, cortisol and similar markers appear to provide little information about normal human cognitive function beyond serving as an index of stress-induced declines in cognition. Decrements in military operational performance can be stress-related, but in many instances are not (Johnson and Merullo, 2000). We will provide data that suggest that another endogenous glucocorticoid, dehydroepiandrosterone sulphate (DHEA-S) is, at least in a population we have recently studied, a better marker of normal cognitive status than cortisol.

Plasma glucose, as discussed in detail below, is also an indicator of impaired cognitive function in diseases such as diabetes. When it is artificially lowered to below physiological levels using the insulin clamp technique, cognitive deficits result. However, it often seems to provide little information about cognitive status in healthy individuals, in part because it is tightly regulated. We will provide data that suggest that other metabolic factors associated with energy and carbohydrate metabolism, in particular free fatty acids (FFA) and triglycerides, may, in healthy individuals, be better markers of cognitive state, and perhaps metabolic status, than glucose.

The Inherent Difficulty of Identifying Biochemical Markers for Cognitive State

Although there is great need for objective markers of cognitive state, there are a variety of reasons why it has been extremely difficult to define reliable markers for brain function in normal humans. The greatest difference in normal human cognitive states is between sleep and waking. Classical electrophysiological techniques (polysomnography), as well as functional measures, such as monitoring physical activity, can distinguish sleep from waking state. However, it is not possible to biochemically distinguish these states. The only biochemical measure that, under certain conditions, corresponds to sleep state is the hormone melatonin, but it is not a marker of sleep-state. If states as disparate as sleep and waking - which exhibit the most extreme differences in human cognitive function - are not biochemically distinguishable, we cannot expect to easily find a marker for more subtle differences in human cognitive state, such as optimal alertness versus sleepiness.

The lack of markers for cognitive state is reflected by the fact that there are no biochemical markers for any common psychiatric or neurological disease. Diagnosis and assessment of most psychiatric and neurologic disorders typically relies on labor-intensive, often subjective, clinical evaluations and self-reports. Common diseases such as depression, schizophrenia, ADHD, PTSD, narcolepsy, Alzheimer's and Parkinson's disease, cannot be diagnosed or their progression followed by a biochemical test. Progress has been made using scanning technologies to assess cognitive function, as well as to diagnose and follow the progression of certain central nervous system (CNS) diseases. However, it is difficult to conceive of how such technologies could be practically employed in military field operations to assess cognitive state until significant technological advances occur.

Why has there been so little progress in discovering biological markers of CNS function? It has been known for many years that specific neurotransmitter systems were involved in various CNS disorders, including depression, schizophrenia and Parkinson's disease. However, no biochemical test has been developed to diagnose or follow the course of these diseases. Clearly the development of biochemical tests to assess brain function and behavior has been hampered by the unique, protected status of the brain. The blood-brain-barrier (BBB) isolates, and thereby protects, the brain by preventing the transfer of metabolites from the periphery into the brain. However, the BBB also isolates the periphery from brain metabolites. Therefore, when biochemical markers are assessed in the periphery, usually no direct information regarding central function is provided. Limited exceptions to this principle include hormones released by the brain into the periphery, and a few substances that cross from the brain to the plasma.

Usually glucose is the major source of energy for the brain and, under certain limited conditions, plasma glucose is a predictor of cognitive state. When plasma glucose is reduced from normal euglycemic levels of about 5.0 mmol l^{-1} (90 mg/dL) to 2.6 mmol l^{-1} (47 mg/dL) in non-diabetic individuals, using a hyperinsulinemic clamp, cognitive function is impaired (Strachan et al., 2001). Although this non-physiologic paradigm demonstrates the importance of glucose to the brain, peripheral glucose is tightly regulated in healthy individuals, and rarely reaches levels below 3.6 mmol $^{-1}$ (Wilson et al., 1998). Studies of sustained military training scenarios, which simulate combat, e.g., Ranger Training, support these clinical observations. In Ranger trainees who are in a chronic state of semi-starvation due to several months of severe undernutrition in harsh field conditions, plasma glucose levels fell to no lower then 3.8 mmol $^{-1}$ (Friedl et al., 2000; Moore et al., 1992).

In military, as well as civilian populations, a consistent relationship between plasma glucose within the normal range and cognitive performance has never been demonstrated. Carbohydrate administration can clearly enhance physical performance when high levels of energy are being expended. However, the data relating cognitive performance, carbohydrate administration and plasma glucose are not consistent. Both beneficial and adverse effects on cognition of increasing plasma glucose and providing carbohydrate have been reported (For a review see Bellisle et al., 1998). Overall, while it is clear that carbohydrate supplementation can, in certain circumstances, alter cognitive function (Lieberman et al., 2002), these effects are probably not associated in any simple manner with plasma glucose levels in healthy, non-diabetic individuals.

NEW MARKERS OF COGNITIVE STATE: STUDIES ON MILITARY POPULATIONS IN WHICH COGNITIVE AND BIOCHEMICAL FACTORS WERE ASSESSED

On several occasions, as part of field studies, we have examined the relationships between cognitive performance and plasma or saliva metabolites. Initially neurotransmitter precursors like tryptophan and tyrosine were of interest as they are actively transported into the brain across the BBB. In an early study, the volunteers were soldiers participating in an evaluation of a lightweight ration and were modestly undernourished for several weeks (Askew et al., 1987). In that study the ratio of plasma tryptophan to the other large neutral amino acids (LNAA), which predicts the rate of transport of tryptophan across the BBB, was correlated with cognitive performance ($r = 0.40$ to 0.44, $p<0.02$). We believe that the tryptophan/LNAA ratio was associated with cognitive performance because tryptophan is the precursor of a critical brain neurotransmitter, serotonin. Levels of other plasma amino acids were not related to cognitive performance (Lieberman et al., 1997). In a previous presentation to the Committee on Military Nutrition Research (CMNR), we discussed these findings and addressed the overall importance of a variety of neurotransmitter precursors (Lieberman, 1999). In the last few years we have focused on hormones and metabolic factors that can be measured in saliva, or that do not require assessment of multiple amino acids (all the LNAA's).

Study I: A Brief, Intense Training Exercise Conducted by an Operational Ranger Unit

Recently, we evaluated cognitive function, and several biochemical markers of stress, of soldiers engaged in a brief (52 h) high-intensity training operation. The exercise was conducted by U.S. Army Rangers and designed to evaluate junior leaders (Lieberman et al., 2002). The scenario simulated combat-like conditions, specifically a high-intensity, light infantry operation in a hostile environment, by combining multiple stressors: near total sleep deprivation, continuous physical activity, substantial physiological, environmental and psychological stress and simulated, combat-like activities. All volunteers (N=31) were Ranger officers (mean age = 32 years) with the rank of Captain, and had served, on average, 9 years on active duty. The exercise was conducted in a hot, humid environment.

The exercise consisted of three phases: a garrison preparation phase, a field exercise and a concluding garrison phase. Cognitive performance, mood and body composition were assessed once during each phase. We used a battery of cognitive tests that were administed on notebook computers and took less than an hour to complete. The battery was designed to assess a wide range of militarily-relevant, cognitive functions. To assess mood we employed the most widely accepted measure of mood state, the Profile of Mood States (POMS), which has been used in hundreds of civilian and military studies (McNair et al., 1971). It is a standardized, validated self-report questionnaire, consisting of 65 mood-related adjectives, which are rated on a five-point scale, in response to the question, "How are you feeling right now?" It takes less than 5 minutes to complete. The adjectives factor into six mood sub-scales: Tension, Depression, Anger, Vigor, Fatigue and Confusion.

Carefully selected measures of mood state are excellent predictors of cognitive performance and sensitive indicators of functional capability. Depressed patients perform poorly, and drowsy normal subjects have impaired cognitive function. Drugs, environmental stress, foods and dietary supplements that affect cognitive performance have repeatedly been shown to have analogous effects on related mood states. Compounds that enhance cognitive performance like amphetamine, caffeine and tyrosine improve corresponding moods, while treatments that degrade performance, like benzodiazepines (e.g. valium), melatonin and antihistamines, invariably impair mood (Dollins et al., 1993; Fine et al., 1994; Lieberman et al., 1986; Newhouse et al., 1989). Advantages of mood questionnaires include; the brief period of time required to administer even comprehensive versions of them, and the fact that no equipment is needed for their administration. In situations like Marine basic training, where volunteers are available for only brief periods of time and a large number of subjects must be tested simultaneously, they are the only practical way of gathering frequent and detailed data on cognitive state.

At both the in-field and post-field testing sessions we observed very large decrements in cognitive performance, including changes in fundamental functions, like vigilance ($p<0.001$; Fig. 1) and choice

reaction time ($p<0.001$), as well as more complex abilities – learning ($p<0.001$), memory ($p<0.001$) and logical reasoning ($p<0.001$; Fig. 1). All mood states assessed were adversely affected, including vigor ($p<0.001$), fatigue ($p<0.001$; Fig. 1) , confusion ($p<0.001$; Fig. 1), tension ($p<0.02$), depression ($p<0.002$) and anger ($p<0.01$) (Lieberman et al., 2002). We also assessed cortisol, testosterone and melatonin in saliva samples collected 3 times/day. As in previous short-duration studies conducted with soldiers exposed to multiple stressors (for example see Opstad, 1994), rather than an increase in cortisol or testosterone, we observed suppression in their circadian pattern of release. Patterns of melatonin release did not change. We did not observe any consistent relationships between hormone levels and impairments in cognitive performance over the course of the exercise, although pre-exercise cortisol did predict, in several instances, pre-exercise, and subsequent, cognitive performance. This association suggests Rangers who perceived the exercise as likely to be stressful, or who were already "stressed" when they reported for the exercise, performed worse than their peers. In this study, conducted with soldiers who were subjected to a variety of stressors, but not severe psychological stress, saliva cortisol, testosterone and melatonin levels provided limited information on cognitive state.

Study II: Marine Basic Training-Relationships between Cognitive and Biochemical Changes in Female Trainees

Recently, our laboratory conducted a comprehensive study of a large group of female trainees enrolled in the 12 week Marine basic training course at Parris Island, S.C. (Bathalon et al., in press). Every 4 weeks, on the same day, plasma was collected and a POMS mood state questionnaire was administered. A variety of other parameters were also regularly assessed. The mood questionnaire was administered in the morning and blood samples were obtained in the afternoon. Mood was assessed to provide information on the cognitive state of the volunteers as they progressed through training. We also attempted to assess the relationship between mood state and biochemical markers of metabolic state, endocrine status and inflammation.

All mood states assessed by the POMS in the female Marine basic trainees improved substantially over the course of basic training (Fig. 2). The trainees began basic training feeling worse than is typical of age-matched females but, by the time they had completed training, their scores were better than the norm (McNair et al., 1971). During training there were also significant changes in a number of biochemical parameters, particularly free fatty acids, triglycerides and DHEA-S (Fig. 3). Other biochemical markers such as glucose and cortisol were more stable (Fig. 3). The changes in FFA and triglycerides were consistent with the changing physiological and nutritional status of the trainees. Over the course of the study the women lost substantial body mass overall (mean = 1.7 kg), especially fat (mean = 4.4 kg) but gained muscle mass (mean = 3.3 kg), as assessed by dual-energy x-ray absorptiometry (DEXA). A gain in muscle-mass would be expected given the rigorous nature of basic training. The trainees' diets also changed, with a significant reduction in total food intake, and reduced fat in the diet, compared to their pre-recruit diet. Levels of stress appeared elevated, as indicated by chronically elevated levels of cortisol, near the upper limits of normal, and high levels of tension on the POMS, particularly during the earlier phases of training (Fig. 2, 3).

There were robust, highly significant correlations between mood and DHEA-S, substance P, FFA and triglycerides (Table 1) over the course of training. Plasma levels of fructosamine, which reflects average blood glucose levels for the last 17 to 21 days, thyroid stimulating hormone (TSH) and substance P also were associated with mood states, but not as frequently or as robustly as DHEA-S, FFA and triglycerides (Table 1). When stepwise multiple linear regression analyses were performed, the most reliable predictor variables for mood were DHEA-S, FFA and triglycerides. The extent of overall individual weight loss over the course of training was only associated with the mood state of vigor, with the greater weight loss associated with less vigor ($r = -0.20$, $p< 0.02$). Weight loss was often a statistically significant predictor variable in the multiple regression analyses, even though the correlations between weight loss and moods were modest ranging from $\pm$ 0.02 - 0.20. It also appeared that the predictive biochemical parameters were associated with a similar underlying factor(s), as they often were individually correlated. When these markers - FFA, triglycerides, fructosamine and DHEA-S were aggregated in multiple regression models, with weight loss

included as a predictor variable, ability to predict mood states was increased and r^2 values as high 0.40 were obtained, indicating the regression model could account for 40% of the overall variance associated with certain mood states.

The magnitude of the relationships we observed between mood states and these biochemical markers, both as individual correlations and within multiple regression models, was surprising. We are not aware of any combination of putative physiological markers for mood or cognitive state where such robust associations have been observed in healthy individuals. The magnitude of the individual relationships between plasma markers and mood (many r values were in the range 0.3-0.45 as shown in Table 1) should be placed in the context of firmly established, clinically significant relationships between other biochemical markers and functional outcomes. Widely accepted markers of disease generally have only modest associations with the underlying disease state they predict. For example, the association of "ratio of high density cholesterol to total cholesterol" with the extent of coronary occlusion in patients with cardiovascular disease is only r = -0.20 (Naito et al., 1980).

The associations we have observed between these peripheral metabolic markers and cognitive state during Marine basic training are of the same magnitude as those we previously observed for the tryptophan/LNAA ratio in soldiers participating in the lightweight ration study discussed above. The tryptophan/LNAA ratio determines the rate of tryptophan transport across the BBB. Tryptophan, because it is a rate-limiting precursor of the neurotransmitter serotonin, serves a critical CNS need (Lieberman et al., 1997; Lieberman, 1999). It should be emphasized that many of the metabolites and hormones evaluated in the Marine basic training study, including glucose, corticotropin-releasing factor (CRF), cortisol and leptin which, based on their known associations with brain function, might have been expected to be associated with cognitive function, but were not (Table 1).

CONCLUSIONS AND RECOMMENDATIONS

There are many obstacles associated with identifying biochemical markers of cognitive state. In the study we conducted with U.S.Army Rangers engaged in a brief, high intensity field exercise, saliva cortisol, melationin and testosterone were not usually associated with performance and mood. However, the preliminary findings from the Marine basic trainee study we describe, suggest that at least one endocrine factor (DHEA-S), and several metabolites associated with energy status, are robust markers for cognitive state in female recruits during basic training. Of course, these associations may be unique to the gender of the volunteers or to the combination of physiological, nutritional and psychological factors the basic trainees experienced. To determine if these relationships generalize to other populations, this study will have to be replicated and extended, including studies of males. Of particular interest will be whether cognitive performance, as well as mood, is associated with these biochemical markers. It will require a substantial effort to address these questions since it is more difficult to assess cognitive performance than mood state in large samples. Furthermore, the unique factors associated with basic training, especially the large changes in mood and biochemical state that occurs, make attempts at replications in other populations of questionable validity. We believe that we observed these biochemical-behavioral relationships because we were evaluating individuals who had unusually robust changes in both metabolism and behavior. Rarely are metabolic and cognitive changes of this magnitude observed in healthy individuals.

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Approved for public release; distribution is unlimited. The views, opinions and/or findings in this report are those of the authors, and should not be construed as an official Department of the Army position, policy or

decision, unless so designated by other official documentation. Human subjects participated in these studies after giving their free and informed voluntary consent. Investigators adhered to AR 70-25 and USAMRDC Regulation 70-25 on the use of volunteers in research. For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law CFR 46. Citation of commercial organization and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

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TABLE 1 The relationship between plasma markers and mood states during Marine basic training in females. Pearson corr
values, between mood, assessed by the POMS, and selected plasma hormones, metabolites and markers of inflammation are p
were 41 volunteers. If the association is significant ($p < 0.05$), a p value is presented. If a marker had five or more signi
highlighted.

HORMONES	FATIGUE	CONFUSION	DEPRESSION	TENSION	ANGER
Dehydroepiandrosterone sulfate	0.36, < 0.001	0.45, < 0.001	0.35, < 0.001	0.44, < 0.001	0.30, < 0.001
Substance P	0.18, 0.03	0.23, 0.005	0.23, 0.007		0.18, 0.03
Free Fatty Acids	0.22, 0.005	0.46, < 0.001	0.24, 0.002	0.44, < 0.001	0.16, 0.05
Triglycerides	- 0.25, 0.001	- 0.45, < 0.001	- 0.35, < 0.001	- 0.44, < 0.001	- 0.29, < 0.001

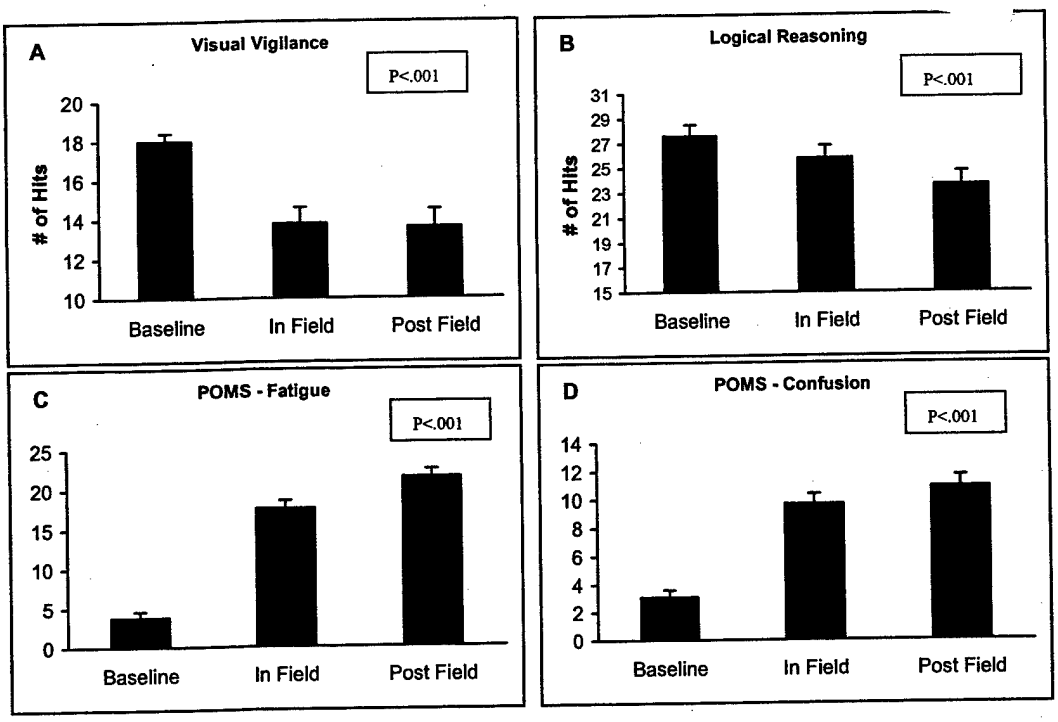
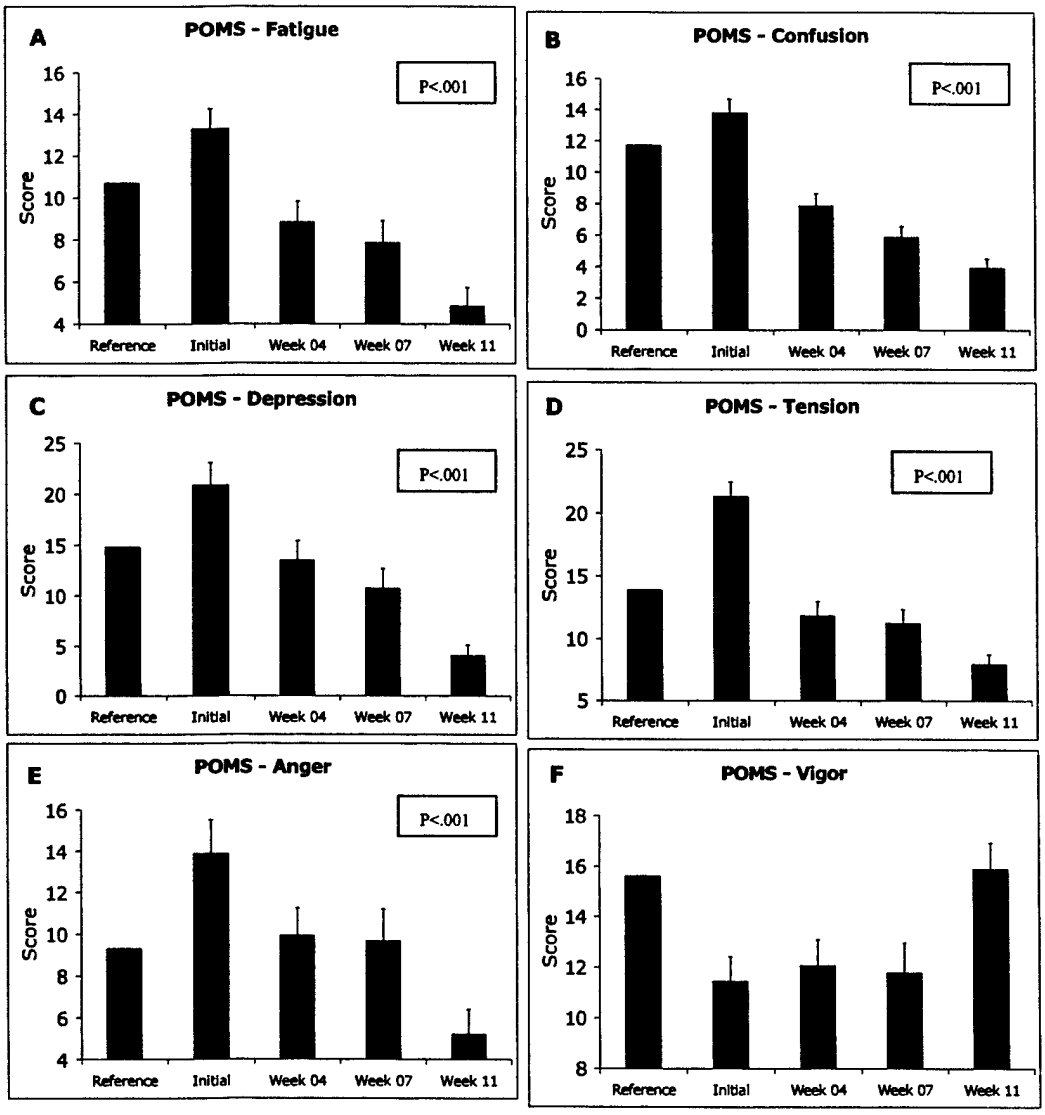


FIGURE 1 Fig. 1, A–C. Changes in cognitive performance and mood (mean + SEM) assessed before, during and immediately after a brief, high intensity Ranger training exercise. Statistical significance over time, as determined by a within-subject ANOVA, is provided (Lieberman et al, 2002).



□□□□□□□□
□□□□□□□□□□□

FIGURE 2 Fig. 2, A-F. Mean (+ SEM) changes in mood state in female trainees as assessed by the POMS over the course of Marine basic training. A reference value for female college students, of approximately the same age as the trainees, is provided for comparison (McNair et al., 1971). Statistical significance over time, as determined by a within-subject ANOVA, is provided.

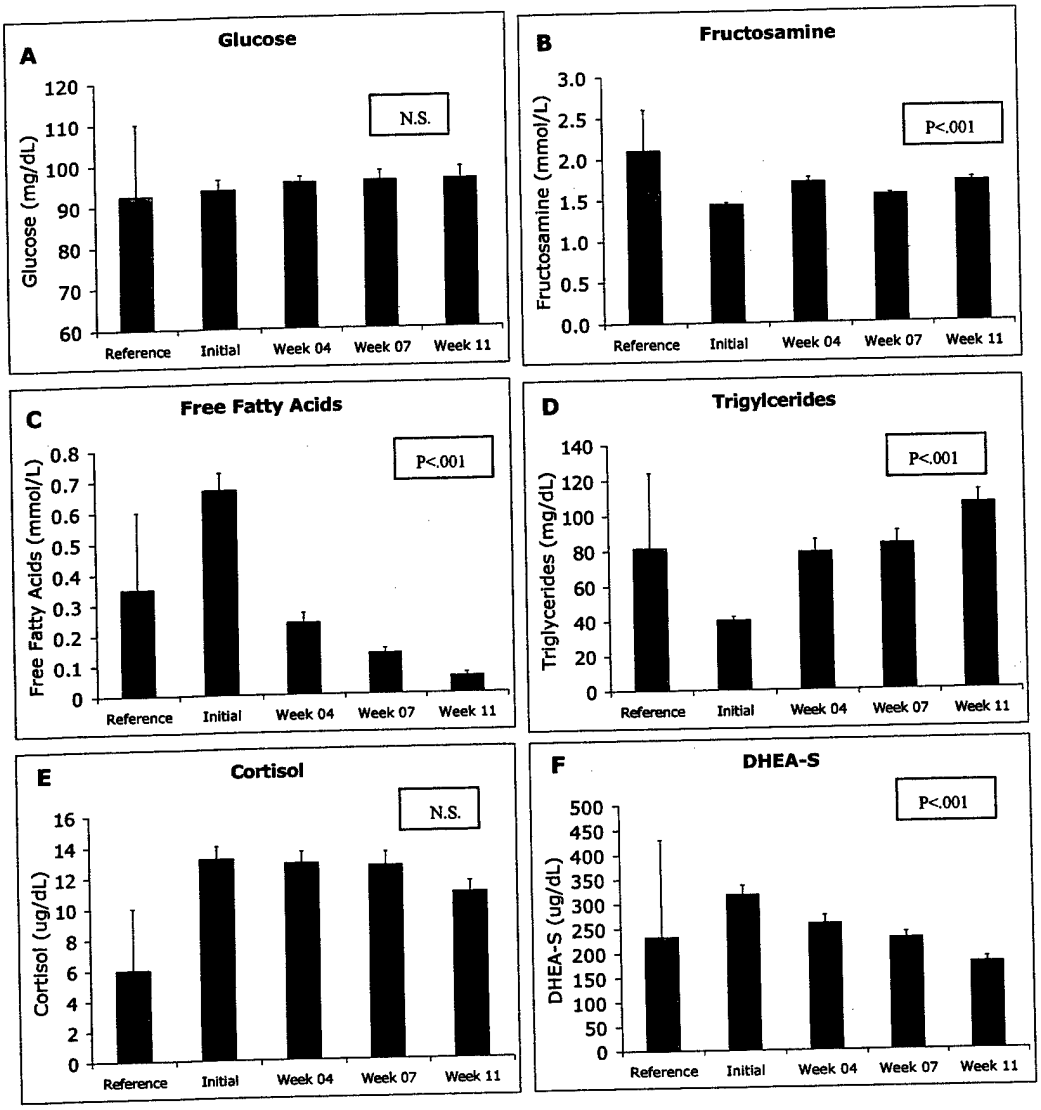


FIGURE 3 *Fig. 3, A-F. Variation in mean (± SEM) plasma concentration of the indicated marker in female trainees over the course of Marine basic training. A reference value (± 2 SD) is provided for comparison. Whenever the data were available the reference value is for females of approximately the same age. Statistical significance over time, as determined by a within-subject ANOVA, is provided.*

UTILITY OF INSULIN-LIKE GROWTH FACTOR-I FOR ASSESSING METABOLIC STATUS DURING MILITARY OPERATIONAL STRESS

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MILITARY RELEVANCE OF MONITORING INSULIN-LIKE GROWTH FACTOR-I

Mission success in military tactical environments dictate that the warfighter be able to perform prolonged physical exertion in the face of food and sleep restriction (i.e., military operational stress). The physiological strain produced by these operational stressors can have deleterious effects on muscle mass, endocrine and metabolic function, as well as physical and mental performance (Friedl, 1999; Friedl et al., 2000; Nindl et al., 2003; Nindl et al., 1997; Nindl et al., 2003; Nindl et al., 2002) (see Figure 1). A goal of the Army Medical Research and Materiel Command's (MRMC) biomedical research program is to identity useful biomarkers that are indicative of nutritional and physiological status that can be assessed rapidly, with minimally or non-invasive collection methods. Once identified, these biomarkers will be used to sustain warfighter readiness and aid in assessing the effectiveness of intervention and recovery strategies.

The growth hormone/insulin-like axis is a central endocrine axis and is thought to mediate many of the somatotrophic changes that are observed when warfighters are exposed to harsh field environments (Florini et al., 1996; Friedl et al., 2000; Nindl et al., 2003; Rosen, 1999; Rosendal et al., 2002). For this reason, periodic assessment of the growth hormone/insulin-like growth factor axis may have utility for sustaining warfighter health and performance. In direct support of Objective Force Warrior's (OFW) vision of revolutionizing soldier performance by aggressively employing science and technology efforts that enhance the warfighter's survivability, lethality, sustainment, and mobility on the modern battlefield, The Military Performance and Military Nutrition Division of the U.S. Army Research of Institute of Medicine have been evaluating IGF-I as a candidate biomarker for assessing nutritional stress. Our research has focused on 1) characterizing temporal response patterns of IGF-I and its family of binding proteins during military operational stress, 2) the influence of macronutrient and energy intake on

the circulating IGF-I system responses to stress, and 3) assessment of minimally invasive and field expedient collections methods for determination of IGF-I.

The purpose of this short review paper is to summarize why IGF-I has been of interest as a potential biomarker and our experimental strategies for evaluating the merits of IGF-I as a biomarker of nutritional and operational stress. The paper initially describes the complex nature of IGF-I regulation and relevance for the military then describes initial work characterizing the IGF-I response to military operational stress. The experimental outcomes suggest that IGF-I has potential value as a biomarker of nutritional strain during operational stress.

Insulin-Like Growth Factor-I Physiology and Regulatory Complexity

The primary source of circulating IGF-I is the liver, but local release from tissues that secrete IGF-I in an autocrine/paracrine manner also contribute. IGF-I itself is a 7.6-kDa polypeptide consisting of 70 amino acids with three intrachain disulfide bonds. Only a small amount (<2%) of IGF-I, however, circulates in free form. Most circulates in either a binary (~20-25%) or ternary complex (~75%). When circulating in the binary form, IGF-I is complexed with one of six binding proteins (BPs; BPs- 1-6), ranging in size from 22.8 to 31.4 kDa. The ternary complex consists of IGF-I, IGF BP-3, and an 80-86 kDa protein called the acid labile subunit (Baxter, 2000; Jones and Clemmons, 1995; Rajaram et al., 1997; Sara and Hall, 1990). An IGF-I specific protease is responsible for breaking the bonds holding the ternary complex together, and making the IGF-I available for receptor binding. The IGF-I complexes are thought to regulate the availability of IGF-I to target tissues as only the free and binary complexes can pass from the vascular compartment into the interstitial space. The different forms of BPs are also thought to play a role in transporting the IGF-I to the target tissue (Baxter, 2000; Sara and Hall, 1990).

IGF-I has several metabolic effects. It is known to promote amino acid uptake, enhance protein synthesis, and attenuate protein degradation (Florini et al., 1996; Rosen, 1999; Thissen et al., 1999). Additionally, IGF-I plays a role in stimulating cell growth and differentiation (Baxter, 2000; Florini et al., 1996).

The appeal of IGF-I as a biomarker is the dynamic nature in which circulating concentrations respond to nutritional stress. Underfeeding and protein-calorie malnutrition result in substantial reductions in IGF-I concentrations and the response persists until the nutritional stress is removed (Friedl et al., 2000; Frystyk et al., 1999; Nindl et al., 2003; Rand et al., 2003; Thissen et al., 1992; Thissen et al., 1999). Additionally, IGF-I concentrations are relatively stable. Unlike hormones such as growth hormone, IGF-I displays little in the way of circadian variability, thus single time point samples are indicative of IGF-I status.

Effects of Military Operational Stress on the Circulating IGF-I System

US Army Ranger Training

Friedl and colleagues performed experimental studies characterizing the physiological responses of soldiers participating in the U.S. Army Ranger Training Course (Friedl et al., 2000; Friedl et al., 1994; Nindl et al., 1997). The data provide insight into the adaptive process that occurs as soldiers cope with sustained physical work, energy restriction and sleep disruption. The U.S. Army Ranger training course is 62-days in length and is designed to teach and evaluate individual leadership and small unit tactics under physically and mentally challenging conditions. The course includes multi-day periods consisting of near-continuous physical activity, energy restriction and sleep deprivation. In the first investigation, energy intake was restricted to 1,300 kcal per day during the field training portion of the course and the periods of underfeeding produced average energy deficits of ~1000 kcal/day over the entire course (Friedl et al., 2000). Average energy expenditures were ~4000 kcal/day. At the end of the course, the participants had

lost 13-16% of their initial body mass, ~65% of their fat mass, and had lost 7% of their initial lean body mass. IGF-I, measured every 2 weeks during the Ranger Course, progressively declined through the first 6 weeks, with no further reduction over the final 2 weeks of the course (Figure 3). At the end of the course, IGF-I values had fallen 62% (pre: 198±54 $ng \bullet ml^{-1}$ vs. post: 75±25 $ng \bullet ml^{-1}$). As Figure 3 illustrates, most of the decrease in serum IGF-I occurred during the initial 2 weeks of the course. The potential of IGF-I as a discriminating variable for assessing nutritional and/or metabolic stress was the separate observation that the soldiers who had the greatest decline in IGF-I were the soldiers that lost the most weight ($r = -0.38$, $P < 0.01$).

A second study with the U.S. Ranger Training Course enabled investigators to study the effects of altering the energy content of the diet on the metabolic and hormonal responses to the course (Friedl et al., 2000). In the second study, the training conditions were nearly identical, but the participants received additional calories during the energy restriction periods embedded within the course (+400 kcal/day). Additionally, to gain information about short-term responses to refeeding (while other course stressors remained undiminished), a blood sample was obtained after a week of access to food that was preceded by multiple days of energy restriction (~1700 kcal/day) coupled with high-energy expenditures (>4500 kcal/day). As illustrated in Figure 3, the addition of 400 kcal/day significantly attenuated the decline in circulating IGF-I concentrations when compared to the group receiving fewer calories. Additionally, the investigators found that the brief period of refeeding was sufficient to temporarily restore IGF-I concentrations to baseline values. When food was again restricted after this brief refeeding period, IGF-I concentrations rapidly fell and remained low through the remainder of the course. These data demonstrate the sensitivity and responsiveness of IGF-I to energy and nutrient delivery. When energy is restricted, IGF-I values fall and remain low until energy restriction is removed. The provision of energy and the restoration of fuel stores are accompanied by an increase in IGF-I.

The traditional evaluation of nutritional status utilizes a global assessment of parameters that include anthropometric measures and the assay of serum proteins (Baxter et al., 1998). The proteins commonly measured include albumin, transferrin, prealbumin and retinol binding protein. Transferrin is indicative of iron binding capability; retinol binding protein is indicative of Vitamin A status and ability to transport Vitamin A, prealbumin (considered by some to be best single marker of malnutrition due to its short half-life) is sensitive to protein malnutrition and zinc deficiency. The strength of these markers is that they provide insight into the nutritional status of the individual. Unfortunately, a number of non-nutritional factors can affect serum levels independent of dietary adequacy. For example, prealbumin levels fall with inflammation, albumin levels are affected by hydration state and oral contraceptive use, transferrin levels decline in response to protein malnutrition but also chronic illness and inflammatory states and with liver disease. In contrast, IGF-I appears to be a more responsive and selective biomarker of energy status due to its rapid response to depletion and repletion (Baxter et al., 1998). The 2-4 hour half-life of IGF-I provides a distinct advantage vs. other traditional serum protein biomarkers (prealbumin ~ 2 d, albumin 20 d, transferrin 20 d).

In a study examining endocrine and metabolic recovery responses, Nindl et al. (1997) measured IGF-I, transferrin, ferritin, and prealbumin before, at the end of The U.S. Army Ranger Training Course, and after 5 weeks of recovery. The five week recovery period produced a rebound effect such that body mass was significantly higher than measured before starting the course. Body composition analysis revealed a 1.1 kg increase in fat free mass and a 4.1 kg increase in fat mass above pre-course values. IGF-I fell ~50% during the course and was 30% above baseline values after 5 weeks recovery. Transferrin levels did not significantly change during Ranger training or during recovery. Prealbumin levels declined 21% during the course (26.8 to 21.3 mg/dl) and returned to baseline levels during recovery period, but the levels at the end of the course (despite accruing an 11% body mass loss) were well above values indicative of malnutrition (< 15 mg/dl). Thus, in this study, IGF-I appeared more sensitive to changes in energy balance and body composition changes than the other markers of nutritional status.

Short-Term Military Sustained Operations

To study the acute responses to energy and nutrient restriction we recently measured the circulating IGF-I and IGF binding proteins pattern of response to 4 days of near-continuous physical work, energy restriction, and sleep disruption (Nindl et al., 2003). The participants had morning fasted blood drawn on days 1, 3, and 4 during a control week that contained physical performance testing but no sustained physical activity, caloric restriction or sleep deprivation. They also had blood samples drawn on days 1, 3, and 4 of the experimental period that included the physical performance tests, near-continuous physical activity (energy expenditure ~ 4,500 kcal/day), energy restriction (~1,600 kcal/d), and sleep deprivation (6.2±1.1 h over 84 h course). Blood was assayed for concentrations of total IGF-I, free IGF-I, IGFBPs 1, 3, and 6 and the acid labile subunit. Additionally, in order to gain further insight into whether this type of stress altered the partitioning of IGF-I among its various molecular complexes, IGF-I and IGFBP-3 were measured before and after immunoaffinity depletion of acid-labile subunit complex (i.e., ternary complex removal) thus yielding estimates of ternary (high molecular weight complexes) vs. non-ternary (low molecular weight complexes) IGF-I (Khosravi et al., 2000). Two days of military operational stress significantly lowered circulating total and free IGF-I values and they remained low with continued operational stress (Nindl et al., 2003). Accompanying the IGF-I reductions were small reductions in IGFBP-3 and large increases in IGFBP-1. These changes in circulating IGFBP levels, however, were not associated with a measurable shift in the quantity of IGF-I circulating in ternary, binary or free forms (Nindl et al., 2003). The importance of these data for metabolic monitoring is that they show the speed with which the IGF-I system responds to energy and/or nutritional restriction. They also illustrate a potential method for investigating changes in the bioavailable IGF-I in response to nutritional stress.

Influence of Dietary Protein Content of Circulating IGF-I During Military Training

Both energy restriction and protein energy malnutrition are known to suppress circulating IGF-I. There are many logistical challenges to sustaining adequate nourishment for soldiers during military field training such as food preparation, storage, delivery, and meals that provide adequate levels of calories and macro- and micronutrients. With increased operational tempo of current military maneuvers, space allocation for food is often sacrificed for weapons, ammunition and other necessary field gear. It would therefore seem essential that the nutrients that are provided during military operational stress consist of an optimal macronutrient mix that may protect against the decline in circulating anabolic and growth factors (Friedl, 1999). The recommended RDA for protein is 0.8 g/kg body mass. Current recommendations for physically active populations are 1.2 to 1.5 g protein/kg body mass (Fielding and Parkington, 2002; Rand et al., 2003). It is common for infantry type units to subsist on one-to-two Meals Ready-to-Eat (MRE) per day during field operations. The MRE is a 1,300 kcal ration comprised of 24 menus. Protein content of the ration ranges from 26 to 60 grams with a mean value of 44 grams. Thus, if a soldier is limited to one-MRE per day their diet is low both in energy and protein content. Even consuming 2 MREs per day, the soldier may still not meet the minimal RDA for protein.

To examine the hypothesis that dietary protein supplementation during military operational stress would attenuate the decline in IGF-I observed when units were fed insufficient energy and protein, we recently conducted a study where dietary protein was manipulated, while controlling both carbohydrate and energy intake. Thirty-five Marines were randomly divided into either a group receiving a low energy-low protein diet (1,600 kcal/day and 0.5 g protein/kg body mass per day) or a group receiving a similar amount of energy but with sufficient added protein to receive approximately 1.0 g protein/kg body mass per day. The group was participating in an 8-day field exercise consisting of sustained physical activity (total daily energy expenditure measured in previous iterations has ranged from 17-25 MJ/d) and sleep deprivation. Morning

fasted blood was obtained before, mid-way and at the end of the course. Preliminary results show trends suggesting that protein supplementation may have attenuated the decline in IGF-I during the course. If more thorough examination of the data supports this conclusion, these data would provide further support for the merit of monitoring IGF-I as a biomarker for metabolic status. Another observation from this study was that IGF-I displayed a different temporal pattern in response to the course than other conventional nutritional status indicators (e.g. ferritin, prealbumin, transferrin, and retinol binding protein). Transferrin and ferritin initially increased during the course but reversed towards baseline values during the latter half of the course. Whereas, retinol binding protein and prealbumin declined over the course but more abruptly during the latter half of the course. Thus, while both IGF-I and the conventional markers responded to the training stress their differential response suggests that they each provide a different index of nutritional status.

Measurement of IGF-I with a Filter Paper Blood Spot Assay

If IGF-I is to be used as a metabolic status indicator during military operational stress, field expedient methods for collection and measurement must be established. Field environments present unique logistical challenges compared to the laboratory. There is more likelihood of sample contamination, and since it is difficult and sometimes impossible to bring the laboratory equipment to remote field environments, sample collection, processing, and transportation become significant logistical hurdles.

A technique that has been used successfully to study malnutrition in underdeveloped countries is chemical analysis of dried blood spots (Diamandi et al., 1998; Mitchell et al., 1987). The technique requires minimal amounts of blood, minimal field processing, and no refrigeration during shipping. Mitchell et al. (1987) originally described measurement of IGF-I from blood spots using a conventional RIA. More recently, Diamandi et al., (1998) described the extraction and measurement of IGF-I and IGFBP-3 from dried blood spots using an enzyme-linked immunoabsorbent assay.

To study whether the dried blood spot methodology could track IGF-I responses to military operational stress, both blood spots and conventional blood samples were collected in a recent field study that manipulated dietary protein intake (described above). We found that IGF-I measured from blood spots declined during the 8-day course and the magnitude of decline was similar to the decline measured using serum samples (Figure 4) (Nindl et al., 2003). Overall, the blood spot IGF-I and serum IGF-I significantly ($p<0.05$) correlated ($r = 0.92$), but the blood spot values were on average 61% lower than serum (Nindl et al., 2003). Diamandi et al. (1998) also reported lower (20-25%) IGF-I values from blood spots when compared to plasma samples. Several possible factors could have contributed to the differences in IGF-I using the two sampling techniques. First, in order to reduce pre-analytical variance and ensure maximal extraction it is essential that complete dryness of the blood spot is maintained until the sample is analyzed. In both our study and that of Diamandi et al. (1998), the blood spots were stored in plastic bags without addition of desiccant. Work by others suggests that moisture can produce a glassing effect whereby hygroscopic blood proteins impede elution. Assaying dried blood spots also assumes an absolute and consistent blood volume is distributed onto each punch. If the volume of blood on filter paper was consistently overestimated, it may have contributed to the bias between the two sampling methods as IGF-I was purposefully measured using different assays. Regardless of the reason for the bias, the outcomes of this study reveal that the blood spot on filter paper technique can be applied for measurement of IGF-I responses to military operational stress. The technique requires minimal blood, and minimal equipment assets for sample collection, processing and shipment. The filter paper blood spot method for IGF-I detected reductions accompanying nutritional stress and may be of potential value

for characterizing the IGF-I response when conventional blood sampling methods are not feasible.

Future Directions and Enablers for Objective Force Warrior

The data collected on the physiological responses to military operational stress support the potential utility of IGF-I as a metabolic sensor of energy status. IGF-I responds rapidly to energy restriction and remains a viable indicator of an altered energy state until the stressor is removed. IGF-I responds rapidly to dietary changes and can be used to evaluate adequacy of protein intake independent of energy intake. Future work, however, must establish whether IGF-I alone or in combination with other biological indices can provide useful information to preserve the health and performance of military personnel during operational stress (Friedl, 2003). In addition, sampling and processing techniques must be established that are safe, reliable, require minimal logistical support and most important, provide rapid feedback to personnel tracking physiological status.

While this review has exclusively focused on the utility of IGF-I measurement during military operational stress, IGF-I may also have merit as a biomarker during fitness and exercise training. Continued scientific efforts should focus on further elucidating the link between alterations in the biological matrix (e.g. muscle, bone, adipose, immune, and neural cells) and ensuing influences on soldier physical performance. A provocative hypothesis is that any changes in the biological matrix are mediated by somatotrophic hormonal responses that act in both systemic and local mechanisms (Nindl et al., 2001; Nindl et al., 2002). A greater understanding of the somatotrophic influences mediating muscle repair and bone remodeling after the microtrauma of physical exertion and viable countermeasures which modulate muscle repair and tissue regeneration after microdamage is essential toward the transformation of the modern Army through Objective Force Warrior. To maintain dominance across the full spectrum of military operations, the 21st century warfighter must posses an optimal level of physical readiness and be able to recover quickly from fatigue and overexertion. IGF-I is emerging as a truly important regulator that is important to health and fitness. IGF-I is a promising outcome measure for military studies on the refinement of medical fitness standards as well as physical training and nutrition policies.

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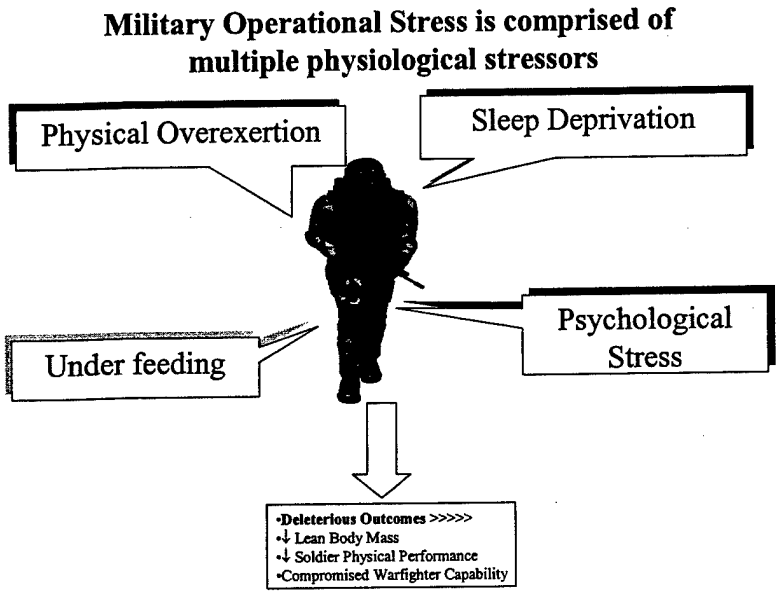
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FIGURE 1 Military operations place multiple stressors on the Warfighter. These stressors typically occur simultaneously. The magnitude of the resulting strain is dependent on the severity of the stressors. The resulting physiological stain can result in deleterious outcomes on lean body mass, soldier physical performance and can compromise warfighter readiness



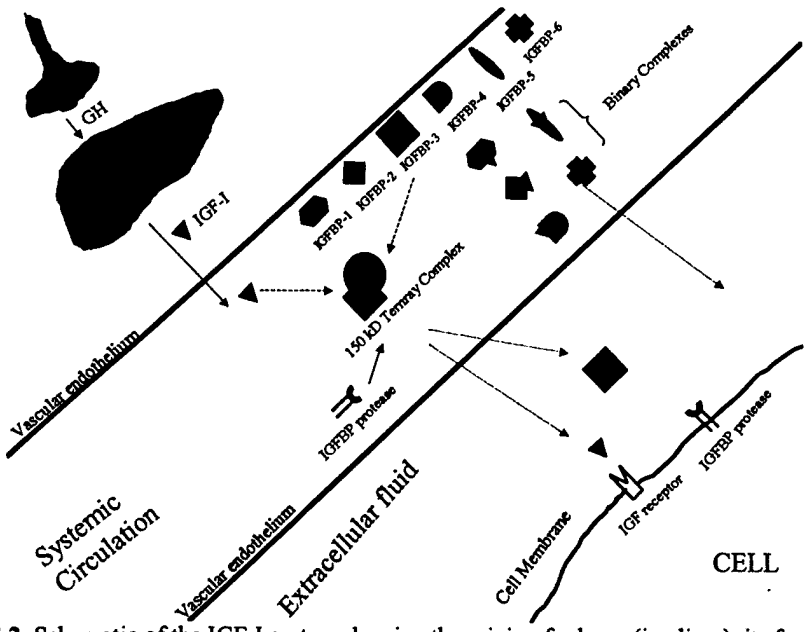


FIGURE 2 Schematic of the IGF-I system showing the origin of release (i.e. liver), its family of binding protiens (BPs 1-6), ternary and binary complexes, and IGF-I trafficking from the circulation to the receptor.

FIGURE 3 Serum IGF-I concentrations over the 8-wk U.S. Army Ranger Training Course. The solid lines represent the values from Study 1 and the dotted line represents the values from Study 2. Study 1 and Study 2 were conducted under identical conditions with the exception that during Study 2, the subjects received 400 more kcal/day than Study 1. Values are mean ± SD. Different letters represent mean values that are statistically different; shaded area represents values below normal for young men. The figure is printed with permission. (See Friedl et al., 2000)

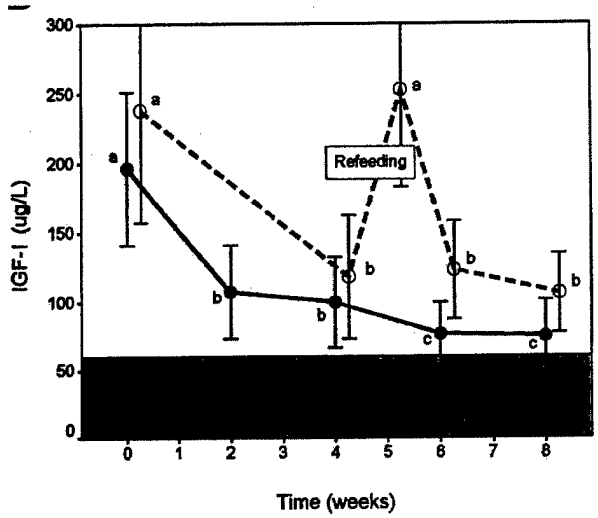
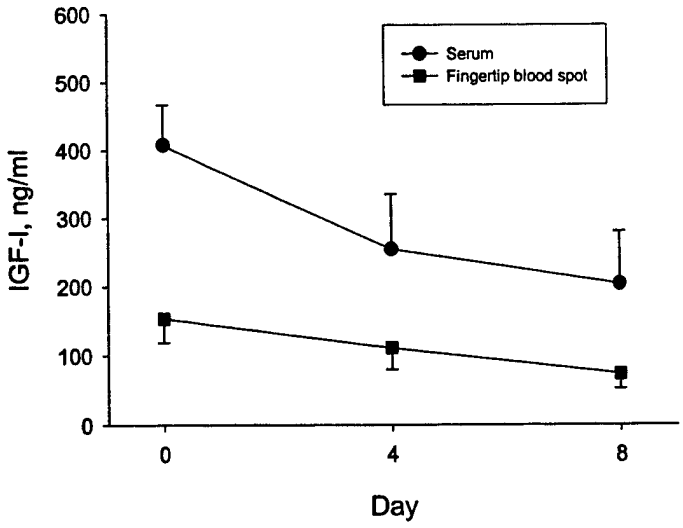


FIGURE 4 Comparison between serum and filter paper blood spot IGF-I concentration during Days 0, 4, and 8 of military operational stress. For both methods, a progressive decline over time was observed (Day 0 > Day 4 > Day 8). Serum IGF-I was greater than filter paper blood spot IGF-I at each respective timepoint. (See Nindl et al., 2003)



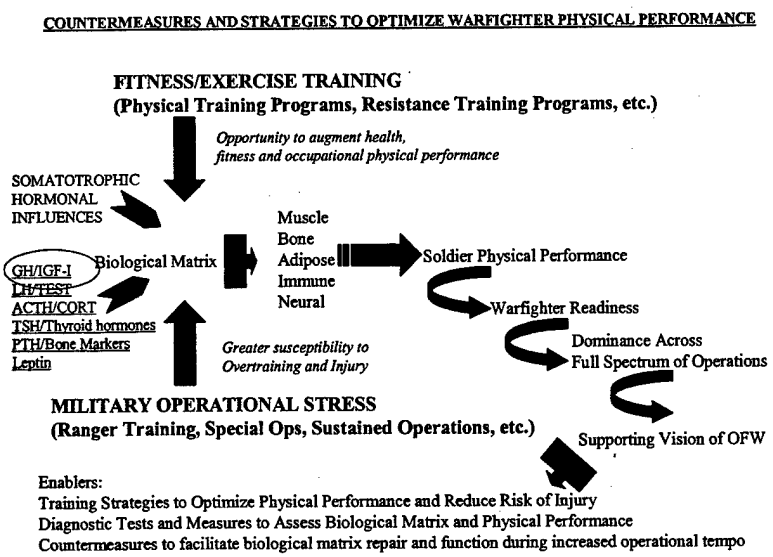


FIGURE 5. A conceptual model depicting how a better understanding of somatotrophic hormonal mediators may benefit soldier physical performance and support the vision of Objective Force Warrior (OFW). Soldiers are exposed to rigorous physical training and military operational stress. These influences can either positively or negatively affect the body's biological matrix. Changes in the biological matrix can affect soldier physical performance (for example declines in muscle mass will inhibit strength and power). Soldier physical performance directly contributes to Warfighter readiness and dominance across the full spectrum of operations. Monitoring IGF-I may have great utility for assessing physical training, evaluating recovery strategies, and sustaining performance during operational stress.

Biomarkers of Bone and Muscle Turnover: Effects of Exercise

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INTRODUCTION

Bone is a hard tissue with multiple components that provide mammals with a structural framework and a never-ending source of calcium for most homeostatic processes. Traditionally the skeleton has been classified into trabecular and cortical elements. As such the cortical shell has been classically viewed as protective with relatively slow remodeling rates, whereas the trabecular skeleton has been considered metabolically active due to its proximity to marrow elements and its large surface area. This, however, is a relatively simplistic model for the skeleton since it is clear there are other major differences for these two components, both in respect to cell constitution and vascular supply. In addition, the regulation of bone growth, modeling and acquisition differs in time, sequence and outcome between cortical and trabecular sites. Thus, as growth occurs, modeling of the skeleton takes place at the growth plate and at periosteal sites along long bones. Muscle insertion also occurs on the periosteum, and repetitive stresses strongly influence periosteal expansion and turnover.

The process of remodeling and subsequent bone acquisition represents a complex consolidative process occurring at the endosteal surface as well as the periosteum, ultimately resulting in attainment of peak bone mass. For the trabecular skeleton that point occurs around the time of linear growth cessation; whereas cortical bone continues to consolidate until the early people reach their thirties. The control over growth and remodeling, as well as skeletal maintenance, has been the subject of intense investigation over the last two decades. However, less attention has been paid to the differential compartments as they relate to growth and remodeling. Recent evidence from our laboratory and others have provided significant insight into the role of periosteal growth and remodeling in the acquisition of bone mass and the potential role of the periosteum in modulating exercise induced skeletal changes. This paper discusses one marker of bone and muscle turnover, IGF-I, insulin-like growth factor- I, and its role in the process of cortical peak acquisition and skeletal homeostasis.

THE PERIOSTEUM AND ENDOSTEUM

The periosteum is a highly specialized surface overlying the cortical envelop of all long bones. Although it contains all the necessary cells for bone remodeling; i.e. osteoclasts, osteoblasts, and osteocytes, the origin of these cells remains in doubt. Because of the prominent vascular supply to the periosteum, and its role in fracture healing, it seems likely that these osteoblasts are, at the least, unique in respect to their signaling and origin from primitive cells outside the bone marrow. Indeed, there is some suggestion that periosteal osteoblasts may be derived from pericytes in the blood vessels of the outer cortical shell. Regardless of their site of origin, it is apparent that periosteal function changes with various stages of life, and that certain periosteal osteoblasts may work in opposition to their counter parts on the endosteum. In fact, there is likely to be differential regulation of these two compartments, and this in turn becomes important for targeting approaches aimed at strengthening bone or preventing stress fractures.

Several recent lines of evidence support differential regulation of periosteal and endosteal bone turnover. First, our group was the first to report that among inbred strains of mice, there are strong genetic differences in peak bone acquisition (Beamer et al., 1996). Initially due to the level of

resolution of our scanning devices in mice, we hypothesized that the differences among inbred strains was purely genetic and not confined to a single skeletal compartment. However, recently we reported that although one inbred strain, C3H/HeJ, had much higher cortical bone mass than did another strain C57BL6, that difference was reversed when we examined trabecular bone mass by uCT analysis (Beamer et al., 2001). Hence, within a given strain, one can find both high and low bone mass, depending on the compartment being measured. Moreover, we had assumed that all bone mass was acquired in the mouse by 16 weeks of age (Beamer et al., 1996). This also proved incorrect! Cortical bone density reached peak at four months of age, but trabecular BMD is more rapidly acquired and maintained by 6 weeks of age (Beamer et al., 1996; Bouxsein, personal communication). These findings confirm there is dual regulation of skeletal compartments in mice.

More emerging evidence supports that thesis. A recent abstract from a group in Sweden confirmed that in humans, these two skeletal compartments work in opposite directions (Alborg et al., 2002). The authors followed more than 100 postmenopausal women for 19 years using single energy X-ray absorptiometry of the distal radius. They noted about a 1.7% per year rate of bone loss, principally from the endosteal surface, in these women over the two decades. By contrast, periosteal circumference increased 0.6% per year and hence expanded by nearly 12% over the two decades of observation. This expansion is associated with an improvement in the cross sectional moment of inertia, and almost certainly results in modest but not complete structural protection against rapid bone loss. The third line of evidence is derived from unloading experiments in C3H animals with high cortical bone mass. Despite significant endosteal bone loss after sciatic neurectomy, cortical expansion becomes a major compensatory pathway which preserves bone strength at least in the short run.

Very recently, Kim et al examined the differential regulation of the periosteum and endosteum in growing rats of both genders (Kim et al., 2003). To begin with they reported that male rats tend to have nearly 25% greater bone width than females, and this is associated with greater bone strength. They noted that GH and androgens in males independently stimulate expansion of bone, but that GH deficiency alone does not significantly reduce bone fragility because of the androgen mediated effects on periosteal growth. By contrast, in females, GH stimulates periosteal expansion, but estrogen inhibits such growth. Hence, gonadectomy in females results in trabecular bone loss but periosteal expansion as the inhibitor of such activity is removed. As such it is clear that under certain hormonal manipulations, as well as mechanical influences, changes in the periosteal envelope differ considerably from that in the endosteum.

What controls periosteal and endosteal remodeling and growth? Since the origin of periosteal osteoblasts is not known, many questions remain about the control mechanisms involved in periosteal expansion during growth and with aging. Utilizing inbred strains of mice, our group has defined the importance of genetic determinants in periosteal and endosteal expansion. And, it is also clear that there are at least two principal regulators of the periosteum: skeletal muscle with its insertion into bone, and systemic hormones which likely make their way through the vascular network in the periosteum to alter the behavior of specific bone cells. As noted above, the sex steroids certainly are considered within this latter category, although many investigators would maintain that both types of regulators work through a single common pathway, the IGF regulatory system.

THE REGULATION OF THE PERIOSTEUM: A ROLE FOR IGF-I?

Several lines of evidence support a major role for circulating IGF-I in determining bone size. These data are principally derived from in vivo manipulations using genetic engineering and inbred strains of mice. IGF-I is a ubiquitous polypeptide that is expressed in most tissues and also circulates in very large concentrations bound to a series of IGF specific binding proteins. Bone is a major site of IGF-I production principally from early and mature osteoblasts. It is stored within the skeletal matrix bound to IGFBP-5 and IGFBP-2 and released during osteoclast mediated bone

resorption. Because the marrow bathes trabecular elements, it is not surprising that the relative content of IGF-I in sites such as the vertebrae is quite substantial. As such, the principal source of IGF-I in these areas is likely to be local synthesis. On the other hand, although the periosteum is rich in osteoblasts, IGF-I content in this region appears to be a function of both circulatory and local synthesis. Impressive in vivo data supporting that contention have recently been published by our group and others.

Technology that permits selective knockout or knock down of ligands and receptors in mice has opened an exciting era for testing functional correlates of peptide growth factors and their signals. Yakar, Rosen et al recently demonstrated that with selective knockout of the IGF-I gene in liver, there are significant skeletal changes (Yakar et al., 2002). The LID mice were generated by using an albumin promoter tied to Cre-Recombinase and mating those mice with another group of mice carrying a floxed IGF-I gene. The resultant animals had normal expression of IGF-I in all other tissues besides the liver, including the skeleton, but a 75% reduction in serum IGF-I. Despite growth curves which were not markedly abnormal, the long bones of the LID mice were shorter and had markedly reduced bone volume, despite normal skeletal IGF-I expression. All the skeletal changes were in the cortical component and reflected a reduction in periosteal circumference as well as cortical thickness. Trabecular bone was entirely normal. These data suggest that alterations in circulating IGF-I affect skeleton modeling and principally the cortical component. Similarly, recent work from Tom Clemens and from our laboratory have shown that knockout of the IGF-I Type I receptor in mature osteoblasts using an osteocalcin specific promoter and Cre lox P recombinase resulted in a dramatic skeletal phenotype of reduced trabecular bone density, slow bone mineralization, but no change in the cortical envelop, periosteal circumference or femur length (Zhang et al., 2002). These data are remarkably similar to over expression studies of IGF-I in bone, in which the animals have significantly enhanced bone density but no change in size, volume or length of their bones (Zhao et al., 2000). Finally, our laboratory has confirmed that in a spontaneous mutant mouse, *little*,which does not make growth hormone and has low serum IGF-I, that periosteal size and circumference are markedly reduced(as is femur length), but that trabecular bone mass is not altered, nor is skeletal expression of IGF-I. In sum, it appears that circulating but not skeletal IGF-I controls periosteal growth and modeling, whereas local IGF-I almost certainly plays an important role in trabecular mineralization and acquisition.

Further support for that tenet comes from work with congenic mice at The Jackson Laboratory. Bouxsein, Rosen et al created a congenic mouse that has a knockdown in serum IGF-I of approximately 20-25% (Bouxsein et al., 2002). This is associated with no change in skeletal IGF-I expression but a significant reduction in periosteal circumference, femoral length, and cortical osteocyte apoptosis. This congenic(6-T) also has reduced free levels of IGF-I compared to parental C57BL6 controls, suggesting that alterations in the circulating concentration of IGF-I can have a significant impact on bone growth and consolidation.

WHAT IS THE ROLE OF EXERCISE, LEAN MASS AND MUSCLE IN PERIOSTEAL GROWTH?

Those particular animal models have allowed us to dissect the regulation of individual skeletal compartments by careful phenotyping. Another approach is to define how the second major regulator of cortical bone, skeletal muscle, affects periosteal growth and expansion. Once again, we can turn to animal models. Currently, at The Jackson Laboratory, a major endeavor is underway to completely characterize a number of phenotypes related to body composition and bone mass in forty different strains of mice. Not surprising, there are major differences not only in bone mass but also IGF-I and body composition among these strains. The "Phenome Project" will provide tremendous insight into the role of muscle and lean mass, as well as adiposity in periosteal and endosteal growth. At the present time several strains have been identified that have similar lean body mass but major differences in bone mineral content. Experimental manipulation of these

mice, followed by public dissemination of this information, will allow investigators to dissect how muscle mass, or repetitive muscle action, affects peak cortical and trabecular bone mass on various genetic backgrounds. Other approaches are likely to include repetitive exercise and muscle stimulation studies to define how muscle determines the structure of bone envelopes. In the meantime, more studies are needed to define how circulating IGF-I may predict risk for failure of the cortical skeleton, principally in respect to stress fractures. Serum IGF-I is regulated by genetic factors, nutritional determinants, age of the individual, growth hormone secretion, insulin status, and systemic cytokine elaboration. As such, this test may prove to be extremely useful as an integrative measure of physiologic homeostasis as well as an indirect indicator of periosteal status.

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Hydration Status Monitoring

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INTRODUCTION

Definition and Documentation

This paper reviews widely used indices of hydration status in human. For the purposes of this review, euhydration will refer to "normal" total body water (TBW), whereas hypohydration will refer to a body water deficit. The term dehydration will be used to refer to the dynamic process of body water loss (i.e., the transition from euhydration to hypohydration) (Greenleaf and Sargent, 1965; Sawka, 1992). The term hypovolemia will define when blood volume is less than "normal".

Impact on Human Performance

Both physical and cognitive performance are impaired proportionally to the magnitude of body water loss incurred (Gopinathan et al., 1988; Sawka, 1988) However, even small losses of body water (1–2% body mass (BM)) have a detrimental impact on physical work and negatively impact human thermoregulation(Sawka, 1988; Sawka et al., 2001). Accordingly, dehydration may be the greatest non adversary threat to military operations.

Fluid Balance, Distribution and Exchange

"Adequate" hydration is essential for maintaining effective military field operations. Several common operational stresses can result in relatively large alterations in TBW content and distribution. During most "normal" conditions, humans have little trouble maintaining optimal fluid balance. However, many factors such as sickness, physical exercise, climatic exposure (heat, cold and/or altitude) and psychological strain can lead to significant disturbances in water balance. Perhaps the best example of this is the combination of heat stress and physical activity. For sedentary persons in temperate conditions, water requirements usually range from 2-4 L per day and water balance is regulated primarily by the kidneys. For physically active persons, exposed to heat stress, water requirements can often more than double (Sawka et al., 2001) and it would not be unusual for physically active, heat stressed individuals to incur water deficits of several liters.

Water is the largest single constituent of the body (50–70% of body weight) and is essential for supporting the cardiovascular and thermoregulatory systems, and cellular homeostasis. TBW is distributed into intracellular fluid (ICF) and extracellular fluid (ECF) compartments. The ICF and ECF contain ~65% and ~35% of total body water, respectively (Guyton et al., 1975). The ECF is further divided into the interstitial and plasma spaces. The average 75 kg male has ~45 L of total body water, therefore ICF contains ~30 L of water whereas the ECF contains ~15 L of water with ~3.4 L in plasma and ~11.6 L in the interstitium. These volumes are not static, but represent the net effect of dynamic fluid exchange and turnover between compartments (Guyton et al., 1975). Exercise-heat stress not only stimulates fluid loss, primarily by sweating, but induces electrolyte imbalances and changes in renal function. As a result, fluid deficits with and without proportionate solute changes can occur. In addition, exercise-heat stress alters transcompartmental and transcapillary forces that redistribute fluids between various compartments, organs, and tissues (Sawka et al., 2001). For these reasons, the accuracy of most methods used to assess hydration status

is limited by the circumstances in which they are measured and the purposes for which they are intended.

Dehydration and Muscle Water

Incomplete fluid replacement decreases total body water and, as a consequence of fluid exchange, affects each fluid space. For example, Nose and colleagues (Nose et al., 1983) determined the distribution of body water loss among the fluid spaces as well as among different body organs durng dehydration. They thermally dehydrated rats by 10 percent of body weight, and the fluid deficit was approtioned between the intracellular (41%) and extracellular (59%) spaces. The distribution of organ fluid loss was muscle (40%), skin (30%), viscera (14%), and bone (14%). However, no significant changes occurred in liver and brain water content. Nose and coworkers (Nose et al., 1983) concluded that dehydration results in water distribution largely from the intra and extracellular spaces of muscle and skin.

The measurement of TBW is the "gold standard" to assess hydration status (Aloia et al., 1998; Lesser and Markofsky, 1979). TBW can be directly measured with doubly labeled water (DLW) or other dilution techniques. The major drawbacks of the DLW and other dilution methodologies are the cost and the technical difficulties associated with isotope analyses. The requirement for an isotope ratio mass spectrometer and sample preparation systems often limits the use of this method in most military scenarios. In addition, to obtain accurate changes in TBW with these methologies, serial measurements are required, which further limits their use for routine assessment of TBW changes for hydration assessment. Although the choice of specific biomarker for assessing hydration status should ideally be sensitive and accurate enough to detect relatively small fluctuations in body water, the practicality of its use (time, cost, and technical expertise) is also of significant importance.

Estimates of hydration status are commonly done using 1) bioelectrical impedance analysis, 2) plasma markers and fluid regulatory hormones, 3) urine indices, 4) changes in body weight, or 5) signs and symtoms. Given consideration to military field operational use, hydration assessment measurements are presented in order of increasing assessability and practicality.

Methods for Hydration Status Monitoring

Bioelectrical Impedance

Recently, bioelectric impedance (BIA) has gained attention because it is simple to use and allows rapid, inexpensive and noninvasive estimates of TBW (O'Brien et al., 2002). In practice, a small constant current, typically 800 uA at a fixed frequency, usually 50 kHz, is passed between electrodes spanning the body. The voltage drop between these electrodes provides a measure of bioimpedance. Prediction equations, previously generated by correlating impedance measures against an independent estimate of TBW, may be used subsequently to convert a measured impedance to a corresponding estimate of TBW (Kushner et al., 1992). Absolute BIA values are well correlated with dilution TBW techniques (Kushner et al., 1992; Van Loan, 1990).

BIA does not have sufficient accuracy to assess dehydration (~7% TBW) and loses resolution with isotonic fluid loss (O'Brien et al., 2002; Van Loan, 1990). In addition, since fluid and electrolyte concentrations can have independent effects on the BIA signal it can often provide

grossly misleading values regarding hydration status (O'Brien et al., 2002). Therefore, BIA has little application for the field assessment of hydration status.

Plasma Markers

Plasma volume changes can be estimated from hemoglobin and hematocrit changes; however, accurate measurement of these variables requires considerable control for posture, arm position, and skin temperature and other factors (Sawka, 1988). If adequate controls are employed, plasma volume decreases in proportion with the level of exercise-heat mediated dehydration. Likewise, plasma volume decreases with dehydration, and this response varies due to the type of dehydration (iso-osmotic or hyper-osmotic), physical activity, physical fitness, and subjects heat acclimatization status (Sawka, 1988).

Plasma osmolality is controlled around a set-point of 280–290 mOsmol/kg in euhydrated volunteers (Senay, 1979). This narrow range increases ~5mOsmol/kg for every 1–2% BM of dehydration incurred (Popowski et al., 2001). Figure 1 presents the effects of body water loss on resting plasma osmolality and plasma volume in heat acclimated persons undergoing exercise-heat mediated dehydration (Sawka and Coyle, 1999). These same levels will be maintained during subsequent physical exercise. If an iso-osmotic dehydration occurs, such as with altitude or cold exposure (O'Brien et al., 1998; Sawka, 1992), then plasma osmolality changes will not follow TBW changes and much larger plasma volume reductions will occur.

Plasma sodium concentration provides an alternative to measuring osmolality (as most of the osmolality changes are usually reflective of sodium changes), however, that linear relationship may not be as strong as expected (Senay, 1979).

Osmolarity is sensed in the hypothalamus by osmoreceptors, and those neurons, in turn, stimulate the production of antidiuretic hormone. When plasma osmolarity is below threshold, the osmoreceptors are not activated and antidiuretic hormone secretion is suppressed. When osmolarity increases above the threshold for ADH release, the osmoreceptors recognize this as the cue to stimulate the neurons that secrete antidiuretic hormone. Figure 2 shows that antidiuretic hormone concentrations rise steeply and linearly with increasing plasma osmolarity (Robertson and Athar, 1976). If hydration status changes are

the result of water loss, the plasma solute concentration (osmolality) will change proportionately. However, the relationship of plasma osmolarity and vasopression concentrations is confounded by exercise, hyperthermia, nausea, and fluid volume changes (Norsk, 1996).

Aldosterone, secreted by the adrenal cortex, is a potent hormone regulating electrolyte balance. Aldosterone acts directly on the kidney to decrease the rate of sodium-ion excretion with accompanying retention of water, and to increase the rate of potassium-ion excretion. Dehydration mediated elevations in aldosterone secretion are confounded by heat acclimation status and exercise (Francesconi et al., 1983). The measurement of plasma volume, osmolality, sodium, aldosterone, and AVP requires phlebotomy (invasive), technical skill, and expensive instrumentation.

Urine

Urinalysis is a frequently used clinical measure to distinguish between normal and pathological conditions. Urinary markers of hydration status include urine specific gravity (USG), urine osmolality (U_{Osmol}), and urine color. Urine specific gravity and osmolality are quantifiable and threshold values can have some value, whereas color is subjective and can be influenced by many factors. It is important to recognize that the accuracy of these urinary indices in assessing chronic hydration status is improved when the first morning urine is used due to a more uniform volume and concentration (Sanford and Wells, 1962; Shirreffs and Maughan, 1998). Likewise, many additional factors such as diet, medications, exercise, and previous climatic exposure can confound these indices.

The most widely used urine index is USG. Measured against water as a standard (1.000 g/ml), USG represents the concentration of particles dissolved in urine and is a reflection of the kidney's ability to concentrate or dilute urine in relation to plasma. Because urine is a solution of water and various other substances, normal values range from 1.010–1.030 (Armstrong et al., 1994; Popowski et al., 2001; Sanford and Wells, 1962). It has been suggested that a USG of ≤ 1.020 represents a state of euhydration (Armstrong et al., 1994; Sanford and Wells, 1962). As a measure of chronic hydration status, USG appears to accurately reflect a hypohydrated state when in excess of 1.030 (Armstrong et al., 1994; Popowski et al., 2001; Sanford and Wells, 1962). However, considerable variability exists and no singular value can be used to determine a specific hydration level (see Figure 3). U_{Osmol} also can provide an approximation of hydration status (Shirreffs and Maughan, 1998) as it is highly correlated with, but more variable than, USG (Armstrong et al., 1994; Popowski et al., 2001).

Endocrine responses to dehydration stimulate water and electrolytes retention by the kidney. However, while the linear rise in plasma osmolality (with hypovolemia) that occurs with dehydration (Popowski et al., 2001) stimulates vasopressin and the tubular re-absorption of water at the kidney, the renal response lags behind changes in plasma osmolality during acute fluxes in body water (2–4 hrs) brought on by dehydration-rehydration (Popowski et al., 2001). In fact, when large volumes of water are consumed, a pale colored urine with low specific gravity is excreted long before euhydration is achieved (Shirreffs and Maughan, 1998) due to rapidly declining AVP levels triggered by the swallowing reflex. When water is consumed in excess of sweat losses during exercise, urine output increases and fluid balance is not restored unless sufficient electrolytes are

also consumed (Maughan and Shirreffs, 1996). Logically, U_{Osmol} is therefore also limited for assessing acute changes in body water (Kovacs et al., 1999; Popowski et al., 2001).

Body Mass

Body Mass (BM) measurements represent the simplest technique for rapid assessment of changes in hydration status. In our laboratory, we observe very small (< 1%) fluctuations in first morning BM when measured over consecutive days in young men taking food and fluid ad libitum. The stability of this measurement, coupled with the known losses of fluid that occur with exercise-heat exposure (primarily eccrine sweat), allows rapid changes in BM (incurred over hours) to be correctly attributed to water loss. Acute changes in BM weight are therefore a popular and reasonable field estimate of dehydration (Cheuvront et al., 2002).

The level of dehydration is expressed as a percentage of starting body weight ($\Delta BW/startBW) \times 100$] rather than as a percentage of total body water (TBW) since TBW ranges from 50–70% of body weight. This technique assumes that 1) starting BW represents a euhydrated state, and 2) 1 ml of sweat loss represents a 1 g change in weight (i.e., specific gravity of sweat is 1.000 g/ml). As an acute measure, first morning BW is still limited by changes in bowel habits. BW is also limited as a tool for long-term assessment of hydration status since changes in body composition (fat and lean mass) that may occur with chronic energy imbalance are also reflected grossly as changes in BW. Clearly, the use of daily body weight should be used in combination with another hydration assessment technique to dissociate gross tissue losses from water losses if long-term hydration status is of interest.

Signs and Symptoms of Dehydration

In the early stages of dehydration, no signs and symptoms are apparent. However, as greater body water (BW) losses occur increased thrist, increased pulse rate, and increased rectal temperature present. In addition, BW loss of 1–5% can be associated with flushed skin, nausea, sleepiness, and reductions in economy of movement. Body water losses of 6–10% are associated with dizziness, headache, tingling in limbs, decreased blood volume, and cyanosis. Severe dehydration, 11–20% BW, results in delirium, numb skin, deafness, and spasticity. Furthermore, death is likely as greater BW loss occurs. Assessment of dehydration via signs and symptoms is easy and quick; however, these estimates are imprecise to accurately determine hydration status. Nevertheless, if any of the signs and symptoms of dehydration present, rehydration should begin immediately.

CONCLUSIONS

Under most conditions, day to day body mass changes (>2%) and first morning urine specific gravity (>1.030) when used together provides an approximate indication that an individual is hypohydrated (see Table 1). However, plasma osmolality changes can provide more reliable information regarding hydration when greater precision is required. Measurement of fluid regulatory hormones for routine hydration assessment are not necessary and are often confounding. Moreover, BIA has limited utility to assess hydration status in the field for reasons previously described. It is possible that other technological advances may allow evaluation of other measures (e.g., muscle water content) that hold promise as hydration indices.

The views, opinions, and/or findings contained in this publication are those of the authors and should not be constructed as an official Department of the Army position, policy, or decision unless so designated by other documentation.

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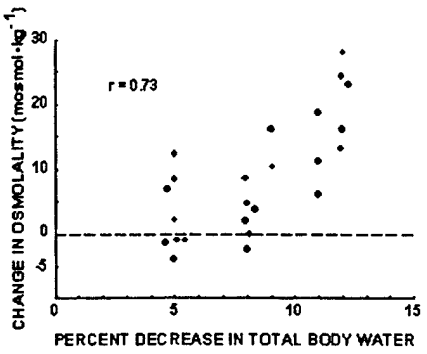
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A



B

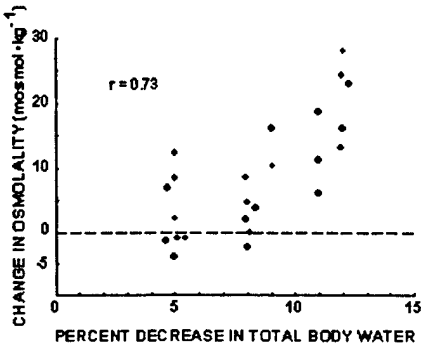


FIGURE 1 The effects of body water loss on resting plasma osmolality and plasma volume in heat acclimated persons undergoing exercise-heat mediated dehydration (Sawka and Coyle, 1999).

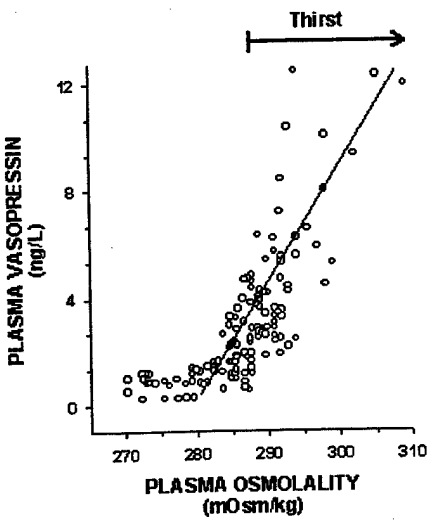


FIGURE 2 Plasma vasopressin concentrations compared to plasma osmolarity (Robertson and Athar, 1976)

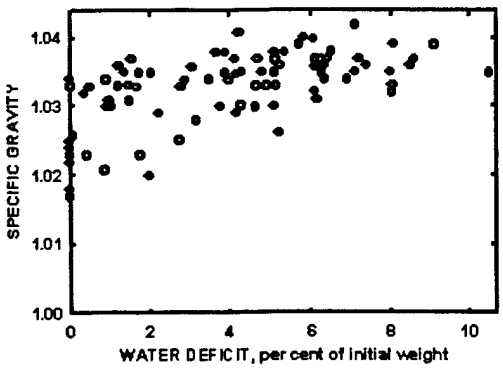

SPECIFIC GRAVITY
1.04
1.03
1.02
1.01
1.00
0
2
4
6
8
10
WATER DEFICIT, per cent of initial weight

FIGURE 3

TABLE 1 Biomarkers for Hydration Assessment

Marker	Advantages	Disadvantages
Signs & Symptoms	Easy, Quick	Imprecise
TBW, Dilution	Valid, Reliable	Pre-measurement, Invasive, Complex
TBW, BIA	Easy, Rapid	Pre-measurement, Imprecise
Plasma Volume	---	Pre-measurement, Invasive,
Osmolality	Often Valid, Reliable	Imprecise
Sodium	Hyponatremia	Invasive, Complex
Fluid Reg. Hormones	Often Valid	Invasive, Imprecise
Urine	Easy, Rapid, Screen	Invasive, Sometimes Confounded, Complex
Saliva	Easy	Imprecise, Easily Confounded
Body Weight	Easy, Rapid	Invalid

AMINO ACIDS AS BIOMARKERS FOR FATIGUE

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ABSTRACT

Muscle fatigue limits physical activity. Fatigue can be defined as the inability to maintain power output. The causes are not known and there is no known marker for predicting the onset of fatigue. The etiology can be of either local or central origin. Physical fatigue originates within the muscle and whereas central is secondary to alterations within the brain. A number of hypothesis related to amino acid metabolism have been proposed to account for the development of fatigue. The most promising marker for identifying subjects prone to fatigue is examination of the plasma aminogram during periods of intensive training.

INTRODUCTION

Muscle fatigue limits physical activity. Fatigue can be defined as the inability to maintain power output. The causes are not known. The etiology can be of either local or central origin. Local fatigue originates within the muscle and whereas central is secondary to alterations within the brain. Central mechanisms postulate the release by exercising muscle of factors which act systemically and so impact the central nervous system. In the context of military performance systemic effects are likely to be of greater significance because of their potential to impact both physical and mental performance.

Muscle fatigue is not the same as muscle soreness. Muscle soreness is the pain occurs about a day after exercise and peaks 2–3 days post exercise (Clarkson et al., 1992). The underlying mechanisms for delayed onset muscle fatigue and fatigue are different. Soreness is believed to be due to a localized inflammatory response (Smith, 1991) and so the appropriate markers are markers for an inflammatory response. Neither is the onset of pain considered as a marker for muscle fatigue. Pain by itself is performance limiting and therefore is not a 'predictor'.

The majority of studies of muscle fatigue have assumed that the fatigue is the results of events localized within skeletal muscle (Davies and White, 1981; Edwards, 1981). Prior studies of muscle fatigue have focused on the relationship of putative marker to the underlying biochemical or histological changes. This review has a somewhat different focus, the use of those markers as predictors for the onset of fatigue, and specifically markers of protein origin.

For a marker to be of practical use certain conditions must be met (Banister et al., 1985). It must apply to all subjects. A statistical relationship is inadequate when applied to the individual (Barron et al., 1985). The measurement must be technically feasible on large number of subjects without costing too much. These criteria limit the assays to 'spot' blood and urine measurements. In line sensors in a selected muscle are not likely to be of much use. An isolated muscle may not reflect the whole musculoskeletal system and the muscle selected may not be one of the muscles that are becoming fatigued.

LOCAL FACTORS

Protein Turnover

Proteins are the machinery of the body. All of the work and all of metabolic functions in the body, from movement to ion pumping, from cell division to obtaining energy from foodstuffs to host defense mechanisms are effected by proteins. The health of the body protein pool is maintained by proteins being in a dynamic state; proteins are continually being ma and broken down (protein turnover). A dynamic state of protein turnover allows a rapid response when a new mix of proteins is required, for example injury or infection where defense proteins need to be mobilized, the removal of any damaged proteins and by altering protein levels, as a means of metabolic regulation. Clearly if the machinery begins to malfunction, performance will decrease.

Unfortunately there is no simple means of assessing the status of protein turnover. The classical marker for protein breakdown is 3-methyl histidine production. Monitoring the urinary 3-MeH is a standard assay for assessing myofibrillar protein breakdown; its limitations are well known (Long et al., 1981; Munro and Young, 1978; Rathmacher et al., 1995; Rennie and Millward, 1983). To obtain interpretable 3-MeH data subjects should be on a meat free diet. Placing combat troops on a meat free diet – or even attempting to control meat intake is not a realistic option and thus monitoring of 3-MeH production is not feasible even if it were shown to somehow correlate with fatigue.

The other side of protein turnover is protein synthesis. There is no non-isotopic method for measuring human protein synthesis. Clinically, the classic method for assessing protein status (and still the most sensitive), and hence indirectly protein turnover, is nitrogen balance. The problems with the nitrogen balance method is that is fraught with errors, the errors tend to be unidirectional towards over-estimating nitrogen retention.

It would be enough to know that there has been a major change in N balance for concern. Classically nitrogen balance is done by measuring input (food) and output (urine, sweat, feces and any increase in the BUN). To measure all of these parameters accurately is difficult in a Clinical Research Center environment; to do so non-invasively in the field is not going to be possible.

Potential markers

None.

NUTRITIONAL FACTORS

Plasma Amino Acids

While not directly correlated with protein synthesis, plasma and tissue free amino acid concentrations and distribution patterns provide useful information on protein metabolism. An important study by Kingsbury et al. compared fasting plasma amino acid patterns in elite athletes from the 1996 British Olympics team during training (Kingsbury et al., 1998). Athletes were divided into three groups, group A, no lasting fatigue after training; group B, group B heavy fatigue at night but recovered after an overnight rest and group C, chronic fatigue with full recovery taking a week or more. The results are summarized in table 1.

Plasma amino acid concentrations were lower in the two groups subject to fatigue (Table 1). There were significant relationships between the fatigue and some of the changes in individual amino acids. Figure 1 shows the distribution of plasma glutamine and histidine. There is virtually no overlap between the fatigue groups and the controls with glutamine. What is important about the findings is the very high discriminatory power of the measurements for identify the subjects prone to fatigue. The study is promising but not definitive because the subjects were not matched by athletic event and no dietary data was collected. An observational, non-randomized follow-up study of increasing protein intake

appeared to be of benefit to the subjects with low glutamine and histidine concentrations. Plasma glutamine and histidine concentrations were increased and as was performance (Kingsbury et al., 1998).

These observations may be indicative of early substrate depletion for the maintenance of protein synthesis. More likely they reflect limitations in energy generation by the TCA cycle Amino acids provide precursor substrates for the TCA cycle (Young and Marchini, 1990). If energy expenditure is increased the need for the replenishment will be increased from amino acids will be increased (Wagenmakers, 1998).

Potential markers

Assuming that the study can be validated under more controlled conditions, it would appear that measurement of the fasting plasma aminogram has the potential of early identification of subjects prone to fatigue.

Energy

Troops in fields situations may suffer from energy deficits either because of limited food availability or very high workloads. In deed, Rigorous military field training can induce energy deficits as high as 1000 kcal/d (Friedl and Hoyt, 1997; Kramer et al., 1997). Such energy deficits lead to weight loss and some loss of lean body mass. Even so, nutrition is not likely to be the cause of fatigue. Glycogen depletion is a natural process, after muscle glycogen has been used, muscle uses fat.

Humans have extensive energy reserves. The average male has enough endogenous fat to withstand starvation for up to 70 days. A controlled study by Zachwieja et al found that moderate short-term deficits (2 weeks, 750 kcal/d) in food intake does not impact performance in otherwise healthy individuals (Zachwieja et al., 2001). Adequately fed humans have sufficient endogenous energy reserves to function normally for extended periods. With extreme depletion or nutritional deprivation the situation will be different, but by that time fatigue will be of minor consequence in the health status of the soldier.

Potential markers

Alterations in fuel metabolism can be detected from either reduced fuel availability in the plasma, and fuels include energy substrates as well as oxygen, or end products of fuel metabolism such as lactate. However since fuel supply to the muscle is not likely to be limiting, monitoring dietary status as a potential predictor for the onset of fatigue is unlikely to be productive.

Other Nutritionally Related Factors

Several plausible mechanisms have been proposed. These include limited or decreased availability of energy fuels, glycogen depletion, depletion of phosphocreatine, proton accumulation, failure of neuro-muscular transmission and actual muscle damage (Davis, 1995). With the exception of proton accumulation (lactate production) and actual muscle damage, there is little supportive experimental evidence.

Potential Markers

Monitoring specific fuels and metabolites within muscle (e.g. glycogen, phosphocreatine) is not likely to have the necessary reliability and sensitivity. There is no certainty that the muscle selected for monitoring is going to be one of the muscles causing the fatigue. Furthermore such measurements are likely to be technically difficult.

MUSCLE DAMAGE

Excessive work leads to actual damage to muscle. Numerous studies have explored the use of plasma levels of muscle derived proteins as indices of muscle damage. The principal markers that have been used are : aspartate amino transferase, lactate dehydrogenase, creatine kinase, myoglobin, fatty acid binding proteins, carbonic anhydrase isoenzyme III, myocyte contractile proteins such as Troponins and myosin heavy chains (Janssen et al., 1989, Sorichter et al., 1999). In rodent studies it has been the clearly shown that the degree of damage estimated from plasma enzyme levels is greater than that found by histological examination. The reason is that plasma enzyme levels reflect a combination of actual muscle damage and transient changes in membrane permeability (van der Meulen et al., 1991). The most frequently used marker for muscle damage has been creatinine kinase.

A direct connection between muscle injury and muscle fatigue has not been proven. A little damage in one or two muscles is enough to increase plasma levels of muscle proteins. But a small degree of damage does not appear to impact performance although ultimately if the muscle damage is severe enough it should lead to impaired performance.

Potential markers

The problem with the use of proteins released from damaged muscle is that they are markers for damaged muscle and not fatigue. Subtle changes in muscle ultra structure may lead to decrease strength and fatigue, but such changes will not necessarily be reflected by increased leakage of muscle proteins into the plasma compartment (Behm et al., 2001). Muscle proteins indicated damage, but cannot predict fatigue in humans because the correlation between damage and fatigue is weak.

THE OVER-TRAINING SYNDROME

Related to muscle fatigue is the over-training syndrome. Over-training is a term that is used to describe the process where the training is excessive and the resulting condition of staleness or burnout (Barron et al., 1985; Hooper et al., 1995). Staleness is characterized by chronic fatigue, poor performance and delayed recovery (Fry et al., 1991; Kuipers and Keizer, 1988; Verde et al., 1992). The major symptom is underperformance (Budgett, 1998).

Potential Markers

At present that are no objective markers for overtraining other than outcome. Parameters that have been investigated include heart rate, blood pressure, enzyme blood levels, hormones and leukocyte numbers. In general, correlations have been observed, but they are weak and of no predictive potential. For example a study by Hooper et al investigated potential markers for overtraining in Olympic caliber Australian swimmers. The only correlations between fatigue (as recorded by the subjects), staleness (failure to improve during training) and blood markers was higher levels of catecholamines ($r^2 = 0.33$) and leukocytes ($r^2 = -0.16$) with fatigue. Both of these parameters are also markers for stress (Hooper et al., 1995). Elsewhere Budgett concluded that 'there is no diagnostic test available' (Budgett, 1998).

CENTRAL FATIGUE

Little is known about the mechanisms for central fatigue. It has not been a very active area for research. But fatigue of central origin it is of potentially great significance to the army because it could also effect mental performance as well as physical activity. Two viable hypotheses have been published; the ammonia hypothesis and the tryptophan hypothesis (Davis, 1995). In both cases, the theory is plausible, there is some experimental supporting evidence, but the evidence is as suggestive at best. The tryptophan hypothesis has attracted the most interest.

The Tryptophan Hypothesis

Newsholme et al proposed that exercise induced change is in the plasma amino acid distribution could induce central fatigue by influencing the synthesis, concentration and release of neurotransmitters, particularly 5-hydroxy tryptamine (5-HT) within the brain (Blomstrand, 2001; Castell et al., 1999). Brain 5-HT is in involved in the control of arousal, sleepiness and mood, so it is therefore conceivable that brain 5 HT levels could be laying to fatigue during and after a vigorous physical activity (Blomstrand, 2001). Indeed there is a considerable amount of evidence from rodent studies showing that inhibiting the action of 5 HT improves endurance (Blomstrand, 2001).

Plasma tryptophan is the precursor for brain 5 HT. The rate limiting step in the synthesis of 5 HT is the transport of tryptophan across the blood brain barrier into the brain (Fernstrom, 1990). The tryptophan transporter system also transport's the other large neutral amino acids, specifically the three branched chain amino acids. The hypothesis proposes that competition for the transport of between tryptophan and the branched chain amino acids occurs. Thus the rate of entry of tryptophan into the brain will depend on be amount of free tryptophan in the blood to the amount of competitive amino acids. During prolonged exercise there is a decrease in the concentration of most amino acids. Most of the tryptophan in the plasma is bound to albumin. Free fatty acids compete for the tryptophan binding sites on albumin. Thus as exercise progresses, fatty acid mobilization occurs and the increased plasma free fatty acid levels displace bound tryptophan from albumin leading to an increase in free tryptophan in the plasma. At the same time the concentration of the branched chain amino acids decreases with prolonged exercise. The net effect is that the ratio of free tryptophan to BCAA increases several fold and more tryptophan is taken up into the brain. At rest only about 10 percent of blood tryptophan is in the free form. Whether the ratio tryptophan to BCAA increases with exercise depends on the type and duration of the exercise.

The tryptophan:BCAA theory predicts that increasing the plasma BCAAs concentration should decrease tryptophan uptake into the brain thereby decreasing 5 HT synthesis and the delaying fatigue. A number of studies have sought to test this prediction. Results have been ambiguous with some studies reporting positive results and others no effect from BCAA supplementation (Blomstrand, 2001). Ingestion of carbohydrates can also lead to lower free tryptophan during exercise. Carbohydrates depress fat mobilization thereby increasing the proportion of the blood tryptophan bound to albumin. One report found improved mental agility of psychological tests of the sustained competitive exercise during which the subjects were given both BCAA and carbohydrates (Hassmen et al., 1994).

Potential markers

The potential markers are plasma tryptophan, plasma branched chain amino acids, plasma albumin together with determination of the amounts of free fatty acids bound to the albumin and the plasma free fatty acid concentration. These are potential markers for the prediction of the onset of fatigue, and so should be considered as 'real-time markers' rather than predictors of future fatigue (e.g. amino acids). Overall the evidence is not very strong, but the hypothesis may be worth a definitive experiment. Part of the reason for favoring further investigation of the tryptophan hypothesis is that the hypothesis leads to a counter-measure, giving supplemental BCAAs. This would be feasible in a field situation.

The Ammonia Hypothesis

All tissues produce ammonia. High concentrations of ammonia in the brain are neurotoxic. With exercise, muscle ammonia production increases (Banister et al., 1985; Eriksson et al., 1985; Yuan and Chan, 2000). There is not however a direct correlation between exercise, blood ammonia levels and the concentration of ammonia in the brain.

The major sources of the increased ammonia production in muscle are the purine nucleotide cycle in which adenosine monophosphate is deaminated to inosine monophosphate by adenylate deaminase the

catabolism of the branched chain amino acids. Ammonia production through both pathways increase with duration and intensity of exercise.

Increasing the plasma ammonia levels with exercise leads to an increase in the tissues and a parallel increase within the brain (Meyer et al., 1980). The mechanisms for the increased brain ammonia are not known, both increased uptake of and decreased export have been proposed (Banister et al., 1985; Yuan and Chan, 2000). Within the brain, ammonia participates in numerous reactions which could lead to neurotoxicity. The levels produced with exercise are comparable with neurotoxic levels (Banister et al., 1985). The difference of course being that the exercise induced increases are transient, they resolve soon after the termination of exercise whereas with disease the elevation is chronic. In the case of clinically induced hyperammonemia, reducing the ammonia load has been of benefit.

Potential markers

The potential marker is the blood ammonia level. A problem is, that while the hypothesis is viable, there is little actual experimental evidence.

SUMMARY

The only amino acid derived parameter with the potential for predicting future fatigue is measurement of the fasting plasma aminogram during a period of strenuous training (Kingsbury et al., 1998). If the findings on Olympic athletes can be reproduced with soldiers, measurement of the fasting plasma aminogram during training could have the potential of early identification of subjects prone to early fatigue. The measurement appears to have both the specificity and technically simplicity needed to be used in a real life situation (Kingsbury et al., 1998). Moreover, Kingsbury et al. (1998) reported that in a small subset of their cohort with low plasma amino acid levels, increasing dietary intake did lead to improved performance. Thus there is also the possibility of treatment. Replicating Kingsbury's results in the population of interest to the army would be important.

The potential gain for army in following up Kinsbury's observations is great, the risks negligible and the cost small. The results are scientifically plausible. Indeed the army suspected that amino acids intake might be a key factor in improving performance and commissioned the committee on military medicine to investigate the role of amino acids in 1999 in improving performance. The British study was published after the committee completed their report. One wonders what would the committee would have concluded had they seen Kinsbury's data?

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Amino Acid	Normal Range	A	B	C
n		21	12	18
Glutamine	480-800	554 (25.2)	356 (16.0) ***	383 (13.6) ***
Histidine	30-150	79 (6.1)	32 (1.2) ***	50 (2.9) ***
Alanine	150-450	422 (24.7)	352 (20.4) *	344 (17.1)*
Threonine	70-220	121 (8.7)	72 (4.7) ***	91 (4.6) **
Serine	90-290	104 (5.3)	109 (5.3)	88 (5.1) *
Lysine	100-300	161 (8.5)	89 (6.1) ***	124 (8.2) **
Tryptophan	30-80	67 (3.5)	44 (3.7) ***	55 (2.9) *
Tyrosine	30-120	62 (3.8)	43 (3.2) ***	55 (4.3)
Valine	90-300	219 (11.4)	151 (8.8)	188 (10.4)
Leucine	65-220	146 (3.9)	127 (5.7) *	137 (9.5)
Isoleucine	26-100	77 (5.3)	59 (2.9) **	69 (4.6)
Arginine	40-120	82 (6.2)	57 (3.6) **	71 (4.9)
Proline	85-290	232 (12.1)	196 (13.8)	188 (18.7)
Ornithine	25-120	59 (3.9)	58 (5.3)	60 (5.5)
Methionine	10-60	35 (2.5)	26 (1.5) *	30 (1.3)
Glutamic acid	25-130	55 (6.3)	102 (4.9) ***	56 (8.7)
Glycine	100-330	227 (10.3)	316 (20.4) ***	199 (9.9)
Phenylalanine	35-100	71 (2.5)	88 (2.9) ***	70 (4.1)
Total AA		2839 (92.1)	2396 (90.1)	2307 (71.6)

TABLE 1 Fasting plasma amino levels of athlete groups during training. Mean with SEM in parentheses. Units are μmol. L^{-1}. Subjects were divided into three groups depending level of fatigue. Group A, no lasting fatigue; group B heavy fatigue at night but with full recovery by the next day and group C (n=18), chronic fatigue and poor performance. * $p<0.05$ vs group A, ** $p<0.01$ vs group A*** and $p<0.001$ vs group A. Adapted from Kingsbury et al., 1998.

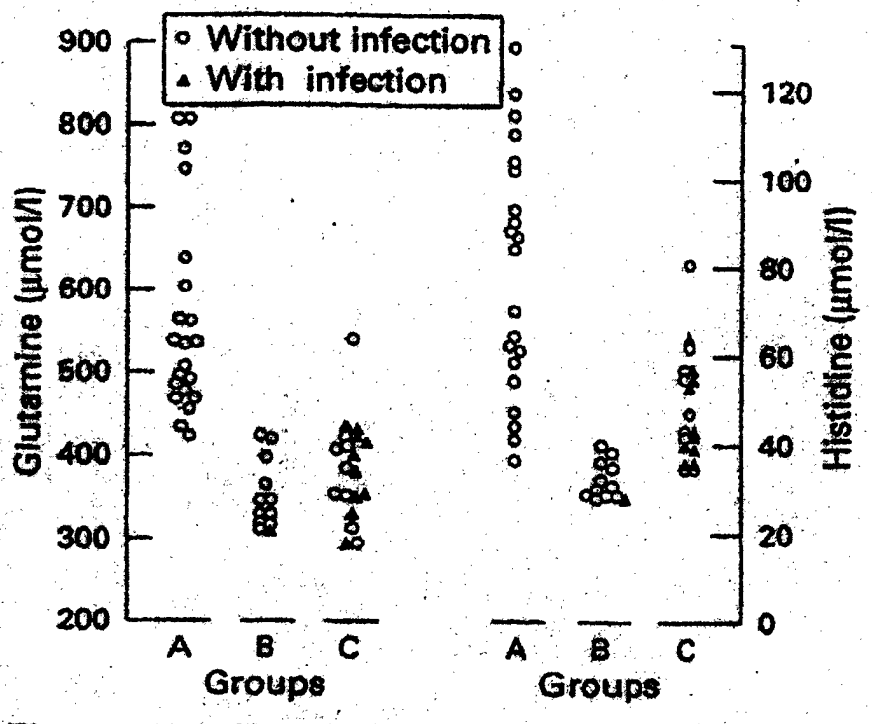


Figure 1 Pre-Olympic plasma glutamine and histidine levels (µmol/l) in groups A, B, and C.

FIGURE 1 Fasting plasma glutamine and histidine levels of athlete groups during training. Units are µmol. L^{-1}. Subjects were divided into three groups depending level of fatigue. Group A, no lasting fatigue; group B heavy fatigue at night but with full recovery by the next day and group C (n=18), chronic fatigue and poor performance (Kingsbury et al., 1998).

AUTONOMIC NERVOUS SYSTEM ACTIVITY AND ITS RELATIONSHIP TO ATTENTION AND WORKING MEMORY

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INTRODUCTION

In the present paper we describe a model of neurovisceral integration in which a set of neural structures involved in cognitive, affective, and autonomic regulation are related to HRV and cognitive performance. Neural network studies in humans have reported increased activity in prefrontal cortex during tasks involving executive function and working memory (Goldman-Rakic, 1998). Compte et al. (2000) have proposed that the prefrontal cortex holds sensory information temporarily online through sustained activity. This continued activation of a neural network is essential for the linking of 'input' with 'output' to achieve flexible responding to changing environments. As such, optimal prefrontal functioning is necessary for the formation of associations and the representation of acquired relationships between disparate pieces of information, including information separated in time (Miller, 2000). In addition, these cortical regions are implicated in inhibitory functions that are known to be critical for the performance of executive function tasks. Relatedly, performance on working memory tasks have been reported to be significantly related to general intelligence as indexed by standard IQ tests. Direct and indirect pathways by which the frontal cortex modulates parasympathetic activity via subcortical inputs have been identified, (Ter Horst, 1999; Ter Horst and Postema, 1997). A number of researchers have hypothesized inhibitory cortical-subcortical circuits (Benarroch, 1993, 1997; Masterman and Cummings, 1997; Mayberg et al., 1999; Spyer, 1989). However, Thayer and Lane (2000) have been the first to tie these circuits to heart rate variability (HRV).

We will provide an overview of the neural structures linking the central nervous system to HRV. Next, we will review a number of studies from our group showing that individual differences in HRV are related to performance on tasks associated with executive function and prefrontal cortical activity. We propose that these findings have important implications for the development of biomarkers related to performance in modern warfighters.

THE CENTRAL AUTONOMIC NETWORK

Investigators have identified functional units within the central nervous system (CNS) that support goal-directed behavior and adaptability. One such entity is the *central autonomic network* (CAN; Benarroch, 1993, 1997). Functionally, this network is an integrated component of an internal regulation system through which the brain controls visceromotor, neuroendocrine, and behavioral responses that are critical for goal-directed behavior, adaptability, and health. Structurally, the CAN includes the anterior cingulate, insular, orbitofrontal, and ventromedial prefrontal cortices, the central nucleus of the amygdala, the paraventricular and related nuclei of the hypothalamus, the periaquaductal gray matter, the parabrachial nucleus, the nucleus of the solitary tract (NTS), the nucleus ambiguus, the ventrolateral medulla, the ventromedial medulla, and the medullary tegmental field. These components are reciprocally

interconnected such that information flows bi-directionally between lower and higher levels of the CNS. The primary output of the CAN is mediated through preganglionic sympathetic and parasympathetic neurons that innervate the heart via the stellate ganglia and vagus nerve, respectively. The interplay of these inputs to the cardiac sino-atrial node produces complex variability that characterizes the HR time series (Saul, 1990). Thus, the output of the CAN is directly linked to HRV. Notably, vagal influences dominate cardiac chronotropic control (Levy, 1990). In addition, sensory information from peripheral end organs such as the heart and the immune system are fed back to the CAN. As such, HRV is an indicator of central-peripheral neural feedback and CNS-ANS integration.

Other functional units within the CNS serving executive, social, affective, attentional, and motivated behavior in humans and animals have been identified (Damasio, 1998; Devinsky et al., 1995; Masterman and Cummings, 1997; Spyer, 1989). One such network has been termed the anterior executive region (AER; Devinsky et al., 1995). The AER and its projections regulate behavior by monitoring the motivational quality of internal and external stimuli. The AER network has been called the "rostral limbic system" and includes the anterior, insular, and orbitofrontal cortices, amygdala, periaquaductal gray, ventral striatum, and autonomic brainstem motor nuclei. Damasio (1998) has recognized a similar neural "emotion circuit", for which there is considerable structural overlap with the CAN and the AER (Thayer and Lane, 2000).

We propose that the CAN, the AER network, Damasio's "emotion circuit" 1998, and related systems (Masterman and Cummings, 1997; Spyer, 1989) represent a common central functional network recognized by different researchers from diverse approaches. This CNS network is associated with the processes of response organization and selection, and serves to control psychophysiological resources in attention and emotion (Friedman and Thayer, 1998a, 1998b; Thayer and Friedman, 1997). Additional structures are flexibly recruited to manage specific behavioral adaptations. This sparsely interconnected neural complex allows for maximal organism flexibility in accommodating rapidly changing environmental demands. When this network is either rigidly coupled or completely uncoupled, the ability to recruit and utilize appropriate neural support to meet a particular demand is hampered, and the organism is thus less adaptive.

It has been proposed that the prefrontal cortex is taken "off-line" during emotional stress to let automatic, prepotent processes regulate behavior (Arnsten and Goldman-Rakic, 1998). This selective prefrontal inactivation may be adaptive by facilitating predominantly non-volitional behaviors associated with subcortical neural structures such as the amygdala to organize responses without delay from the more deliberative and consciously guided prefrontal cortex. In modern society, however, inhibition, delayed response, and cognitive flexibility are vital for successful adjustment and self-regulation, and prolonged prefrontal inactivity can lead to hypervigilance, defensiveness, and perseveration.

ATTENTIONAL REGULATION AND EXECUTIVE FUNCTION

Attentional regulation and the ability to inhibit pre-potent but inappropriate responses is also important for health and optimal performance in a complex environment. Many tasks important for survival in today's world involve cognitive functions such as working memory, sustained attention, behavioral inhibition, and general mental flexibility. These tasks are all associated with prefrontal cortex activity (Arnsten and Goldman-Rakic, 1998). Deficits in these cognitive functions tend to accompany aging, and are also present in negative affective states and dispositions such as depression and anxiety. Stress can also impair cognitive function and may contribute to the cognitive deficits observed in various mental disorders and in extreme environments. It is also possible that autonomic dysregulation contributes to deficits in attention and cognitive performance. A series of experiments in our lab have been conducted to examine this issue, and are described below.

In a recent experiment, Johnsen et al. (2003) examined inhibitory responses in an emotional Stroop paradigm. Dental phobics were first exposed to recorded scenes of dental procedures and then administered the emotional Stroop test. In addition to the traditional color congruent and color

incongruent words, phobic subjects also were asked to respond to neutral words and dental-related words (e.g., "drill" and "cavity") which were threatening to them. All subjects exhibited longer reaction times to the incongruent color words and the dental-related threat words, and thus, displayed a difficulty in inhibiting pre-potent responses. However, greater HRV was associated with faster reaction times to these words, consistent with the link among vagally mediated HRV, inhibitory ability, and frontal lobe function. These results support the idea that vagally mediated HRV is associated with efficient attentional regulation and greater ability to inhibit pre-potent but inappropriate responses.

Subsequent studies further examined executive function and working memory in healthy individuals in a military context. In the first experiment, subjects performed a number of tasks involving continuous performance including a simple reaction time task, a choice reaction time task, and three tasks that involved delayed responding and working memory (Hansen et al., in press; Johnsen et al., 2002). The California Computerized Assessment Package Abbreviated version, (CalCAP; Norland Software, Los Angeles, Calif., Miller, 1999) was chosen as a continuous performance task. CalCAP is recognized as a test of sustained attention and consisted of four sub-tests, two with non-executive components (simple reaction time and response latencies to specific stimuli components) and two with executive components (detection of identical stimuli and a simple addition task). The test was self-explanatory and needed only minimal supervision by the investigator. In addition a modified version of a working memory test (WMT) developed by Hugdahl et al. (2000) based on Baddeley and Hitch's research (1974) was chosen. This test consisted of a continuous flow of digits and subjects were to detect identical digits to the one presented two trials previously. The stimuli were numbers from 1 to 9. These latter three tasks involved aspects of delayed responding and working memory, and have been shown to be associated with prefrontal activity (Goldman-Rakic, 1998). HRV and cortisol responses were recorded, and subjects were grouped into low and high HRV groups.

Performance on tasks involving simple and choice reaction times did not differ between these groups. However, on tasks associated with prefrontal activity, subjects in the low HRV group performed more poorly in terms of reaction time, number of errors and number of correct responses than those in the high HRV group. In addition, the groups did not differ in baseline, morning, or evening cortisol, but the low HRV group showed larger cortisol responses to cognitive tasks that lasted into the post-task recovery period. Stress is associated with an increased cortisol release, and cortisol plays a major role in immune function through its association with proinflammatory cytokines (Kiecolt-Glaser et al., 2002). Cortisol is also known to impair function on cognitive tasks associated with prefrontal cortex (Lupien et al., 1999). Thus, the low HRV group was less stress tolerant as indexed by cortisol responses and more impaired cognitively than the high HRV group.

In another study in the series, military subjects performed the same tasks as above, but half did so under threat of electric shock (Hansen et al., 2002). Again, subjects were divided into two groups based on resting HRV levels. In the shock threat condition, task performance involving delayed responding and prefrontal activity was significantly impaired in the low HRV group. Thus, persons with high HRV were more stress tolerant and less affected by the threat compared to those with low HRV. In yet another study, HRV was manipulated by having half of the subjects in a physically active group undergo mild de-training for four weeks. Aerobic capacity and HRV were significantly reduced in this group compared to those that maintained their fitness and HRV levels. All subjects again performed the above cognitive tasks: once before the four-week de-training period, and once after. The de-trained, lower HRV group failed to show the expected learning effect associated with repeated performance of the cognitive tasks, and thus, did not reap the typical benefit of previous task exposure.

CONCLUSIONS

Taken together, these results support the usage of HRV to index efficient allocation of attentional and cognitive resources needed for efficient functioning in a challenging environment in which delayed responding and behavioral inhibition are key. In addition, these data show that low HRV marks increased risk to stress exposure. Significantly, these results provide a connection among stress-related cognitive

deficits, high negative affect, and negative health consequences via the common mechanism of autonomic imbalance and low parasympathetic activity.

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Biomarkers for Brain Hypometabolism Due to Sleep Deprivation

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INTRODUCTION

Both acute and chronic sleep deprivation (roughly surge and sustained operations respectively) degrade cognitive performance (Belenky et al., in press). The neurobiological basis of this cognitive performance degradation appears to be a global decrease in brain energy metabolism, with greatest decreases occurring in prefrontal cortex (Thomas et al., 2000). The prefrontal cortex governs highest-order cognitive processes including anticipation, planning, situational awareness and common mental models, the ability to envision the desired end state and the paths to achieving it. In military operations, these functions translate into the ability to adapt at all levels of command and control to take advantage of tactical, operational, and strategic opportunities in real-time.

Surge operations and sustained operations differ in their effects on performance and thus presumably on underlying neurobiology. During surge operations (less than 4 hours of sleep per 24 hours) performance degrades in a linear fashion while brain metabolism declines over the first 24 hours and then stabilizes at this lower level (Thomas et al., 2000; Thomas et al., in preparation). Because recovery from surge operations is rapid and generally complete within 24 to 48 hours with adequate (8 hours per night) recovery sleep, it is assumed that brain metabolism also recovers completely. The effects of sustained operations (more than 4 but less than 7 hours of sleep per 24 hours) on performance has received far less attention, and therefore are less well understood – however, results from a recently completed study by our group indicate that with less than 8 hours sleep per night performance degrades over the first few days and then stabilizes at a lower sub-maximum level of performance (Balkin et al., 2000; Belenky et al., in press). Unlike surge operations, recovery from sustained operations can take days or weeks (Belenky et al., in press). The effects of sustained operations on brain metabolism are not known, but our performance data suggest that sustained operations are associated with a more enduring down-regulation of brain metabolic capacity.

As both military and civilian industrial endeavors become increasingly continuous (24 hour per day) operations, the potential for sleepiness-related incidents—ranging from operational inefficiencies to errors resulting in serious accidents—is increasing. The task of determining how – or what – to measure to predict human performance degradation is difficult and complex. Because brain hypometabolism is assumed to underlie performance deficits, the former would be the "gold standard" biological signal to monitor. Biomarkers of brain metabolism changes during sleep deprivation include blood flow (e.g., Braun et al., 1997) and glucose metabolism (Thomas et al., 2000). Clearly, however, these markers are not fieldable—and to date evidence indicating that they are *predictive* of performance degradation is lacking. Since in most operational settings changes in actual performance are of concern, the question could be rephrased as, "Are measures of actual performance as good as (or perhaps better than) measures of brain hypometabolism?" If the answer to the latter is positive, the question then becomes, "What constitutes a promising metric of general sleep-related performance capacity for use in the operational environment?" To this end, we tested, compared, and judged several candidate measures across seven consecutive days in which subjects were allowed 9, 7, 5, or 3 hours in bed per night. This design constituted an in-laboratory simulation of sustained operations (as defined above).

MATERIALS AND METHODS

General Design and Procedures

A complete description of the study subjects, design, and procedures can be found in Balkin et al. (2000). Briefly, 66 Commercial Motor Vehicle (CMV)-licensed drivers (16 women, 50 men; age range 24-62 years) participated. They spent 14 days in the laboratory. The first two days were adaptation/training (T1, T2) and the third served as baseline (B). Subjects were allowed 8 hours in bed (TIB) from 2300-0700 on the nights prior to T2 and B. Beginning on the fourth day and continuing for a total of seven days (E1-E7) subjects were assigned to one of four sleep conditions: nine hours TIB (2200-0700); seven hours TIB (2400-0700); five hours TIB (0200-0700), or three hours TIB (0400-0700)]. On the eleventh day and continuing for a total of three "recovery" days (R1-R3) subjects were again allowed to sleep from 2300-0700 (8 hours TIB). Data from these recovery days are not reported here.

Cognitive/Psychomotor Tests

Subjects performed a series of cognitive and alertness tests daily including Psychomotor Vigilance or "PVT" (Dinges and Powell, 1985); synthetic work (Elsmore, 1994); simulated driving ("StiSim" - see Balkin et al., 2000); running memory; grammatical (logical) reasoning; Stroop color naming; serial addition/subtraction; 10-choice reaction time (RT); time estimation or "interval reproduction," code substitution; subjective sleepiness via the Stanford Sleepiness Scale (Hoddes et al., 1973); objective sleepiness via a sleep latency test (SLT) (Carskadon et al., 1986); 4-choice RT (Thorne et al., 1985); and an oculomotor function test or "FIT." A detailed description of these tests can be found in Balkin et al. (2000).

Data Analyses

Analysis of variance

Data were first analyzed using conventional analysis of variance (ANOVA; Kirk, 1995) techniques: a mixed ANOVA for Sleep Group (between subjects) x Day (within subjects) was applied to all data, with additional factors for time of day as appropriate. Greenhouse-Geisser corrections (Kirk, 1995) were applied to repeated measures effects. Significant Sleep Group x Day interactions were followed by simple effects analyses for sleep group at each day. Significant sleep group simple effects were then analyzed using *Post-hoc* Tukey Honestly Significant Difference (HSD) comparisons (Kirk, 1995) among all possible pairs of sleep groups (maximum of 6 comparisons: 3-hr v. 5-hr, 7-hr, and 9-hr; 5-hr v. 7-hr and 9-hr; 7-hr v. 9-hr). All performance data were normalized by converting to percent baseline.

Effect size analysis

Data were also explored by generating an effect size estimate (also known as a d statistic) for the relationship between nightly sleep time and each task/dependent variable listed above independent of sleep group assignment (Balkin et al., in preparation). Variability of the effect size was estimated using a bootstrap procedure to determine whether the effect size differed from zero. The bootstrap procedure also provided estimates of confidence intervals.

RESULTS

Analysis of Variance (ANOVA)

Analysis of variance revealed that nightly total sleep time (TST) increased significantly in the 9-hour group and decreased significantly in the 3-, 5-, and 7-hour groups across the sleep restriction/augmentation phase (E1—E7) compared to baseline (B) (Group x Night, $p < .05$). TST significantly differed among all sleep groups on nights E1 through E7 (Tukey HSD, $ps < .05$).

Table 2 summarizes the number of significant post-hoc comparisons among sleep groups for each task and dependent variable baseline (BL) through experimental day 7 (E7) for which both the Sleep Group x Day interaction and significant simple effects of sleep group at each day were significant. The tasks/dependent variables are rank-ordered by total number of significant post-hoc contrasts summed across baseline and E1—E7. As indicated in Table 2, by this criterion, PVT relative speed was most sensitive.

Effect Size Analyses

Figure 2 shows results of the effect size analysis. Using this technique, the SLT accounted for the largest percentage of variance in nocturnal sleep during the experimental phase (45%), followed by PVT speed (21%), StiSim lane deviations (19%) 4-choice RT speed (13%) and SSS (10%); the effect sizes for these tasks/dependent measures were statistically significant ($ps < .05$). Note that although StiSim accidents showed a relatively large effect size, the confidence intervals for this measure also were large; thus, the effect size was nonsignificant. On the other hand, effect sizes for StiSim lane position (7%), 10-choice RT number correct (7%), serial addition/subtraction speed (5%), and 4-choice RT correct (3%) were relatively small but significant since the confidence intervals were relatively narrow.

DISCUSSION AND CONCLUSIONS

Although it is assumed that biomarkers of brain hypometabolism (which is assumed to underlie performance deficits) would be the preferred biological signal to monitor to predict sleep deprivation-induced performance impairments, such markers are currently not fieldable – therefore, in the present paper the question of what constitutes a promising metric of general sleep-related performance capacity for use in the operational environment was addressed.

Of the various measures compared, the most sensitive (as reflected by the number of statistically significant post-hoc comparisons from the ANOVA) was the Psychomotor Vigilance Test (PVT, Dinges and Powell, 1985). The most sensitive test as reflected by the effect size analysis was the sleep latency test (SLT). Although the rank ordering of tasks differed somewhat between the ANOVA and effect size analysis, in general those tests found to be most sensitive by one technique also ranked highly using the other technique. Tasks in the top rankings for both included the PVT, simulated driving lane deviations and lane position, SLT, SSS subjective sleepiness self-ratings, and serial addition/subtraction speed.

That the sleep latency test (SLT) accounted for the most variance by the effect size technique is perhaps not surprising since it could be argued that the SLT is the most "direct" measure of sleep loss in that it actually gauges sleep (onset) itself. However, under most circumstances the SLT is not practical – and more important, sleep latency does not necessarily predict performance. PVT speed most frequently

mirrored the gradations in total sleep times—and, by inference, the differential levels of recuperation that result from spending 3, 5, 7, or 9 hours in bed, over 7 consecutive nights. That PVT speed did not account for a greater proportion of variance in nocturnal sleep time (effect size analysis) may indicate that total sleep time, rather than PVT speed, is not a particularly sensitive index of recuperation processes. It may be that some other index of sleep-mediated recuperative processes, such as slow-wave activity might better predict performance.

The present results suggested relatively poor sensitivity of the FIT for detecting sleepiness. It is possible that sensitivity could have been increased by increasing FIT test duration. In its current configuration, the FIT is a short (45-second) test. Even extremely sleepy subjects can perform adequately for short periods of time, suggesting that *any* short-duration task will lack sensitivity. For example, had the PVT been administered only for 45 seconds, it likely would have been relatively insensitive – and in fact, our analyses of PVT data across time on task indicate that decrements do not become evident until the 3rd or 4th minute on task. The SLT may also constitute a 20-minute vigilance task whose sensitivity would be decreased by shortening the test to 1-2 minutes.

In the near-term, progress in developing the means to measure and monitor the effects of sleep loss in the operational environment will require further, similar studies--systematic, head-to-head comparisons of the sensitivity and reliability of multiple measures (with consideration of the likelihood that these measures could be obtained the operational environment of interest). At the core of these near-term (within 2 to 5 years) studies will be performance metrics, with a vision toward integration of newer, "high-risk / high-payoff" technologies such as analyses of changes in gene expression across sleep deprivation/sleep restriction – and how such changes in gene expression relate to specific performance metrics. Also needed in the near-term are studies describing the exact relationship between sleep deprivation-induced brain hypometabolism and specific aspects of cognitive performance to determine whether there is actually a need for measuring hypometabolism directly. That is, does a marker of brain hypometabolism (blood flow, metabolism) confer some predictive advantage beyond that of performance measures? Do markers of brain hypometabolism better determine individual differences in response to sleep loss? Far-term (10-20 years out) studies will consist of aggregate measures of sleep/wake history over weeks -- analogous to glycosylated hemoglobin as an index of blood glucose control over a period of weeks.

In both the near-term and far term, investigations into the underlying neurobiology of sleep and wakefulness are critical –for example, no chemical has yet been identified in the blood that accumulates during sleep deprivation and causes performance impairments. The analogous state of affairs would be alcohol-induced impairment, where alcohol levels are measurable in exhaled air, and levels of alcohol have been correlated with degree of performance impairment. The latter is the end result of a long and complex (and still ongoing) process.

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		Number of significant post-hoc contrasts among sleep groups (max = 6 per day)									Actual Group n (max possible)						
TASK	DEPENDENT MEASURE	B	E1	E2	E3	E4	E5	E6	E7	TOTAL	3 (18)	5 (16)	7 (16)	9 (16)	TOTAL (66)	*	^
Total Sleep Time	*Abs. Minutes of Sleep*	*NS*	*6*	*6*	*6*	*6*	*6*	*6*	*6*	*42*	*18*	*16*	*15*	*16*	*65*		
PVT	Rel. Speed	n/a	2	3	4	4	3	4	4	24	14	13	14	16	57		
PVT	Rel. Speed - 2 times of day	n/a	2	3	4	4	4	4	3	24	16	15	15	16	62		
STISIM	Rel. SD of Lane Tracking	n/a	NS	4	2	2	3	2	3	16	10	13	13	12	48		
STISIM	Rel. Lane Position	n/a	1	2	3	2	2	2	2	14	10	13	13	12	48		
Stanford Sleepiness Scale	Rel. Sleepiness Score	NS	NS	2	3	1	2	2	1	11	17	15	15	13	60		
Wilkinson 4-choice RT	Rel. Speed	n/a	NS	1	2	2	2	1	3	11	14	15	15	10	54		
Running Memory	Rel. Speed	n/a	1	NS	1	0	3	2	3	10	17	14	15	14	60		
Modified MSLT	Abs. Latency to Sleep (minutes)	NS	NS	4	0	NS	4	2	0	10	16	14	15	9	54		
Stroop	Rel. Speed	n/a	1	NS	NS	1	1	1	2	6	17	15	14	15	61		
Serial Addition/Subtraction	Rel. Speed	n/a	NS	NS	1	1	1	1	2	6	17	14	15	13	59		
Running Memory	Rel. Accuracy	n/a	NS	NS	NS	NS	1	1	3	5	17	14	15	14	60		
Serial Addition/Subtraction	Rel. Accuracy	n/a	NS	NS	NS	NS	1	2	2	5	17	14	15	13	59		
Grammatical Reasoning	Rel. Accuracy	n/a	0	0	NS	NS	NS	NS	NS	0	17	14	15	13	59		
Time Estimation	Rel. Coefficient of Variation	n/a	NS	NS	NS	0	0	0	0	0	17	13	15	13	58		
Wilkinson 4-choice RT	Rel. Accuracy	n/a	NS	NS	0	NS	NS	0	0	0	14	15	15	10	54		
10-Choice RT	Rel. Speed (Group x Day p = 0.0	n/a	NS	NS	0	1	0	3	2	6	17	13	15	13	58		
Stroop	Rel. Accuracy	n/a	NS	NS	NS	NS	NS	NS	NS	0	17	15	14	15	61	*	
Grammatical Reasoning	Rel. Speed	n/a	NS	NS	NS	NS	NS	NS	NS	0	17	14	15	13	59	*	
10-Choice RT	Rel. Accuracy	n/a	NS	NS	NS	NS	NS	NS	NS	0	17	13	15	13	58	*	
SYNWORK	Rel. Composite Score	n/a	NS	NS	NS	NS	NS	NS	NS	0	17	15	11	15	58	*	
Code Substitution	Rel. Accuracy ("IREscore")	n/a	NS	NS	NS	NS	NS	NS	NS	0	16	13	13	8	50	*	
STISIM	Abs. Number of Accidents	NS	NS	NS	NS	NS	NS	NS	NS	0	13	14	13	13	53	*	
FIT	Rel. CA	n/a	NS	NS	NS	NS	NS	NS	NS	0	13	8	10	11	42	*	^
FIT	Rel. Eye Closure	n/a	NS	NS	NS	NS	NS	NS	NS	0	13	8	10	11	42	*	^
FIT	Rel. Pupil Diameter	n/a	NS	NS	NS	NS	NS	NS	NS	0	13	8	10	11	42	*	^
FIT	Rel. INDEX	n/a	NS	NS	NS	NS	NS	NS	NS	0	13	8	10	11	42	*	^
FIT	Rel. Saccadic Velocity	n/a	NS	NS	NS	NS	NS	NS	NS	0	13	8	10	10	41	*	^

TABLE 1 **Number of significant post-hoc contrasts among sleep groups for each task and dependent measure. Tasks are rank-ordered by the total number of significant post-hoc Tukey HSD contrasts found.**

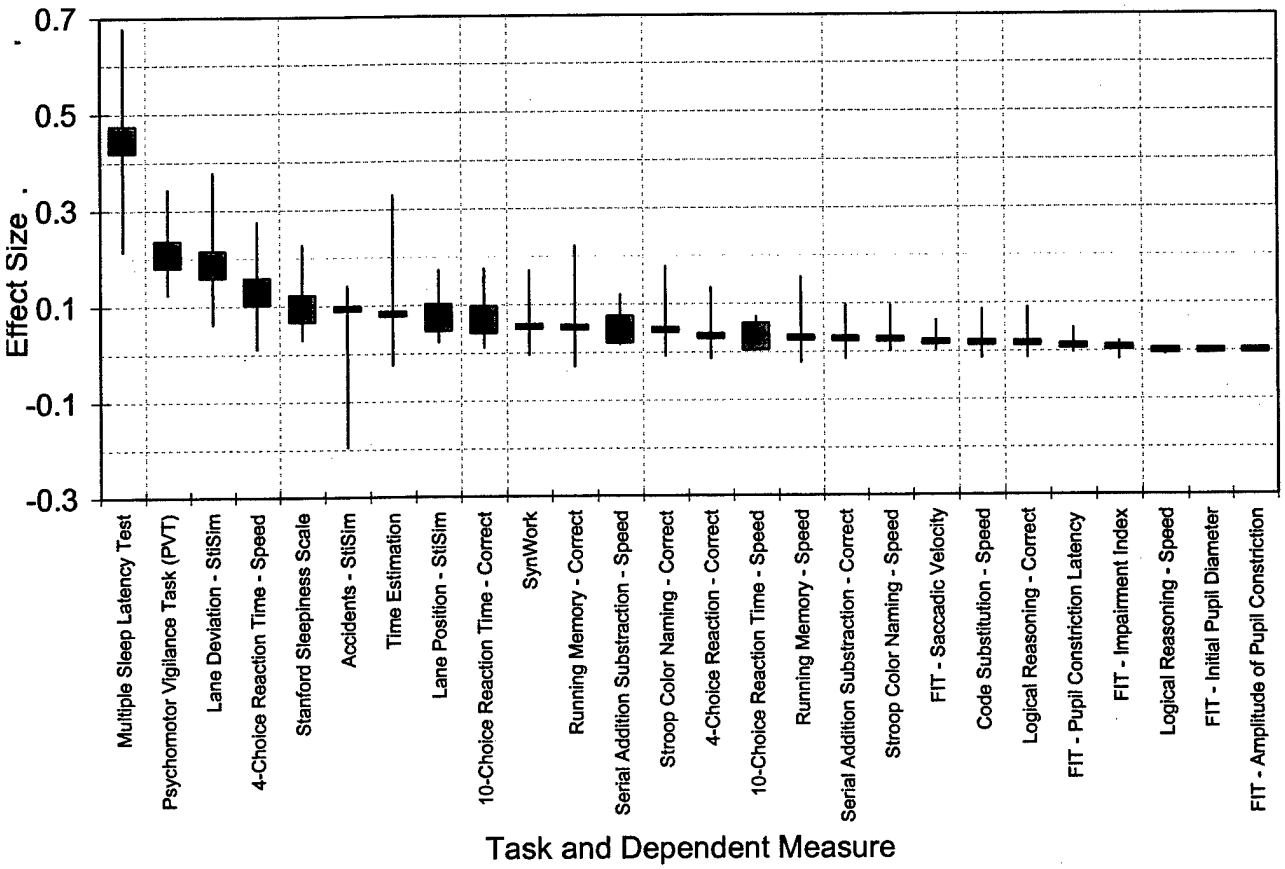


FIGURE 1 Effect size for each task and dependent measure. Significant effect sizes are denoted by filled gray squares; nonsignificant effect sizes are denoted by a solid dash. Vertical lines indicate confidence intervals.

Biomarkers for Change in Protein Turnover of Muscle

Robert Wolfe and Elisabet Børsheim

The net gain or loss of muscle protein represents the balance between the rates of synthesis and breakdown. Consequently, when considering potential markers for changes in protein turnover in muscle it is necessary in fact to evaluate potential candidates in terms of the ability to reflect changes in the net balance between synthesis and breakdown.

The fundamental processes that control the balance between muscle protein synthesis and breakdown are shown in Figure 1. Amino acids that can potentially be used for incorporation into protein (i.e., synthesis) can be derived from transport from the plasma, breakdown, or in the case of certain (non-essential) amino acids, from de novo synthesis. In turn, the amino acids in the precursor pool for synthesis can also be transported back to the plasma and carried away by venous blood.

There is interplay between all of these factors in the context of normal daily activity, including exercise and eating. Since amino acids provide a readily measurable component of the system, it is worthwhile to consider in depth the possible utility of measures of blood amino acid concentrations as indices of the status of the overall system, in particular the balance between synthesis and breakdown. In that regard, it is pertinent to first consider the role of muscle in the overall regulation of whole body protein metabolism.

Many tissues of the body, such as the skin, heart, brain and liver, have a constant demand for amino acids. Protein breakdown is always occurring in these tissues, and without a sufficient rate of synthesis to balance the rate of breakdown the amount of protein would quickly diminish. Since these tissues and organs do not have significant reserves of protein, even transient periods of net catabolism might have significant physiological consequences. Therefore, these tissues are normally able to extract sufficient amino acids from the blood to maintain synthesis at a rate sufficient to match the rate of breakdown, thereby avoiding a net loss of protein. Muscle, on the other hand, serves as a reservoir for amino acids. At least 15% of muscle mass can be lost without physiological consequences. Thus, muscle catabolism serves to provide plasma amino acids when none are available from absorption of dietary intake. In other words, there is a net negative protein balance in muscle in the post-absorptive or fasted state in order to provide the amino acids needed by other tissue organs in which the maintenance of protein mass is more essential for survival. Consequently, when amino acids are being absorbed, the muscle protein pool is the principal target of repletion, since other tissues received adequate amino acids via the blood to maintain protein balance in the absence of intake. Thus, muscle can be considered as a reservoir for amino acids that functions to keep amino acids available, via the plasma, for protein synthesis throughout the body.

The muscle performs its role as a reservoir quite efficiently. Even after 50-60 days of fasting in obese individuals plasma essential and non-essential amino acids are maintained constant (Drenick et al., 1964). Further, intracellular concentrations of essential amino acids are regulated so as to remain constant unless there is a major perturbation in one or more of the factors controlling those concentrations (i.e., synthesis, breakdown, or transport). For example, when extracellular concentrations of amino acid were increased 40% by a primed-constant infusion, the intracellular essential amino acid concentrations remained constant, even though synthesis was stimulated (Bohe et al., in press). Further, when plasma amino acid concentrations were doubled by intravenous infusion, intracellular essential amino acid concentrations actually fell slightly, but significantly (Bohe et al., in press). Only when the rate of infusion of amino acids increased sufficiently to exceed the capacity of synthesis to increase proportionately did the intracellular concentration of amino acids increase (Bohe et al., in press). In an analogous response, when plasma amino acids were artificially lowered 40% below the basal level by hemodialysis, intracellular essential amino acid concentrations were maintained unchanged (Kobayashi et al., in press).

Thus, under normal physiological conditions, changes in concentrations of essential amino acids cannot provide insight into the rates of muscle protein synthesis, breakdown, or the balance between them.

In severe stress, the stimulus for net protein catabolism provides extra amino acids required for processes such as wound healing, immune function, and synthesis of acute phase proteins in the liver. In severe stress, such as burn injury, the signal for breakdown may exceed the increased requirement for amino acids, such that intracellular, and sometimes plasma concentrations of essential amino acids increase. For example, intracellular concentrations of phenylalanine, leucine and lysine are all elevated in burn patients (Biolo et al., in press), although all may not be elevated in plasma. Interpretation of individual essential amino acids may be complicated by specific aspects of its metabolism. For example, phenylalanine is generally elevated in critically ill patients, but the plasma phenylalanine is clouded by the fact that its clearance not only reflects the uptake for the process of synthesis, but the liver clears phenylalanine and metabolizes it to tyrosine. Thus, in critically ill patients an isolated increase in phenylalanine may reflect liver failure as much as net muscle protein breakdown.

The non-essential amino acids alanine and glutamine are the principal means by which nitrogen is transferred from muscle to the liver for eventual excretion as urea. Thus, when net muscle breakdown is accelerated, an increased production of alanine and/or glutamine would be expected. In fact, alanine release from muscle may be elevated by several fold in severely burned patients (Jahoor et al., 1986), and even after exercise alanine release is accelerated (Wolfe et al., 1984). However, plasma concentrations of alanine are not elevated even when flux rates are elevated several fold, probably due to the concurrent stimulation of gluconeogenesis that occurs in response to stress (Wolfe et al., in press). In fact, alanine concentration may actually fall in severe sepsis (Gore and Wolfe, submitted). Consequently, alanine does not provide useful information about net muscle protein breakdown.

Depletion of the intramuscular glutamine pool occurs in stress states. Normally the intramuscular concentration of glutamine is greater than the sum of all other amino acids. In severe stress the intracellular concentration may fall by as much as 90% (Mittendorfer et al., 1999). Nonetheless, plasma concentrations are generally maintained or even fall. Thus, whereas plasma (and interstitial) concentrations of glutamine provide little insight into the net muscle protein balance, monitoring of the intracellular concentration of glutamine could likely provide reasonable insight as to whether the individual was under significant physiological stress. Current technology requires muscle biopsy to accomplish this measurement.

Muscle myofibrillar protein breakdown has been estimated using indirect measures such as 3-methylhistidine (3-MH) excretion (Young et al., 1973). 3-MH is produced by the posttranslational methylation (by protein-histidine N-methyltransferase) of specific histidine residues in the actin of all muscle fibers and in the myosin of type II fibers. It is released during protein breakdown, and is not reutilized for protein synthesis or metabolized in man, but is excreted in urine. The skeletal muscle mass comprises the largest fraction of tissue bound 3-MH in the body. Thus, urinary excretion of 3-MH in its free and acetylated form has been used as a measure of the rate of muscle myofibrillar protein breakdown. Concerns about the validity of 3-MH excretion as an indicator of muscle protein breakdown relate to the contribution of non-muscle sources to urinary 3-MH, e.g., gut smooth muscle and skin. Also, dietary protein intake can contribute to urinary 3-MH. Thus, urinary excretion of 3-MH may be problematic to use as a measure of skeletal muscle protein turnover. Determination of arterial-venous differences of 3-MH across muscle may be more useful in that regard. Nonetheless, even if it were to provide a precise measure of the rate of muscle protein breakdown it would not be useful as an indicator of net muscle balance. When a large body of literature is considered, it is clear that changes in breakdown and synthesis normally occur in the same direction, and the magnitude of the individual responses (i.e., synthesis and breakdown) determines the nature of any change in net balance.

The best example of the lack of a obligatory relation between breakdown and net balance, particularly with relation to potential military applications, can be seen in Figure 2. Resistance exercise caused an improvement in net muscle protein balance, i.e., reduced the fasting rate of muscle catabolism. However, the rate of breakdown actually increased - the improvement in net balance was due to an even

greater increase in synthesis (Biolo et al., 1995a). Thus, whereas monitoring 3-MH could potentially give some indication of the rate of muscle protein breakdown, it is the balance between synthesis and breakdown that determines gain or loss of muscle mass, and knowledge of breakdown alone provides little insight into the net balance.

Taken together, this discussion lends to the conclusion that measurement of plasma (or urinary) levels of amino acids or other potential markers of synthesis or breakdown cannot be expected to be reliable indicators of the balance between muscle protein kinetics or breakdown. Increasing levels of invasiveness enable more detailed information to be obtained.

With oral ingestion of a ^{15}N-alanine bolus and collection and analysis of urinary ammonia enrichment it is possible to calculate whole body protein turnover. Coupled with ingestion of labeled 3-MH and measurement of the decay in enrichment, it is possible to distinguish the contribution of changes in muscle protein breakdown to the overall change in whole body protein turnover. In many, but not all, circumstances, changes in whole body turnover reflect changes in muscle protein turnover.

With increasing levels of invasiveness, it is possible to more directly obtain quantitative information. With the use of isotopically labeled tracer infusion and muscle biopsies and peripheral venous blood samples it is possible to quantify rates of muscle protein synthesis, and if arterial samples are added, breakdown can also be measured (Zhang et al., 1996). When arterial-venous sampling across the leg is coupled with biopsies, all of the factors shown in Figure 1 can be quantified (Biolo et al., 1995b). Unfortunately, these invasive procedures are necessary, because more readily accessible means of estimating muscle protein metabolism are unreliable.

SUMMARY

Changes in muscle mass occur because of an imbalance between the rates of protein synthesis and breakdown. Thus, the complication in finding a pertinent marker is that it must reflect the balance between two distinct processes. Consequently, the only reliable means of estimating changes in muscle protein turnover in a physiologically relevant manner is with the use of stable isotope tracers. Further, use of these tracers must be coupled with invasive procedures such as muscle biopsies and/or arterial and deep venous catheterizations to gain information about changes in muscle protein metabolism.

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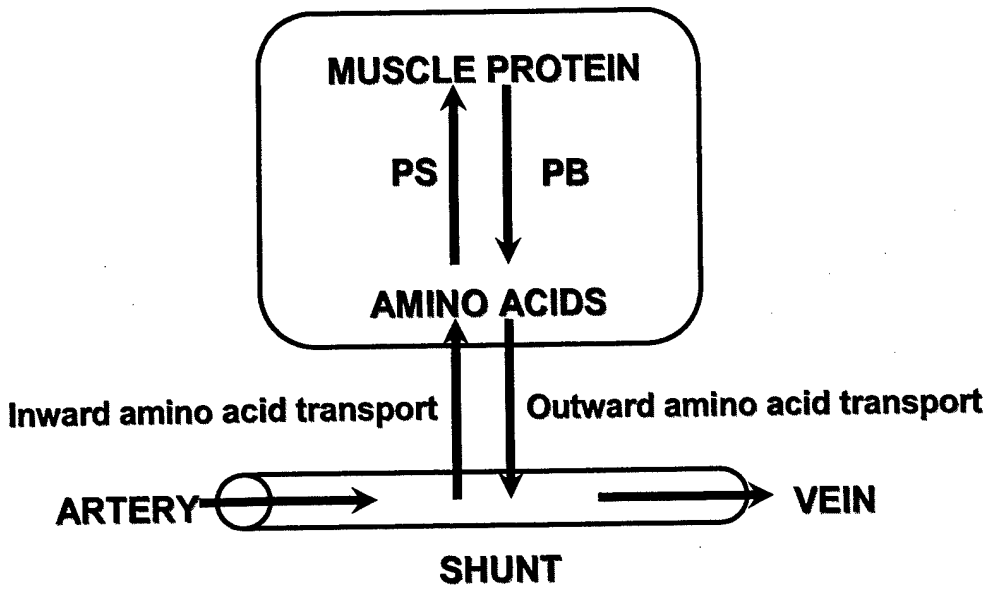
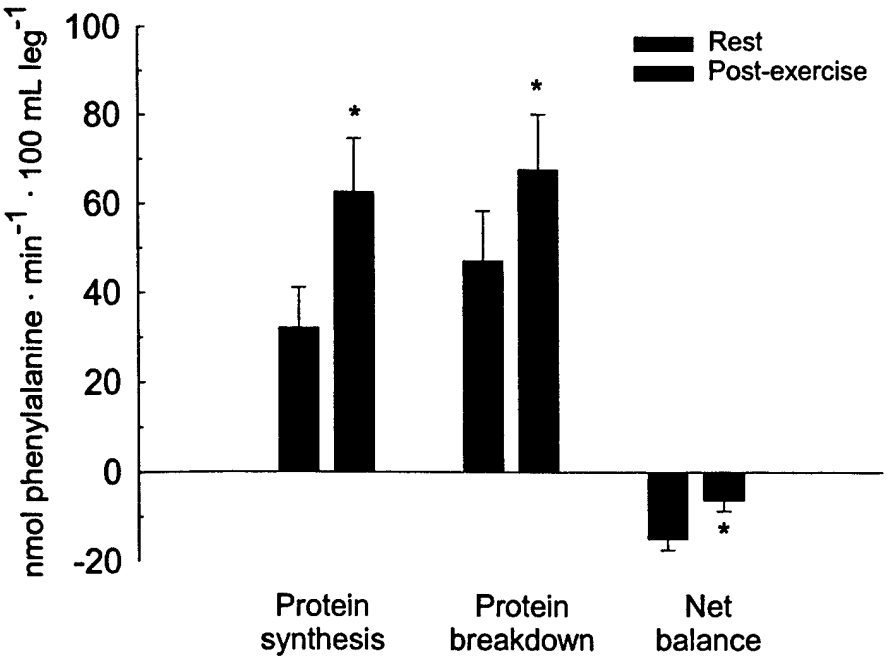


FIGURE 1 Interaction between amino acids and protein kinetics

FIGURE 2 **Muscle protein synthesis, breakdown and net protein balance at rest (black bars) and ~3h after a heavy resistance exercise bout (gray bars) in untrained male volunteers. Values are mean ± SE. *, significant difference ($p<0.05$) post-exercise vs rest. Adapted from Biolo et al. 1995 a.**



Biomarkers of Physiological Strain During Exposure to Hot and Cold Environments

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INTRODUCTION

Soldiers experience thermal (heat and cold) stress arising from the combined effects of environment, clothing insulation and body heat production. Alterations in body temperatures (core, skin & muscle) above and below "normal" levels can lead to thermal illness/injury and degrade performance. Humans regulate core temperature within a narrow range (35° to 41 °C) through both behavioral and physiological responses to thermal stress. When conscious actions to minimize or avoid thermal stress by modifying activity levels, changing clothes and seeking shelter do not completely negate thermal stress, physiological responses are activated that enhance dissipation or conservation of body heat stores, as appropriate, through alterations in metabolic rate, blood flow between the core to the skin, and sweating. Activation of these responses works to maintain temperature homeostasis, but also results in physiological strain. In this brief review, human physiological responses elicited in response to exposure to extremes of hot and cold will be summarized with a view to identifying potential biomarkers of physiological strain. Further, an example of how such biomarkers can be used collectively to assess physiological strain and warn of impending health and performance degradation during exposure to heat and cold will be presented.

CORE TEMPERATURE

Thermal strain is most commonly assessed by the measurement of body core temperature. There is no one "true" core temperature because of temperature difference among different sites in the core. Core temperature is often measured at the esophagus, rectum, mouth, tympanum, and auditory meatus. Measurement methods employed for each of these sites and the relative advantages and disadvantages of each are and discussed in detail by Sawka et al. (1996) and summarized in Table 1. In brief, most thermal physiologists consider esophageal temperature to be the most accurate and reliable non-invasive index of core temperature for humans, followed in preference by rectal temperature and gastrointestinal tract temperature measured using ingestible temperature sensor pills, the latter of which is ideally suited for ambulatory monitoring outside of laboratories (O'Brien et al., 1998). Oral (sublingual), tympanic and auditory meatus temperatures are widely used as reflections of core temperature, but all are influenced to some degree by head and face skin temperatures, as well as ambient temperature, and sensitive to inaccuracies related to proper placement of the sensor.

HEAT STRAIN

Heat Stress

Heat stress increases requirements for sweating and circulatory responses to dissipate body heat. When the ambient temperature is warmer than skin, the body gains heat from the climate, which increases heat the body must dissipate. In addition, exercise increases metabolic rate, and thus increases the rate

that heat must be dissipated to keep core temperature from increasing to dangerous levels. Climatic heat stress and exercise interact synergistically.

The Wet Bulb Globe Temperature (WBGT) is widely used as a quantitative index of climatic heat stress for use in regulating permitted physical activity level and strategies to minimize the risk of heat injury. WBGT is an empirical index of climatic heat stress but does not quantify physiological strain. It is calculated as: outdoor WBGT = 0.7 natural wet bulb + 0.2 black globe + 0.1 dry bulb, or indoor WBGT = 0.7 natural wet bulb + 0.3 black globe. High WBGT values can be achieved either through high humidity, as reflected in high wet bulb temperature, or through high air (dry bulb) temperature and solar load, as reflected in black globe temperature. While useful, WBGT underestimates the risk of heat injury for humid conditions, and the index was originally developed for predicting resting comfort conditions and does not consider clothing or exercise intensity (metabolic rate), so it cannot predict heat exchange between a person and the climate or the physiological strain of thermoregulation (Sawka and Young, 2000). The National Weather Service uses a similar index, referred to as the Heat Index, which, in theory, provides the temperature sensed by the body, when the ambient temperature and humidity are combined (National Weather Service, 2003). This index, like the WBGT, does not consider the level of physical activity or clothing in estimating strain.

Thermoregulatory Responses to Heat Stress

During exercise, core temperature initially increases rapidly and subsequently increases at a reduced rate until heat loss equals heat production and steady-state values are achieved. The core temperature increase represents the storage of metabolic heat that is produced as a by-product of skeletal muscle contraction. At the beginning of exercise, the metabolic rate increases immediately, while thermoregulatory effector responses that enable heat dissipation respond more slowly, but eventually heat loss increases sufficiently to balance metabolic heat production allowing a new steady-state core temperature to be achieved. Within a range of conditions known as the "prescriptive zone," the magnitude of the increase in core temperature is independent of climatic conditions and proportional to the metabolic rate (Sawka et al., 1996).

Outside the prescriptive zone, the increase in core temperature is no longer independent of ambient conditions (Sawka and Young, 2000). During compensable heat stress, thermoregulatory responses may still dissipate heat at a rate allowing a steady-state core temperature to be maintained, albeit at a higher level than within the prescriptive zone. However, there are biophysical limits to heat exchange between the climate and the body, and the relative contributions of dry and evaporative heat exchange to total heat loss varies with climatic conditions. As ambient temperature increases, the gradient for dry heat exchange diminishes and evaporative heat exchange becomes more important. When the ambient temperature equals or exceeds skin temperature, evaporative heat exchange will account for virtually all heat loss. Evaporation is limited by the vapor pressure of water in air, thus, increasing humidity constrains evaporative heat loss. Uncompensable heat stress occurs when the maximal evaporative cooling capacity of the ambient environment exceeds the amount of evaporative cooling required to dissipate metabolic heat production, and a steady-state core temperature cannot be achieved.

Core temperature provides a reliable physiologic index to predict the incidence of exhaustion from heat strain (Sawka and Young, 2000). **Figure 1** presents the relationships between core temperature and incidence of exhaustion from heat strain for heat- acclimated persons exercising in uncompensable or compensable heat stress. During uncompensable heat stress, exhaustion was rarely associated with a core temperature below 38°C, and exhaustion always occurred before a temperature of 40°C was achieved, whereas during compensable heat stress, there are many reports of individuals whose core temperatures exceed 40°C at exhaustion (Sawka and Young, 2000). For example, Joy and Goodman (1968) reported that 35 of 63 (56%) elite soldiers were still performing military tasks when core temperature reached 39.5°Celcius, and Pugh and colleagues (1967) observed that the core temperature of 7 out of 47 marathon runners exhibited core temperatures >40°C (highest value was 41°C) immediately upon completion of the

race. Thus, increasing core temperatures may be useful for predicting onset of heat exhaustion within a group of individuals, but the relationship between core temperature and time to exhaustion is greatly influenced by the environment (compensable versus uncompensable heat stress) and individual variability due to fitness and other factors.

Other commonly measured physiological responses indicative of thermal strain during heat stress include skin temperature, sweating rate and heat rate. Increases in both skin temperature and sweat rate do occur with increasing heat strain, but both skin temperature and sweat rate vary considerably depending on the site of the body where the measurements are made. Further, the ambient air/water temperature surrounding the body can influence temperature measured at the skin, unless steps are taken to carefully insulate the sensor from the environment. Similarly, sweating rate at a given metabolic rate varies with environmental conditions, fitness, hydration and acclimatization status of the individual. Therefore, while skin temperature and sweat rate are useful measurements for laboratory studies of thermoregulation, these variables are probably of limited value for use as generalized biomarkers for monitoring an individual's heat strain.

Heart rate, on the other hand, is easy to measure and is a useful index of thermal strain. During exercise, metabolic rate and heat production may be ten times their levels at rest, and delivery of heat to the skin to achieve core-to-skin heat transfer sufficient for thermal balance must increase proportionately, in order to reestablish thermal balance. Since skin temperature increases in warmer environments, the core-to-skin temperature gradient becomes relatively narrow in hot environments, and skin blood flow must be rather high to achieve sufficient heat transfer to maintain thermal balance during exercise. During exercise in the heat, the primary cardiovascular challenge is to provide simultaneously enough blood flow to exercising skeletal muscle to support its metabolism, and enough blood flow to the skin to dissipate heat. High skin blood flow often is associated with reduced cardiac filling and stroke volume, which require a higher heart rate to maintain cardiac output. Therefore, elevation of the heart rate response to exercise is an index of the increased cardiovascular strain required for thermoregulation during heat stress. The ease of measuring heart rate makes it a good candidate for monitoring thermal strain during exercise-heat stress.

Metabolic Responses to Heat Stress

Exercise in the heat also reportedly increases plasma or muscle lactate levels, and accelerated muscle glycogenolysis during exercise is sometimes observed suggesting that glycolytic metabolism has been increased (Young, 1990). Whether this metabolic effect reflects Q_{10} effects, reduced oxygenation due to reduced perfusion of metabolically active tissue, reduced hepatic removal of plasma lactate or some combination of those effects remains contentious. However, changes in blood lactate levels too nonspecific to useful as an index of thermal strain.

There is growing evidence in both humans and animals of a role for serotonin (5-HT) accumulation in the brain for the genesis of fatigue from exercise hyperthermia (Cheuvront and Sawka, 2001). Monitoring changes in brain 5-HT levels is not feasible, but peripheral measurements of prolactin (PRL) concentrations are an accepted marker for brain serotonergic activity. The most recent findings indicate that an increase in PRL in response to exercise-heat strain is only observed above a core temperature threshold of 38°C. Thus, while PRL release may provide useful information regarding the development of serotonergic fatigue, the apparent existence of a 38°C temperature threshold for PRL suggests that PRL may be a useful metabolic marker to denote early thermal strain in the heat.

COLD STRAIN

Cold Stress

Humans usually rely on behavioral strategies like wearing clothing or remaining in shelters to protect themselves against the cold. However, the nature of most outdoor winter-time military activities

limits the efficacy of behavioral strategies. When behavioral thermoregulation provides inadequate protection from the cold, physiological responses are elicited.

When ambient temperature is colder than body temperature, the resulting thermal gradient favors body heat loss. Besides ambient temperature, wind speed, solar radiation and humidity, also influence the heat loss potential. No single cold stress index integrates all these effects with respect to the heat loss potential of the environment, but one, the Wind Chill Index (WCI) , has achieved widespread acceptance and use. The WCI estimates the environmental cooling rate from the combined effects of the wind and air temperature. Lacking any better tool for quantifying cold stress, these tables are useful to help guide decisions concerning the conduct or cancellation of outdoor activities, but the computational formula for the WCI probably overestimates the risk of tissue freezing as wind speed increases while underestimating the effect of decreasing air temperature. Further, the WCI estimates the risk of tissue freezing only for the exposed skin of sedentary persons, and wearing windproof clothing greatly reduces wind chill effects.

Water has a much higher thermal capacity than air, and the cooling power of the ambient environment is greatly enhanced under cold-wet conditions. During water immersion, conductive and convective heat transfer can be 70-fold greater than in air of the same temperature, depending on the water depth or body surface immersed in the water, and the individual's metabolic rate. Thus, even when water temperatures are relatively mild, persons swimming, wading streams, swamps or through surf can lose considerable amounts of body heat. Furthermore, when clothing becomes wet due to rain or accidental immersion, its insulative value is compromised, and wetting of the skin facilitates heat loss by conduction, convection and evaporation.

Physiological Responses

Since the exposed body surface loses heat faster than it is replaced, skin temperature declines upon exposure to cold. When skin temperature falls below about 35 °C, a peripheral vasoconstriction is elicited, mediated by increased sympathetic nervous activity decreases peripheral blood flow and reduces convective heat transfer between the body's core and shell (skin, subcutaneous fat and skeletal muscle). This effectively increases insulation, retarding heat loss and defending core temperature, but at the expense of a decline in temperature of peripheral tissue that can contribute to the etiology of cold injuries. If tissue temperature falls below 0 °C, freezing tissue injury will ensue, the severity of which will be related to the extent of freezing. Thus, monitoring skin temperature during cold exposure can provide information regarding the likelihood of developing freezing tissue injury.

The vasoconstrictor response to cold is pronounced in the hands and fingers making them particularly susceptible to cold injury and a loss of manual dexterity. In these areas, another vasomotor response, cold-induced vasodilation (CIVD), develops characterized by transient increases in blood flow to the cooled finger to periodically rewarm skin following the initial decline during cold exposure. The CIVD is thought to be beneficial in maintaining dexterity and preventing cold injury, suggesting that by monitoring the presence or absence of such a response during cold exposure might be useful for predicting cold effects, but no clear evidence exists to support that notion.

The other major physiological mechanism elicited during cold exposure is an increased metabolic heat production that helps offset heat losses. Muscle is the principal source of this thermogenic response in humans. Shivering, an involuntary series of rhythmically repeated muscle contractions, may start immediately, or after several minutes of cold exposure, usually beginning in torso muscles, then spreading to the limbs. During muscular contraction, approximately 70% of total energy expended is liberated as heat, certain animals can increase in metabolic heat production by noncontracting tissue in response to cold exposure, e.g., nonshivering thermogenesis, but no clear evidence indicates that humans share this mechanism.

As cold stress becomes more severe, shivering intensity increases and more muscles are recruited to shiver. Oxygen uptake increases as a result of the increasing metabolic requirement of shivering, and the increase in oxygen uptake is related to the intensity of shivering. As mentioned above, heat losses and body cooling are generally more pronounced during cold-water immersion than during exposure to cold

air, and the stimulus for shivering is greater in the water. As a result, whole body oxygen uptake usually increases more during immersion in cold water, often reaching 25–45 percent maximal oxygen uptake or higher, than during exposure to cold air where oxygen uptakes of 15 percent of maximal are more common (Sawka and Young, 2000). This might suggest that measuring oxygen uptake could provide a means to assess shivering intensity, and this is the case for inactive, non-exercising persons. However, muscular contractions associated with exercise also increase heat production, and this heat production can mitigate the need for shivering (see Figure 2).

At low exercise intensities in the cold, metabolic heat production is not high enough to prevent shivering. Thus, oxygen uptake is higher, with the increased oxygen uptake representing the added requirement for shivering activity. As metabolic heat production rises with increasing exercise intensity, core and skin temperatures are maintained warmer and the afferent stimulus for shivering declines causing the shivering-associated component of total oxygen uptake during exercise to also decline. At high intensities, exercise metabolism is high enough to completely prevent shivering, and oxygen uptake during exercise is the same in cold and temperate conditions. The exercise intensity at which metabolic heat production is sufficient to prevent shivering will depend on the severity of cold stress, which, in any given environment, will vary among individuals (see below). As a result, the utility of using oxygen uptake/metabolic rate measurements as an quatitative index of shivering activity is limited. On the other other hand, more direct measurements of muscular contractile activity via actigraphy, accelerometry or even EMG might provide useful quantitative indices of shivering activity.

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Cold exposure also influences metabolism. For example, the increased sympathetic nervous activity that mediates the cold-induced vasoconstrictor response described above also results in a pronounced rise in circulating norepinephrine concentrations. Increased norepinephrine concentrations are thought to promote glycogenolysis and glycolytic metabolism (Young, 1990), and some evidence suggests that glycogenolysis and blood lactate accumulation during light intensity exercise can be higher in the cold than in temperate conditions. The increased glycogen use during low intensity exercise has been attributed to the additional metabolic cost of shivering, but it is also possible that high circulating norepinephrine levels favor a shift in energy substrate metabolism favoring carbohydrate utilization. Unfortunately, a myriad of exercise, environmental and dietary factors can cause norepinephrine and lactate concentrations to increase, and muscle glycogen breakdown to accelerate, so these responses are too nonspecific to provide any useful information about thermal strain during cold exposure.

Factors Modifying Thermoregulatory Responses to Cold

Although measuring skin temperature and shivering activity during cold exposure are feasible, and monitoring skin temperature might provide a means to predict the danger of freezing tissue injury, neither of these indices appear entirely reliable as indices of whole body thermal strain. For example, while blunted shivering contributes to the impaired ability to maintain core temperature during cold exposure observed with exertional fatigue (Young et al., 1998) or prolonged cold exposures (Castellani et al., 1998), there are patterns of cold acclimatization in which shivering responses to cold also become blunted but, simultaneously, other adjustments develop to mitigate heat loss and enhance body heat conservation (Young, 1996). Also, fatter persons shiver less but experience smaller declines in body temperature than lean persons exposed to the same cold conditions because subcutaneous fat provides significant insulation against heat loss in the cold (Gagge and Gonzalez, 1996). Thus, differences in shivering response to cold may not always reflect important differences in thermal strain. Similarly, while the decline in skin temperature during cold exposure does reflect the cold-induced vasoconstrictor response, it is well known that the steady-state skin temperature maintained during exposure to a given cold condition can be influenced by the thickness of subcutaneous fat, fitness level, acclimatization state and level of exercise or activity, not to mention clothing (Gagge and Gonzalez, 1996; Young, 1996). Thus, if only a single parameter is to be monitored to assess overall thermal strain in the cold, core temperature probably provides more meaningful information than measurements of either shivering or skin temperature.

Integrative Approach to Predicting Thermal Strain

Measuring/monitoring any single parameter to reflect thermal strain may be of limited value. To address this limitation, indices that integrate information from several parameters have been developed. For example, the WBGT and the Wind Chill both attempt to combine multiple climatic measurements into a single value reflective of the environmental stress level. Those indices predict the capacity of the environment to induce physiological strain, but not the strain actually experienced. However, Moran et al. (1998, 1999) have described an approach to integrate multiple physiological parameters into a single value reflective of the thermal strain experienced during exposure to heat or cold stress.

Two separate equations, one for use with heat stress and the other for cold stress, have been derived using a similar conceptual basis (Moran et al., 1998; Moran et al., 1999). The equations are constructed to compute the strain value, which can range from 0 (no/little strain) to 10 (very high strain) from the measured values of the physiological input parameters. Both equations assume that core temperature (both absolute and the change from normal, resting level) is of fundamental importance in assessing the strain. Further, the Physiological Strain Index (PSI) equation derived to predict strain in heat stress conditions incorporates a heart rate parameter, because it was assumed that, with heat stress, cardiovascular strain associated with meeting thermoregulatory requirements would contribute to the overall physiological strain. PSI is calculated as follows:

$$\mathbf{PSI = 5(T_{Ct} - T_{C0})/(39.5 - T_{C0}) + 5(HR_t - HR_0)/(180 - HR_0)}$$

Where T_{Ct} and HR_t are simulataneous measurements of core temperature and heart rate at a particular time during the heat stress exposure, and T_{C0} and HR_0 are initial (pre-stress) measurements. The weighting factors for core temperature and heart rate are the same, reflecting the assumption that each contributes equally to the strain. The Cold Strain Index (CSI) derived to predict physiological strain during exposure to cold, replaces the heart rate parameter with a skin temperature parameter, since heart rate is little affected by cold, per se, whereas skin temperature does change quickly in response to the environmental stress and is known to provide afferent stimulus for shivering and vasoconstriction. The parameter weighting used in CSI differ from those in PSI, and were chosen to mimic the weightings used to calculate mean body temperature from core and skin temperature (Pandolf and Moran, 2002). Thus, CSI is calculated as:

$$CSI = 6.67(T_{Ct} - T_{C0})/(35 - T_{C0}) + 3.33(T_{SKt} - T_{SK0})/(20 - T_{SK0})$$

where, again, T_{Ct} and T_{SKt} are the simultaneously measured values for core and skin temperature at a particular time during cold exposure, and T_{C0} and T_{SK0} are the initial (pre-stress) values.

Moran evaluated the PSI values calculated using databases from six independent experimental studies in which human volunteers experienced exercise/heat stress, and reported that the PSI very adequately reflects the heat strain experienced for different climatic conditions, clothing ensembles, hydration states, exercise intensities and between subjects of differing ages and genders (Pandolf and Moran, 2002). A similar approach to evaluate CSI calculated using databases from three independent experimental studies in which human volunteers were exposed to different cold air or cold water immersion conditions also indicated that CSI effectively depicted cold strain (Pandolf and Moran, 2002), but the authors acknowledged that the evaluation of CSI needed to consider a wider range of ambient conditions. Further development of CSI appears necessary to consider the effects of exercise on the calculated strain value (Castellani et al., 2001).

SUMMARY

Climatic heat stress and exercise interact synergistically, and may strain physiologic systems to their limits, impairing performance and increasing heat injury susceptibility. Heat stress increases requirements for sweating and circulatory responses to dissipate body heat, and these physiological adjustments combined with rising body temperatures may have metabolic effects. Core body temperature

and heart rate are considered reliable physiological parameters for monitoring heat strain, while monitoring skin temperature and sweat rates probably provide less important information due to the wide variability in those responses. The possibility that changes in peripheral metabolites such as circulating prolactin levels may provide information about central nervous system heat strain remain to be definitively examined.

In the cold, the ability to maintain body heat balance and normal body temperatures will depend primarily on the severity of climatic cold stress and clothing insulation, and to a lesser extent on the influence of physiological responses. Exposure to cold elicits shivering thermogenesis, but the response to a given environment varies widely among individuals, depending on their clothing, acclimatization, activity level and body composition. Thus, monitoring the intensity of shivering may not provide useful information regarding cold strain being experienced by individuals exposed to cold. Cold-induced vasoconstriction decreases blood flow to peripheral tissues, favoring conservation of body heat at the expense of a decline in skin temperature and increased susceptibility to cold injury, thus monitoring skin temperature, particularly in unprotected skin regions exposed to cold or areas receiving poor circulation can provide prediction regarding development of freezing tissue injury. Changes in core temperature provide a reliable index of whole-body cooling and cold strain experienced by individuals, and reduced core temperature can degrade the ability to achieve maximal metabolic rates and submaximal endurance performance.

Possibly, no one parameter can provide a complete assessment of thermal strain under all conditions. Information from multiple physiological parameters is likely to be the best approach to quantitatively assessing thermal strain to predict injury or performance degradation. More research is needed to identify the most appropriate parameters to assess physiological strain during exposure to heat and cold strain, and to formulate the appropriate weighting and calculations to integrate the information from these multiple inputs.

DISCLAIMER

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TABLE 1 Core Temperature Measures

Site	Advantage	Disadvantage
Esophageal	Accurate, rapid response	Uncomfortable, affected by swallowing
Rectal	**Accurate, measurement ease**	Slow response, uncomfortable, cultural objections
Auditory Canal - Tympanic Membrane	Measurement ease	Inaccurate (biased by skin and ambient temperature), uncomfortable
Oral	Measurement ease	Inaccurate (affected by mouth breathing)
"Pill"	Accurate, measurement ease	Pill movement influences measurement, signal "cross talk" between subjects in close proximity

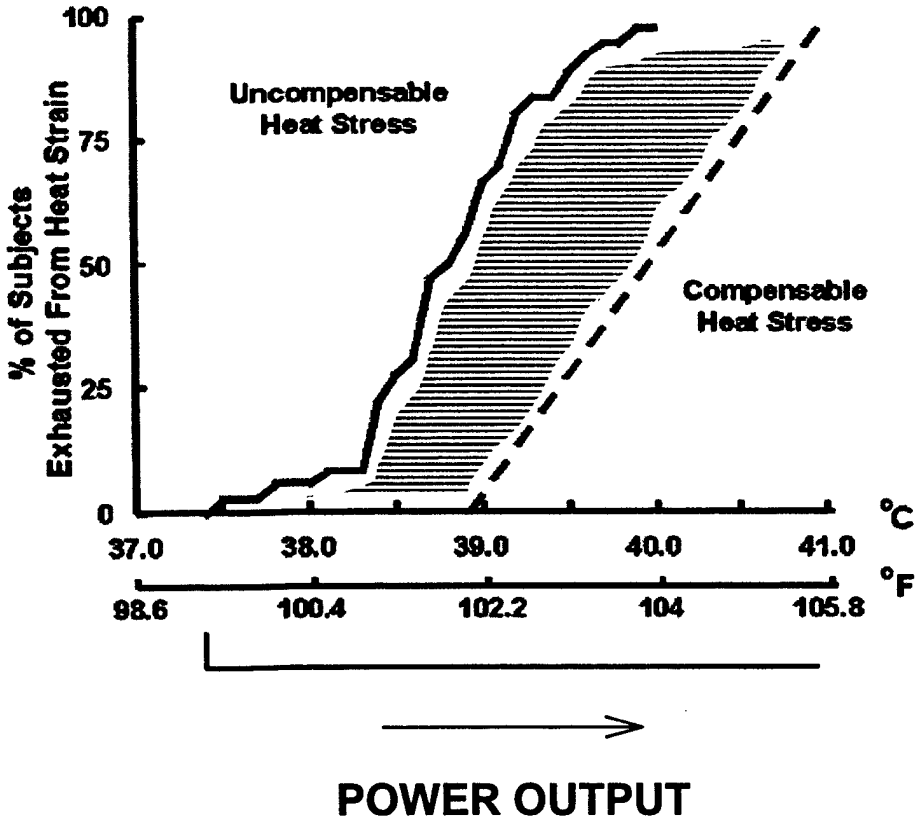


FIGURE 1 Relationships between core temperature and incidence of exhaustion from heat strain

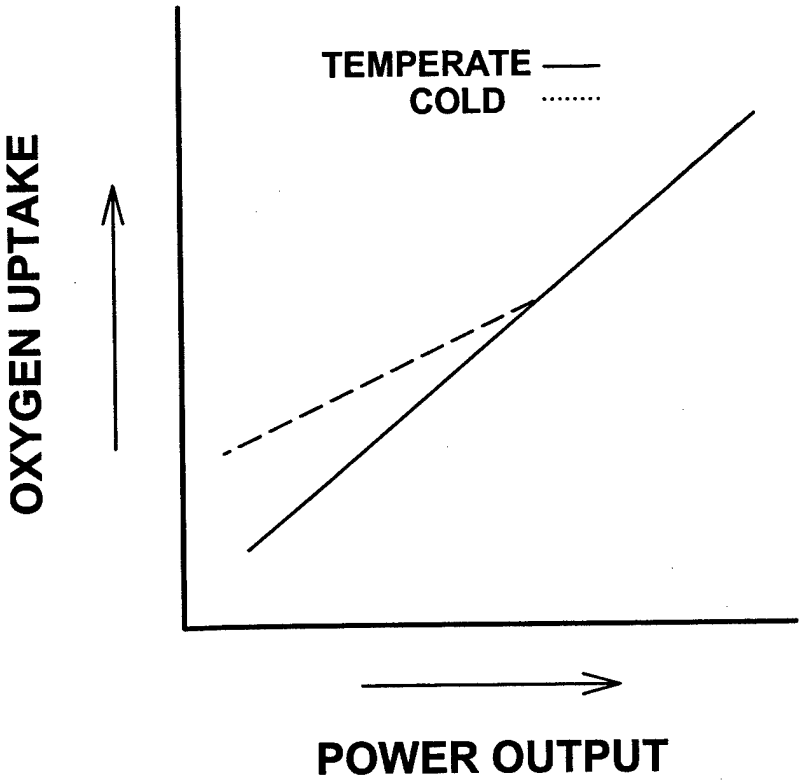


FIGURE 2 Effect of cold-induced shivering on oxygen uptake during exercise at different intensities.

CAFFEINE FOR THE SUSTAINMENT OF MENTAL TASK PERFORMANCE

Formulations for Military Operations



INSTITUTE OF MEDICINE

Caffeine for the Sustainment of Mental Task Performance

Formulations for Military Operations

Committee on Military Nutrition Research

Food and Nutrition Board

INSTITUTE OF MEDICINE

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Knowing is not enough; we must apply.
Willing is not enough; we must do.
—Goethe



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Dedication

The Committee on Military Nutrition Research dedicates this report to the late Gail Butterfield, a diligent and enthusiastic member of the committee who made invaluable contributions to this study and numerous other studies during her six years of service. Her unique background in nutrition and physiology was a special asset to the committee's work. She was dedicated to contributing to the health and nutritional well-being of America's military personnel, both active-duty members and veterans.

Reviewers

This report has been reviewed in draft form by individuals chosen for their diverse perspectives and technical expertise, in accordance with procedures approved by the National Research Council's Report Review Committee. The purpose of this independent review is to provide candid and critical comments that will assist the institution in making its published report as sound as possible and to ensure that the report meets institutional standards for objectivity, evidence, and responsiveness to the study charge. The review comments and draft manuscript remain confidential to protect the integrity of the deliberative process. We wish to thank the following individuals for their review of this report:

E. Wayne Askew, University of Utah
Fergus M. Clydesdale, University of Massachusetts
Joseph T. Coyle, Harvard Medical School
David Dinges, University of Pennsylvania School of Medicine
Harold Goforth, Point Loma College
Steven R. Hursh, Science Applications International Corporation

Although the reviewers listed above have provided many constructive comments and suggestions, they were not asked to endorse the conclusions or recommendations nor did they see the final draft of the report before its release. The review of this report was overseen by Catherine E. Woteki, University of Maryland at College Park, appointed by the Institute of Medicine, who was responsible for making certain that an independent examination of this report was carried out in accordance with institutional procedures and that all review comments were carefully considered. Responsibility for the final content of this report rests entirely with the authoring committee and the institution.

Preface

This publication is the latest in a series of reports prepared by the Committee on Military Nutrition Research (CMNR) of the Food and Nutrition Board (FNB), Institute of Medicine, National Academies. Other reports in the series have included such issues as food components to enhance performance; nutritional needs in hot, cold, and high-altitude environments; body composition and physical performance; nutrition and physical performance; cognitive testing methodology; fluid replacement and heat stress; and antioxidants and oxidative stress. These reports are part of the response that the CMNR provides to the commander of the U.S. Army Medical Research and Materiel Command (USAMRMC) regarding issues brought to the committee by the Military Operational Medicine Research Program at Fort Detrick, Maryland, and the Military Nutrition Division of the U.S. Army Research Institute of Environmental Medicine at Natick, Massachusetts. Typically, reports in this series review the scientific background of an issue, and provide direct responses to questions posed by USAMRMC and specific recommendations from CMNR.

HISTORY OF THE COMMITTEE

The CMNR was established in October 1982, following a request by the assistant surgeon general of the Army that the Board on Military Supplies of the National Academy of Sciences set up a special committee. The purpose of this committee was to advise the U.S. Department of Defense on the need for and conduct of nutrition research and related issues. The CMNR was transferred to the FNB in 1983. The CMNR's current tasks are as follows:

- to identify nutritional factors that may critically influence the physical and mental performance of military personnel under all environmental extremes;
- to identify deficiencies in the existing database;

- to recommend research to remedy these deficiencies as well as approaches for studying the relationship of diet to physical and mental performance; and
- to review and advise on standards for military feeding systems.

Within this context, the CMNR was asked to focus on nutrient requirements for performance during operational missions rather than requirements for military personnel in garrison (the latter were judged to be not significantly different from those of the civilian population).

Although the membership of the committee has changed periodically, the disciplines represented consistently have included human nutrition, nutritional biochemistry, performance physiology, food science, and psychology. For issues that require broader expertise than exists within the committee, the CMNR has convened workshops or utilized consultants. The workshops provide additional state-of-the-art scientific information and informed opinion for the consideration of the committee.

ORGANIZATION OF THIS REPORT

Chapter 1 of this report provides background information on the military interest in caffeine and the history of its use, and Chapter 2 briefly reviews caffeine metabolism and pharmacology. Chapters 3 through 6 review the recent scientific literature organized around the Army's task questions of efficacy, safety, formulations, dosage, ethical considerations, and alternatives. The CMNR's summary responses to questions, conclusions, and recommendations are presented in Chapter 7. The workshop agenda and abstracts are presented in Appendix A. Appendix B contains CMNR recommendations concerning caffeine from the report, *Food Components to Enhance Performance* (IOM, 1994). Biographical sketches of CMNR members and the workshop speakers are given in Appendix C. Speakers invited to the workshop were also requested to submit a brief list of selected background papers. Their recommended readings, relevant citations collected by CMNR staff prior to the workshop, and citations from each chapter are included in the references.

ACKNOWLEDGMENTS

It is my pleasure as chairman of the CMNR to acknowledge the contributions of the FNB staff. Their dedication in the planning and organization of the workshop and in editing this report made it possible for the committee to provide an in-depth response to the Army's request. In particular, I wish to acknowledge the superior efforts of Mary I. Poos, the staff officer for the CMNR. She worked diligently with committee members in securing the expert panel of speakers and organizing the program for the workshop into coherent sessions.

She also conducted extensive reviews and summaries of the scientific literature and performed major edits of the report to ensure clarity and accuracy.

I also wish to commend the workshop speakers for their excellent contributions in preparing abstracts and participating through their presentations and discussions at the workshop. Their willingness to take time from very busy schedules to prepare and deliver outstanding presentations made it possible for the committee to conduct the review and prepare this report. Their thoughtful responses to CMNR members' and workshop participants' questions also contributed immeasurably to the quality of the review. It would be neglectful not to mention the many experts who attended this open meeting at their own initiative and expense. Their questions and comments contributed in no small measure to broadening the exchange of scientific information.

I express my deepest appreciation to the members of the CMNR who participated extensively during the workshop and in discussions and preparation of the summary and recommendations in this report. I continue to be stimulated by the committee's dedication and willing contribution of time and expertise to the activities of the CMNR. I thank all of you for your continuing contributions to this program.

JOHN E. VANDERVEEN, *Chair*
Committee on Military Nutrition Research

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Executive Summary

The goal of any employer, regardless of the field of endeavor, is optimal job performance without compromising the health and well-being of the worker. Intermittent or prolonged physiological and psychological stressors that employees bring to the workplace have an impact not only on their own performance but also on those with whom they work and interact. These stressors are compounded by the physical and mental stressors of the job itself. Military personnel in combat settings endure highly unpredictable timing and types of stressors, both personal and job-related, as well as situations that require continuing vigilance for extended periods of time.

Changes in military operations over the last 50 years have required continued assessment and adoption of technologies that will sustain or enhance physical and cognitive performance of the individual service member. This urgency in maintaining and enhancing performance is fostered by increased reliance on the individual's cognitive skills in the operation and maintenance of complex military equipment in an increasing variety of environmental conditions. Today's military relies heavily on the use of computer-controlled systems that require highly trained and alert individuals. There is also greater reliance on rapid mobility to enable deployment at any time to achieve the nation's military objectives. The urgency to maintain and enhance performance is driven by personnel reductions and shortfalls in recruitment goals—resulting in the need to have the individual perform for longer periods of time with less sleep, shorter transition times, less recovery time between missions, and less reliance on traditional logistical support.

These scenarios can have severe impacts on the individual's level of fatigue, alertness, response time, mood, judgment, reliability in decision making, and other cognitive skills. Increased likelihood of decrements in cognitive function coincides with greater dependence on the individual's performance, and both have a profound impact on the success or failure of a mission.

BACKGROUND

At the request of the U.S. Army Medical Research and Materiel Command (USAMRMC) in 1992, the Committee on Military Nutrition Research (CMNR) of the Institute of Medicine's Food and Nutrition Board reviewed the scientific literature, held a workshop, and produced a report on *Food Components to Enhance Performance* (IOM, 1994). In that report the CMNR recommended that the military pursue additional research on the mechanisms of effects of caffeine on cognitive performance, mood, and alertness, focusing on maximizing positive effects when performance is already degraded.

Specifically, the committee recommended:

> Caffeine definitely should be considered in developing performance-enhancing rations or ration components. Caffeine is safe as a component of food at doses required to overcome sleep deprivation and has been included in diets in coffee and many soft drinks. Since many soldiers may not normally drink coffee, a mechanism for including caffeine in another ration component—that can be selectively used when the situation requires—should be evaluated. It appears that doses of 300–600 mg/70 kg person will achieve the desired stimulus in those not habituated to caffeine; additional research needs to be conducted to determine the effects of this level of caffeine in those with higher habitual intakes. (IOM, 1994, p. 50)

THE COMMITTEE'S TASK

Recent surveys indicate that more than 90 percent of the military population consumes caffeine at some level on a daily basis. Typically, older personnel consume more caffeine than younger ones, and males consume slightly more than females. The majority of caffeine (approximately 70 percent) is consumed as coffee, 23 percent as soda, 5 percent as tea, and slightly less than 2 percent as chocolate, with the remainder coming from medications. The variety of amounts and sources of caffeine consumed confounds the ability to determine risk and to make risk management decisions on the use of caffeine for maintenance and enhancement of cognitive performance in military operations. Thus, the military requested the CMNR's assistance in the decision-making process. The request for this review of caffeine's effects on mental performance and its application to

military operations originated with Army scientists from the U.S. Army Research Institute of Environmental Medicine and USAMRMC. In October 1998, a subgroup of the CMNR participated in a series of conference calls with USAMRMC and CMNR staff to identify the key areas that should be reviewed and to solicit suggestions for the names of scientists who were active in the research fields of interest.

On February 2–3, 1999, the CMNR convened a workshop in response to a request from Army representatives to provide information on the safety, efficacy, and appropriate doses and formulations of caffeine for transition to field application during military operations. The purpose of the workshop was twofold: first, to evaluate the relevant caffeine research completed since the 1992 CMNR workshop "Food Components to Enhance Performance", particularly research conducted by the military on the ability of caffeine to counteract mental task performance deficits engendered by sleep deprivation, and, second, to review military research on the pharmacokinetics and effectiveness of caffeine-supplemented food bars versus caffeinated chewing gum, and assist the Department of Defense (DOD) in the transition of this research to military application.

The USAMRMC provided specific information and questions for the committee's response. These are included in the later section, Response to Military Questions.

METHODS

One purpose of the study was to organize a workshop to review the scientific data on the efficacy of caffeine in maintaining physical and cognitive performance in military operations, caffeine safety, appropriate formulations for administration during military operations, and to identify any ethical or other considerations. Another purpose was to review the effectiveness of caffeine compared to other compounds that have central nervous system-stimulating effects.

The research presented in this report addresses these issues. Information from the speaker presentations and the published scientific literature, as well as the deliberations of the CMNR, were used in the preparation of this report.

NOTE: It is important to emphasize that the responses to the questions and recommendations in this report are specific to military operations and are not necessarily applicable to the needs of the civilian population. Mental alertness and vigilance in situations of sleep deprivation may be necessary during military operations in order to achieve mission objectives. In the civilian environment, taking large doses of caffeine to offset lack of sleep, especially in situations where public safety and health could potentially be compromised, cannot be justified.

CAFFEINE USE

Caffeine (1,3,7-trimethylxanthine) and the related methylxanthines theobromine (3,7-dimethylxanthine) and theophylline (1,3-dimethylxanthine) are alkaloid compounds widely distributed in plants throughout the world. More than 60 different plant species containing caffeine have been identified. The primary sources of these compounds are coffee (*Caffea arabica*), kola nuts (*Cola acuminata*), tea (*Thea sinensis*), and chocolate (*Cocoa* bean).

Caffeine is the most widely consumed psychoactive or central nervous system stimulant in the world. In addition to its natural occurrence in some foods, caffeine is used as a food additive and as a drug or a component of many pharmaceutical preparations. When administered in the amounts commonly found in foods, beverages, and drugs, it has measurable effects on certain types of human performance.

As a food additive, caffeine is generally considered safe based on a long history of use and on extensive research conducted over more than a century throughout the world. However, despite this long history of use, modern epidemiological techniques have raised concerns about associations between continued use of high levels of caffeine and long-term health.

Amounts of caffeine in commonly used beverages and other products vary a great deal (Table S-1) from as low as 2 mg/8 oz of chocolate milk, to as much as 300 mg/6 oz of strong espresso coffee.

Caffeine intakes in the United States have been estimated based on the available product usage and food consumption data. Mean per capita caffeine intake for all U.S. adults was approximately 3 mg/kg body weight (BW) (equivalent to 180–210 mg for a 60–70-kg person). Mean daily intake for adult consumers of caffeine products was 4 mg/kg BW, and for the ninetieth percentile of caffeine users, intakes approximated 5–7 mg/kg BW.

CAFFEINE METABOLISM

Pharmacology

Caffeine is rapidly and completely absorbed in humans, with 99 percent being absorbed within 45 minutes of ingestion. Peak plasma concentrations occur between 15 and 120 minutes after oral ingestion, and may be influenced by route of administration, the form of administration, or other components of the diet. Once caffeine is absorbed, it is distributed rapidly throughout body water. However, caffeine is also sufficiently lipophilic to pass through all biological membranes and readily crosses the blood–brain barrier. The mean half-life of caffeine in plasma of healthy individuals is about 5 hours, although its half-life may range between 1.5 and 9.5 hours. This wide variation in reported half-life may

TABLE S-1 Caffeine Content of Some Common U.S. Food Products

Item	Average (mg)	Range (mg)
Coffee (5-oz cup)[a]		
Brewed, drip method	120	90–150
Percolated	90	64–124
Instant	75	30–120
Decaffeinated	3	1–5
Espresso (6-oz cup)	240	180–300
Teas (loose or bags, 5-oz cup)[a]		
1-minute brew	21	9–33
3-minute brew	33	20–46
Tea products		
Instant (5-oz cup)	20	12–28
Iced (12-oz glass)	29	22–36
Carbonated beverages	24	20–40
Colas and pepper drinks (12 oz)		
National brands, packaged	42	36–48
National brands, fountain	39	32–48
Store brands, packaged	18	5–29
Citrus drinks (12 oz)		
National brands, packaged	52	43–56
Store brands, packaged	38	26–52
Chocolate products		
Cocoa beverage (8 oz)	6	3–32
Chocolate milk beverage (8 oz)	5	2–7
Milk chocolate (1 oz)	6	1–15
Dark chocolate, semisweet (1 oz)	20	5–35
Baker's chocolate (1 oz)	35	35
Chocolate-flavored syrup (1 oz)	4	4

[a] Note these caffeine amounts are based on a 5-oz cup of beverage. Servings today are more likely to be 8 or 12 oz and caffeine intake should be calculated accordingly.

SOURCE: Adapted from FDA (1980a); Grand and Bell (1997); IFT (1983); Lieberman (1992).

be due to individual variation in excretion rates, or whether the individual smokes (decreases half-life) or uses oral contraceptives (increases half-life).

The pharmacological effects of caffeine (similar to those of other methylxanthines) include mild stimulation and wakefulness, ability to sustain intellectual activity, and decreased reaction times. The fatal acute oral dose of caffeine in humans is estimated to be between 10 and 14 g (150–200 mg/kg). Ingestion of caffeine in doses up to 10 g has caused convulsions and vomiting, with complete recovery in 6 hours. Side effects have also been observed in humans at caffeine intakes of 1 g (15 mg/kg), progressing from mild effects including

restlessness, nervousness, and irritability, to more serious effects such as delirium, emesis, neuromuscular tremors, and convulsions.

Physiology

Physiological effects of caffeine include cardiovascular, respiratory, renal, and smooth muscle effects, as well as effects on mood, memory, alertness, and physical and cognitive performance. Caffeine's effect on cognitive function appears to be mediated via several mechanisms: the antagonism of adenosine receptors, the inhibition of phosphodiesterases, the release of calcium from intracellular stores, and antagonism of benzodiazepine receptors. Caffeine's action in blocking adenosine receptors and inhibiting phosphodiesterase appears to be the most important mechanism of action with respect to physiological and behavioral effects.

RESPONSE TO MILITARY QUESTIONS

1. Efficacy: Does CMNR stand by its earlier recommendation that there are sufficient data to recommend a caffeine product to enhance performance? What are the specific indications for use and contraindications for use?

Military personnel face many situations in which extended wakefulness may be required including sentry duty, deployment-related activities, air transportation during emergencies, radar and sonar monitoring, submarine duty, and combat. As part of their duties in these situations, individuals may have to perform complex cognitive tasks. The performance of these tasks is compromised during periods of extended wakefulness.

Caffeine has been shown to induce a variety of positive effects that have contributed to its extensive use worldwide. Caffeine use has been associated with enhanced physical performance, increased alertness, and a countermeasure to the effects of sleep deprivation. Extensive research has been done on each of these effects.

Caffeine use is associated with a reproducible increase in endurance time in physical activities of moderate intensity and long duration with doses of 2–9 mg/kg, in both naive and habituated, trained and untrained test subjects. High-altitude exposure may augment the positive effects of caffeine on endurance performance. Exercise performance is dramatically reduced by altitude exposure, and maximal effort may be diminished by as much as 25 percent. Ingestion of caffeine (4 mg/kg) increased the time to exhaustion at 4,300 m, but not at sea level. This effect was present even after 2 weeks of acclimatization.

Although there is some debate about whether caffeine enhances cognitive performance or simply restores degraded psychomotor performance in rested

individuals, a number of studies have demonstrated that caffeine enhances cognitive performance independent of its ability to reverse symptoms of withdrawal and sleep deprivation. Caffeine enhanced accuracy and reduced reaction time on auditory and visual vigilance tasks in a dose-related manner. Moreover, caffeine significantly increased self-reports of vigor and decreased reports of fatigue, depression, and hostility on the Profile of Moods Scale. In a simulated military situation involving a tedious task that required sustained attention for proficient performance (i.e., sentry duty), caffeine eliminated the vigilance decrement that occurred with increasing time on duty, reduced subjective reports of tiredness, and did not impair rifle firing accuracy. Caffeine also increased the number of correct target identifications in both males and females.

Conclusions

Although there is considerable variation in both the doses tested and subjects' responses to the effects of caffeine on cognitive function, overall research shows that caffeine in the range of 100 to 600 mg is effective in increasing the speed of reaction time without affecting accuracy and in improving performance on visual and audio vigilance tasks. A number of studies have also reported improved performance on long-term memory recall, but not short-term word recall. These enhancing effects of caffeine on cognitive performance are most pronounced when functions are impaired or suboptimal (e.g., as a result of sleep deprivation).

Furthermore, caffeine in amounts ranging from 200 to 600 mg/d enhances endurance performance in a variety of activities. Limited research has shown caffeine to be especially useful in restoring decrements in physical performance that occur at high altitudes. Food and fluid intake must be monitored carefully when caffeine is used for this purpose, since they are frequently suboptimal in operational situations, especially in extremes of hot and cold environments and at altitude.

Recommendations

Caffeine in doses of 100–600 mg may be used to maintain cognitive performance, particularly in situations of sleep deprivation. Specifically it can be used in maintaining speed of reactions and visual and auditory vigilance, which in military operations could be a life or death situation.

A similar dose range (200–600 mg) of caffeine is also effective in enhancing physical endurance and may be especially useful in restoring some of the physical endurance lost at high altitude.

2. Safety: What are the medical risks to individuals associated with ready availability of caffeine, including acute health risks, long-term health risks, potential interaction with other drugs or factors specific to military operations, and potential problems of habituation of use?

The effect of caffeine on various aspects of health has been and continues to be an active area of scientific research, in spite of the fact that caffeine has been used by people around the world for more than 1,000 years without apparent ill effects. Extensive research has been done to evaluate the impact of caffeine consumption on the incidence of cardiovascular disease, reproduction and pregnancy outcomes, fluid homeostasis, and osteoporosis. It has been shown that ingestion of very high doses of caffeine can produce undesirable effects on mental function. Additionally, caffeine use has been associated with physical dependence, which may be reflected in performance decrements during withdrawal under some circumstances.

Potential Health Risks

Hypertension

Results summarized in recent reviews suggest that caffeine-naive individuals experience a small increase in blood pressure after acute dosing with caffeine. During chronic administration of caffeine, tolerance appears to develop, and chronic, long-lasting changes in blood pressure are usually not seen in individuals who consume caffeine routinely.

A recent critical review of 30 years of research on the blood pressure effects of coffee and caffeine concluded that the acute pressor effects of caffeine are well documented, but that at present there is no clear epidemiological evidence that caffeine consumption is causally related to hypertension. One potential risk should be noted, however. A number of studies have demonstrated that caffeine consumption produces a transient elevation in blood pressure and that this occurs regardless of whether or not the individual is a habitual user of caffeine. Thus, high caffeine intake may be an additional risk factor for hypertension at the individual level due to long-lasting stress or genetic susceptibility to hypertension.

Heart Disease

In general, controlled clinical attempts to demonstrate effects of caffeine on increasing heart rate or inducing arrhythmia have been unsuccessful. A meta-analysis of 11 prospective, longitudinal cohort studies showed no increased risk of coronary heart disease associated with consumption of up to 6 cups of coffee per day. Thus, increased risk of cardiovascular problems resulting from the use of caffeine supplements by the military would not appear to be of major concern.

Reproduction

Caffeine consumption has been suggested as the cause of numerous negative reproductive outcomes, from shortened menstrual cycles to reduced conception, delayed implantation, spontaneous abortion, premature birth, low infant birthweight, and congenital malformations. As with most other aspects of caffeine consumption, there is a paucity of reliable data concerning the effects of caffeine on reproductive processes.

Recent reviews of human studies suggest that some of the initial reported associations between caffeine and reduced fertility, teratogenicity, and other fetal and maternal effects in humans may be explained by confounding factors such as associated cigarette smoking, alcohol consumption, reporting inaccuracies, and other methodological errors. A recent, well-controlled study using serum paraxanthine levels to quantitate caffeine exposure demonstrated that women who had spontaneous abortions also had significantly higher serum paraxanthine. However, the odds ratio for spontaneous abortion was not significantly increased except in subjects with extremely high paraxanthine levels (> 1,845 ng/mL). The authors concluded that moderate consumption of caffeine was not likely to increase the risk of spontaneous abortion.

Osteoporosis

Caffeine consumption has also been proposed as a risk factor for osteoporosis. In the large number of studies that have been conducted, there appears to be no consistent trend linking caffeine consumption and negative effects on bone mineral density or incidence of fracture. Early studies also indicated a significant effect on acute calcium diuresis; however, subsequent work indicated that this acute phase of excretion was accompanied by a later decrease in excretion of calcium in the urine. Later studies found either no significant effect of caffeine on calcium balance or negative balance only in subjects consuming less than half of the currently recommended intake of calcium.

Fluid Homeostasis

Caffeine is a diuretic and has been found to increase urinary excretion within 1 hour of treatment. Significant increases have been observed in 3-hour urine output as well as in 24-hour urine output as a result of caffeine consumption in amounts of 250 to 642 mg. Currently, data are inconsistent with respect to whether caffeine creates a total body water deficit. The deficit may depend on the amount of caffeine consumed, the individual's history of caffeine use, and the total solute load of any accompanying food or beverage. However, the risk of water deficit may be increased when caffeine is used in situations already known to put personnel at risk of dehydration such as in hot or desert environments (IOM, 1993) or in cold environments (IOM, 1996).

Behavioral Effects

One potential risk of high doses of caffeine, which needs further substantiation, is a dose-related decrement in mental functioning. A number of researchers have found that high doses of caffeine can adversely affect mental performance. Although a relatively low dose of caffeine (250 mg) produced favorable subjective effects (e.g., elation and pleasantness) and enhanced performance on cognitive tasks in healthy volunteers, higher doses (500 mg) led to less favorable subjective reports (e.g., tension, nervousness, anxiety, restlessness) and less improvement in cognitive performance than placebo. Negative effects may be more pronounced in nonusers than in regular users of caffeine. Excessive intake of caffeine (caffeinism) may be mistaken for anxiety disorder.

Physical Dependence and Withdrawal

The use of caffeine by humans is generally not associated with abuse or addiction. Tolerance develops to some of the effects of caffeine when caffeine-containing beverages are consumed regularly. Withdrawal symptoms often occur with the abrupt removal of caffeine from the diet. The frequency of occurrence of withdrawal varies anywhere from 4 to 100 percent. The symptoms of cessation, when they do occur, are not long-lasting and are generally mild. These include headaches, drowsiness, irritability, fatigue, low vigor, and flu-like symptoms. This withdrawal phenomenon could conceivably lead to decrements in performance during military operations.

Caffeine and Stress

Among the variables that may contribute to differences in caffeine sensitivity are baseline levels of stressor exposure and genetically mediated stress reactivity. Stress may include physical stressors (e.g., exercise) physiological stressors (e.g., heat stress, infection, sleep deprivation), or psychological stressors. After stressor exposures, stress-responsive neurohormonal and neurotransmitter systems are activated. Caffeine alters the degree of responsiveness of these stress-responsive systems to stressful stimuli. The degree to which responsiveness is altered varies according to previous caffeine consumption (habitual users versus nonusers).

Conclusions

The acute pressor effects of caffeine are well documented, but at present there is no clear epidemiological evidence that caffeine consumption is causally related to hypertension. However, high caffeine intake may be an additional risk factor for hypertension at the individual level. In borderline-hypertensive men,

the use of caffeine in situations of behavioral stress may elevate blood pressure to a clinically meaningful degree.

Since military scenarios in which use of caffeine supplements might be desirable would frequently occur when personnel are also under acute mental and/or physical stress, this could be a concern to personnel with family histories of hypertension.

Increased risk of cardiovascular problems resulting from the use of caffeine supplements by the military would not appear to be of major concern.

Results of studies of the effects of caffeine on reproduction have been very mixed, and many studies showing increased reproductive problems have been confounded with other life-style factors, particularly smoking. The most convincing evidence relates to caffeine and the small increased risk of spontaneous abortion. However, since this requires caffeine consumption during the first trimester of pregnancy, it is unlikely to be a major concern for sustained military operations.

The preponderance of research on caffeine and osteoporosis has found no effect. Although caffeine can increase calcium diuresis, this is compensated by lower than normal calcium excretion later. The use of caffeine in this case is less of a concern than is low calcium intake.

Caffeine may increase risk of dehydration which may be an issue for military personnel in operational environments where dehydration may already be a concern, such as desert environments, or where thirst mechanisms are inadequate such as in cold or high-altitude environments.

High doses of caffeine (> 600 mg) can cause decrements in cognitive function. Caffeine can also potentiate the effects of stress.

Recommendations

Use of caffeine under conditions of sustained military operations would not appear to pose any serious, irreversible acute or chronic health risks for military personnel in situations where increased doses might be recommended.

Caffeine use in sustained operations in hot or cold environments or at high altitudes may increase the risk of dehydration, so fluid and food intake of personnel should be closely monitored in these situations.

Female military personnel should be advised of the potential for a small increased risk of spontaneous abortion in the first trimester of pregnancy.

3. Dose and Warning Labels: What dose level should be recommended to habituated caffeine users and to nonusers? What warnings should be provided on such a product in the context of ethical, religious, and potential caffeine habituation concerns?

The effective dose of caffeine will vary from individual to individual, depending on a variety of factors including time of day, usual caffeine intake, and

whether the individual is rested or fatigued. Levels of caffeine in the range of 100 to 400 mg have consistently demonstrated reductions in reaction time and enhanced performance on vigilance tests without adverse effects. In some studies with rested subjects, levels of caffeine in excess of 500 mg in a single dose have shown negative effects on mood and behavior (this may be more likely in those who do not normally consume caffeine). The levels of caffeine that have consistently enhanced physical endurance in humans range from about 200 to 600 mg.

In sleep-deprived individuals, similar to those engaging in sustained operations, a range of 100 to 600 mg of caffeine appears to improve performance (e.g., vigilance, mood, higher cognitive functions) with few acute adverse behavioral effects.

Important ethical considerations for requiring the use of caffeine during sustained operations include: providing personnel with adequate information on use of the product, development of a product that would allow individual control of the dosage, and provision of information on potential negative effects that may be experienced if higher than recommended amounts are consumed.

Individuals who are regular moderate to heavy users of caffeine may experience headaches, fatigue, and other adverse effects if denied access to caffeine in anticipation of the later need to use a supplement.

Conclusions

A caffeine dose of 100–600 mg can be expected to improve vigilance and enhance cognitive performance. A delivery mechanism that provides caffeine in 100-mg increments could be used to allow individuals of smaller body size, nonhabituated caffeine users, and those with a heightened sensitivity to caffeine to use the product.

The recommended dosing interval should take into consideration that too-frequent dosing might produce a build-up of caffeine and its metabolite paraxanthine sufficient to precipitate negative effects, or inhibit sleep onset in some individuals when sleep is desired.

The committee concludes that there is no specific need to include warning or cautionary statements on the product labels, and the dosage recommended is well within the range of caffeine consumption in the general population.

Recommendations

A caffeine delivery vehicle that provides caffeine in 100-mg increments with a total content not exceeding approximately 600 mg would appear to be the most appropriate dose for use in sustained military operations. No differential dosing is recommended for habitual and first-time caffeine users. However, a single dose should not exceed 600 mg, and 400 mg may be adequate for rested individuals performing sustained vigilance tasks.

Since the average half-life of caffeine in the blood of adult men given 280 mg (4 mg/kg BW) is between 2.5 and 4.5 hours, a dosing interval of no less than 3–4 hours is suggested.

Any product that is used as a vehicle for providing caffeine to military personnel should be prominently labeled, including a statement on the principal display panel that the product contains added caffeine and should be used only to maintain performance when involved in sustained operations.

The label should also indicate the amount of caffeine per unit of product (or per serving) and instructions for use. This information is vital for commanders to make decisions about directives for use and for personnel to adapt consumption to their individual needs.

An in-depth training program on the benefits, directions for use, and potential side effects of caffeine should be designed for command personnel. Military personnel should be given adequate training to ensure the benefits of caffeine supplementation and avoid any potential side effects. Such training should include the use of caffeine during periods of sleep deprivation and altered work–rest cycles in nonoperational situations.

Military personnel who are habitual consumers of caffeine should not be restricted from caffeine use in preparation for the need of a caffeine supplement.

4. Alternatives: Are there practical alternatives to caffeine that would better serve the intended purpose of enhancing or maintaining performance in fatigued service members?

Sleep is the most effective means of reconstituting the decrements in cognitive functioning brought on by sleep deprivation. Thus, in situations where it is feasible, sleep should be promoted. There is a dose effect for the restorative effects of sleep duration on cognitive performance. Any amount of sleep from as little as a 15-minute nap can restore some degree of function, although the longer the sleep episode, the greater the amount of cognitive function restored.

Alternatives

Combination of Caffeine and Naps

The most effective nonprescription alternative to caffeine administration alone is a combination of caffeine and naps. In a series of studies, the combination of a short nap and caffeine significantly decreased driving impairment, subjective sleepiness, and drowsiness as measured by electroencephalogram activity. The combination of a nap and caffeine also increased alertness during long periods of sleep deprivation as compared to either caffeine or nap treatments independently.

Amphetamine

Amphetamine has been found to "improve subjective feelings of fatigue, confusion, and depression while increasing feelings of vigor". Amphetamine is, however, a controlled substance and its use would require an individual medical evaluation to determine risk factors and health status before a prescription could be issued. It is possible that with appropriate supervision and control, amphetamine could provide benefits to individuals with unique skills and whose performance is critical to the safety of complex military hardware and personnel.

The potential for abuse of amphetamine is considerable. Appropriate monitoring of its dispensation and use may add unnecessary burdens to personnel involved in the intense and demanding tasks that are directly related to the success of sustained operations (SUSOPS). Although amphetamine (20 mg) was more effective than caffeine at 300 mg in reversing the negative effects on alertness during sleep deprivation, it had deleterious effects on recovery sleep, which also may be important in the ultimate success of demanding and constantly changing SUSOPS. Therefore, considerable caution is warranted, and use of this stimulant should be restricted to circumstances when such measures are considered essential to the success of highly sensitive operations.

Modafinil

Modafinil is a controlled wakefulness-promoting drug developed to counteract excessive daytime sleepiness (EDS) in narcolepsy. This drug appears to be useful in reducing EDS without affecting voluntary naps or nocturnal sleep initiation. These properties suggest that this compound may be useful in extending high levels of vigilance in SUSOPS. The limited research to date on the effects of modafanil in simulated military situations indicates potential for this drug.

Conclusions

Providing the opportunity and environment for adequate sleep would be ideal, but obviously impractical, for continuous military operations. Combining naps with judicious caffeine use may be the best remedy for sleep deprivation-induced decrements in cognitive function in military situations where adequate sleep cannot be obtained. When naps are not an option, caffeine alone could be used.

The use of amphetamine is superior to caffeine in offsetting decrements in cognitive performance; however, the risks outweigh the benefits for most situations. It is a controlled substance, has a high abuse potential, and interferes with recovery sleep. In addition, it is assumed that the majority of combat personnel would not have previous experience with the drug.

The drug modafanil, developed as a treatment for narcolepsy, shows considerable promise. It appears to be as effective as amphetamine in offsetting

performance degradation, does not interfere with initiation of recovery sleep, is not an appetite suppressant, and appears to have a much lower abuse potential.

Recommendations

It is recommended that the military have in place a doctrine related to the importance of sleep prior to extended missions, the importance of naps whenever possible during operations, and the timing of naps for maximum effectiveness.

Of the psychostimulant compounds that have been thoroughly tested, caffeine would be the compound of choice. Many personnel would have personal experience with the compound, it is not a restricted substance, it does not interfere with recovery sleep following periods of sleep deprivation, and it has very low abuse potential.

Additional research should be conducted on the drug modafinil to further explore its potential for sustaining cognitive performance during military operations.

5. Formulation: (a) Does the inclusion of other components (e.g., glucose) improve the beneficial effects of caffeine in sustained operations, as previously suggested by the committee? (b) Is there a better approach to caffeine delivery than the nutrient bar (HOOAH) currently produced for the military?

The evidence of the utility of adding glucose or other components to caffeine to further enhance performance is unclear. Caffeine enhances the availability of free fatty acids and decreases glycogenolysis, whereas carbohydrate increases the availability, and presumably the use, of glucose.

There may be nutritional reasons (e.g., provision of food energy, nutrients, or fluid) for including caffeine in a food or beverage form. Various approaches to caffeine delivery for SUSOPS were considered, including a food/energy bar, caffeinated chewing gum, tablets (both sustained release and regular), and beverages. There is good evidence that caffeine consumed as a liquid is absorbed rapidly and completely from the gut, with virtually all (99 percent) of the administered dose absorbed in about 45 minutes. However, evidence on absorption of caffeine from a food matrix, such as energy bars, was not available.

A caffeine delivery vehicle that is most appropriate in one setting may not be so in another. Caffeine in a food matrix or beverage may be advantageous when it is important to deliver nutrients, fluid, or other food constituents simultaneously. Chewing gums are more appropriate if rapid absorption and action are needed, or weight or bulk is a concern. Caffeine in a fluid matrix or gel may be more appropriate when dehydration is a concern.

Conclusions

Although evidence of a potentiating effect of carbohydrates on caffeine effectiveness is equivocal, there are other reasons to consider providing supplemental nutrients along with the caffeine. Inadequate food and fluid intake is a common problem during military operations.

The use of a caffeinated chewing gum would appear to provide the most rapid absorption. Environmental circumstances and individual characteristics may make one caffeine delivery vehicle appropriate in some circumstances and inappropriate in others.

Recommendations

Definitive research is needed regarding the combined effects of caffeine and carbohydrate on performance since data currently available are inconclusive.

If a food bar or some other solid food matrix is used, the rapidity and extent of caffeine absorption and action must be evaluated.

Under certain circumstances, such as heat stress or desert operations, chewing gums may offer practical operational advantages over a food/energy bar. Thus, two delivery vehicles should be considered. Based on the DOD preference to provide needed supplements to personnel in food form rather than in pill form, a caffeinated chewing gum or a caffeine-supplemented food/energy bar would be suitable delivery vehicles.

1

Basic Concepts

MILITARY INTEREST IN CAFFEINE

Optimal job performance without compromising the health and well-being of the worker is the goal of employers regardless of the field of endeavor. Intermittent or prolonged physiological and psychological stressors that employees bring to the workplace have an impact not only on their own performance but also on those with whom they work and interact. The internal stressors an individual brings to his or her job are compounded by the day-to-day physical and mental stressors of the job itself. Military personnel in combat settings endure highly unpredictable timing and types of stressors as well as situations that require continuing vigilance for long periods of time.

The U.S. military's concerns about the individual war fighter's ability to avoid performance degradation and the need to enhance mental capabilities in highly stressful situations have led to an interest in devising military ration components that could enhance physical and cognitive performance.

Previous Committee on Military Nutrition Research Recommendations

In 1992 the Committee on Military Nutrition Research (CMNR) was asked by the U.S. Army Medical Research and Materiel Command to evaluate the potential of selected amino acids, carbohydrates, structured lipids, choline, carnitine, and caffeine to enhance performance. The committee was asked to address two questions: first, whether the use of diet components or supplements to enhance physical and mental performance in "normal" healthy, young adult

soldiers was a fruitful approach and, second, which food components, if any, would be the best candidates for enhancing military mental and physical performance. In response to this request the committee held a workshop, reviewed the scientific literature, and published the report, *Food Components to Enhance Performance* (IOM, 1994), in which it recommended continued research on the mechanisms of the effects of caffeine on cognitive performance, mood, and alertness. It was noted that particular attention should be paid to maximizing positive effects when performance is already degraded.

Specifically, the committee recommended:

> Caffeine definitely should be considered in developing performance-enhancing rations or ration components. Caffeine is safe as a component of food at doses required to overcome sleep deprivation and has already been included in diets of military personnel via coffee and many soft drinks. Since many soldiers may not normally drink coffee, a mechanism for including caffeine in another ration component that can be selectively used when the situation requires should be evaluated. It appears that doses of 300–600 mg/70 kg person will achieve the desired stimulus in those nonhabituated to caffeine; additional research needs to be conducted to determine the effects of this level of caffeine in those with higher habitual intakes. (IOM, 1994, p. 50)

The Current Situation

Changes in military operations over the last 50 years have forced continued assessment and adoption of technologies that will sustain or enhance physical and cognitive performance of the individual service member. This urgency in maintaining and enhancing performance is fostered by increased reliance on the individual's cognitive skills in the operation and maintenance of complex military equipment in an ever-increasing variety of environmental conditions. Today's military relies heavily on the use of computer-controlled systems that require highly trained and alert operators. In addition, there is greater reliance on rapid mobility to enable deployment at any time to achieve the nation's military objectives. The urgency of maintaining and enhancing performance is also driven by constant pressure, due to personnel reductions, to have the individual perform for longer periods of time with less sleep, shorter transition times, less recovery time between missions, and less reliance on traditional logistical support.

These scenarios can have severe impacts on the individual's level of fatigue, alertness, response time, mood, judgment, reliability in decision making, and other cognitive skills. Increased likelihood of decrements in cognitive function is coupled with greater dependence on each individual in accomplishment of the mission. Both of these factors have a profound impact on the success or failure of a military operation.

In its effort to sustain and enhance the performance of personnel, the military's emphasis should be placed on providing adequate levels of nutrients, water, life support equipment, clothing, and, to the extent possible, sleeping regimens, appropriate rest areas, and work patterns. After these efforts have been put in place, the potential use of dietary supplements and selected pharmaceuticals is an appropriate consideration.

HISTORY OF CAFFEINE USE

In addition to its natural occurrence in some foods, caffeine is used as a food additive and as a drug or a component of many pharmaceutical preparations. It is the most widely consumed psychoactive or central nervous system (CNS) stimulant in the world (Curatolo and Robertson, 1983). When administered in the doses commonly found in beverages and drugs, it has measurable effects on certain types of human performance. It is readily available to both the civilian and the military populations as a beverage (coffee, tea, maté), food (cocoa products), food additive (soft drinks, bottled water), and pharmaceutical (over-the-counter pain and weight-loss medications, numerous prescription drugs). No other substance has this combination of uses.

As a food additive caffeine is generally considered safe based on its long history of use and on extensive research conducted throughout the world for more than a century. However, despite this long history of use, modern epidemiological techniques have raised concerns about associations between continued use of high levels of caffeine and long-term health.

Caffeine (1,3,7-trimethylxanthine) and the related methylxanthines, theobromine (3,7-dimethylxanthine) and theophylline (1,3-dimethylxanthine), are widely distributed in plants throughout the world. More than 60 different plant species containing caffeine have been identified, and history suggests that it may have been consumed, in one form or another, as far back as the Paleolithic period (Barone and Roberts, 1996). The primary sources of these compounds are coffee (*Caffea arabica*), kola nuts (*Cola acuminata*), tea (*Thea sinensis*), and chocolate (*Cocoa* bean). Although the actual discoverer of caffeine as a stimulant is unknown, legend has it that it was first discovered in Ethiopia in the third century AD when a shepherd noticed that his goats became very frisky and agitated after eating coffee berries or "beans". The shepherd tried chewing some of the berries and noted their stimulant properties. An abbot at a nearby monastery brewed the beans in hot water and found that the beverage helped him to stay awake during long nights of prayer. Cultivation of the coffee plant may have begun as early as the sixth century AD, probably in Ethiopia. Elsewhere in Africa, coffee berries were crushed and mixed with fat to serve as a food to stimulate warriors in battle. By approximately 1000 AD, coffee reached Yemen, where the beverage became very popular and drinking it a social ritual among Muslims. From there it spread to Europe and the Americas. All stable indige-

nous cultures having access to caffeine-containing plants have developed drinks or foods containing these stimulant products. The earliest recorded use of caffeine-containing beverages dates back to the Tang Dynasty of China (618–907 AD) where tea was a popular drink believed to prolong life.

Caffeine Content of Common Food Sources

The amount of caffeine in commonly consumed beverages and other products varies a great deal (Table 1-1), from as little as 5 mg/8 oz of chocolate milk, to as much as 300 mg/6 oz of strong espresso coffee. Since early times the adverse effects of very large doses of caffeine, especially in those who are not used to the product, have been noted. The reported signs and symptoms include nervousness, anxiety, insomnia, irregular heartbeats, excess stomach acid, and heartburn (Duke, 1988).

Caffeine Intake of Adults

Based on the available product usage data and food consumption data, Barone and Roberts (1996) estimated caffeine intakes in the United States, United Kingdom, Denmark, and Australia. The per capita daily caffeine intake for all U.S. adults was approximately 3 mg/kg body weight (BW) (for a 60–70-kg person). For adults who actually consumed caffeine products, mean daily intake was 4 mg/kg BW, and for the ninetieth percentile of caffeine users, intakes approximated 5–7 mg/kg BW.

Caffeine intake was higher in the United Kingdom, with per capita daily consumption being 4 mg/kg BW and 7.5 mg/kg BW for the ninetieth percentile of caffeine users. Consumption was highest in Denmark: 7.0 mg/kg BW for all adults and 14.9 mg/kg BW for the ninetieth percentile of caffeine users.

THE COMMITTEE'S TASK

Surveys indicate that more than 90 percent of the military population consumes caffeine at some level on a daily basis. A small-sample survey reported by Lieberman (1999) indicated that mean caffeine intake among military personnel was 340 mg/d. The majority of those sampled consumed 200 mg/d or less; however, consumption levels were highly variable and thus physiological effects cannot be generalized. Typically, older personnel consumed more caffeine than younger ones, and males consumed slightly more than females. The majority of caffeine (about 70 percent) was consumed as coffee, 23 percent as soda, 5 percent as tea, and slightly less than 2 percent as chocolate, with the remainder coming from medications. These factors make it difficult to determine risk and to make risk management decisions on the use of caffeine for maintenance and enhancement of cognitive performance in military operations.

TABLE 1-1 Caffeine Content of Some Common U.S. Food Products

Item	Average (mg)	Range (mg)
Coffee (5-oz cup)[a]		
Brewed, drip method	120	90–150
Percolated	90	64–124
Instant	75	30–120
Decaffeinated	3	1–5
Espresso (6-oz cup)	240	180–300
Teas (loose or bags, 5-oz cup)[a]		
1-minute brew	21	9–33
3-minute brew	33	20–46
Tea products		
Instant (5-oz cup)	20	12–28
Iced (12-oz glass)	29	22–36
Carbonated beverages	24	20–40
Colas and pepper drinks (12 oz)		
National brands, packaged	42	36–48
National brands, fountain	39	32–48
Store brands, packaged	18	5–29
Citrus drinks (12 oz)		
National brands, packaged	52	43–56
Store brands, packaged	38	26–52
Chocolate products		
Cocoa beverage (8 oz)	6	3–32
Chocolate milk beverage (8 oz)	5	2–7
Milk chocolate (1 oz)	6	1–15
Dark chocolate, semisweet (1 oz)	20	5–35
Baker's chocolate (1 oz)	35	35
Chocolate-flavored syrup (1 oz)	4	4

[a] Note these caffeine amounts are based on a 5-oz cup of beverage, servings today are more likely to be 8 or 12 oz and caffeine intake should be calculated accordingly.

SOURCE: Adapted from FDA (1980a); Grand and Bell (1997); IFT (1983); Lieberman (1992).

The military requested the committee's assistance in this decision-making process. The CMNR was requested to evaluate the relevant caffeine research, including all relevant studies performed since the 1992 workshop, and address in a brief report the following proposal and questions to assist the Department of Defense in the transition of research to military application. Specifically, the military provided the following information and questions for the committee's response.

A specific transition opportunity could take the following form: a "HOOAH" food bar (a nutrient-dense energy bar developed by the Army) containing 600 mg of caffeine, scored in 150-mg increments, with labeling that provides specific guidance for use of up to one food bar (600 mg) to offset deficits in cognitive

function and situational awareness produced by inadequate restorative sleep and during military operations at night. The label should also contain warnings, especially for infrequent or noncaffeine users, that no more than one scored segment (150 mg) should be used in the first hour and should be discontinued if undesirable changes in hand steadiness, pulse, and respiration occur. This performance-enhancing ration component could be provided separately or as part of operational rations. Alternatives to be considered include coffee, caffeinated soft drinks, modifications of the HOOAH bar dose, caffeinated chewing gum, caffeine pills, amphetamine pills (dexedrine), and sustained-release caffeine. The intent is to provide a pharmacological/dietary supplement strategy to significantly counter performance deficits in special circumstances when doctrinal and behavioral solutions (adherence to appropriate work–rest cycles, naps, etc.) are not possible or break down. The key questions to be addressed:

1. ***Efficacy:*** Does the committee stand by its earlier recommendation that there are sufficient data to recommend a caffeine product to enhance performance, and what are the specific indications for use (e.g., vigilance activities following inadequate sleep) and contraindications for use (e.g., tasks involving fine motor coordination)?

2. ***Safety:*** What are the medical risks to individuals associated with ready availability of caffeine, including acute health risks (e.g., cardiac arrhythmia, caffeine psychosis), long-term health risks (e.g., hypertension, hypercholesterolemia), potential interactions with other drugs (e.g., ephedra-containing supplements) or factors specific to military operations (e.g., heat stress, stress reactions), and potential problems of habituation of use (e.g., tolerance, caffeine dependence)?

3. ***Dose and warning labels:*** What dose level(s) should be recommended to (a) habituated caffeine users and (b) nonhabituated users? What warnings should be provided on such a product in the context of ethical, religious, and potential caffeine habituation concerns?

4. ***Alternatives:*** Are there practical alternatives to caffeine, which would better serve the intended purpose of enhancing performance in fatigued service members (e.g., amphetamine)?

5. ***Formulation:*** (a) Does the inclusion of other components (e.g., glucose) improve beneficial effects of caffeine in sustained operations (SUSOPS), as previously suggested by the committee? (b) Is there a better approach to caffeine delivery than the HOOAH bar (e.g., is it better to have more rapid absorption and action using caffeinated chewing gum, longer duration of action using sustained-release caffeine products, or pill or beverage formulations)?

A workshop was organized to review the scientific data on the efficacy of caffeine in maintaining physical and cognitive performance in military operations, its safety, and appropriate formulations for administration during military

operations and to identify any ethical or other considerations. Another purpose of this workshop was to compare the effectiveness of caffeine to other pharmaceuticals that have CNS effects.

The research presented at this workshop addressed many of these issues. Information from the speaker presentations and the published scientific literature, as well as the deliberations of the CMNR, were used in the preparation of this report.

NOTE: It is important to emphasize that the responses to the questions and recommendations in this report are specific to military operations and are not necessarily applicable to the needs of the civilian population. In particular, it is recognized that mental alertness and vigilance in situations of sleep deprivation may be necessary during military operations in order to achieve mission objectives. In the civilian environment, taking large doses of caffeine to offset lack of sleep in situations where public safety and health could potentially be compromised, such as in the operation of aircraft, motor vehicles, heavy equipment, delicate life-saving procedures, and the like, is not justified.

2

Pharmacology of Caffeine

As stated in Chapter 1, caffeine is the most widely used central nervous system (CNS) stimulant in the world. It has numerous pharmacological and physiological effects, including cardiovascular, respiratory, renal, and smooth muscle effects, as well as effects on mood, memory, alertness, and physical and cognitive performance. This chapter provides a brief summary of the metabolism and physiological effects of caffeine

Caffeine (1,3,7-trimethylxanthine) is a plant alkaloid with a chemical structure of $C_8H_{10}N_4O_2$ (see Figure 2-1) and a molecular weight of 194.19. In pure form, it is a bitter white powder. Structurally, caffeine (and the other methylxanthines) resembles the purines. The mean half-life of caffeine in plasma of healthy individuals is about 5 hours. However, caffeine's elimination half-life may range between 1.5 and 9.5 hours, while the total plasma clearance rate for caffeine is estimated to be 0.078 L/h/kg (Brachtel and Richter, 1992; Busto et al., 1989). This wide range in the plasma mean half-life of caffeine is due to both innate individual variation, and a variety of physiological and environmental characteristics that influence caffeine metabolism (e.g., pregnancy, obesity, use of oral contraceptives, smoking, altitude). The pharmacological effects of caffeine are similar to those of other methylxanthines (including those found in various teas and chocolates). These effects include mild CNS stimulation and wakefulness, ability to sustain intellectual activity, and decreased reaction times.

The fatal acute oral dose of caffeine in humans is estimated to be 10–14 g (150–200 mg/kg body weight [BW]) (Hodgman, 1998). Ingestion of caffeine in

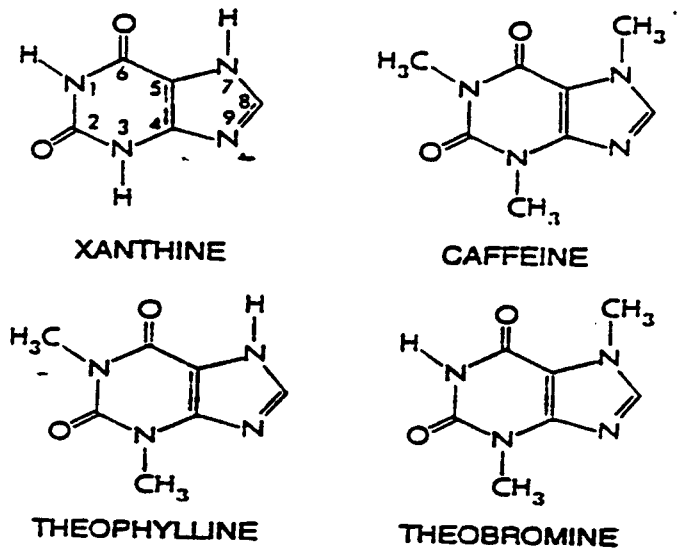


FIGURE 2-1 Chemical structure of methylxanthines.

doses up to 10 g has caused convulsions and vomiting with complete recovery in 6 hours (Dreisbach, 1974). Extreme side effects were observed in humans at caffeine intakes of 1 g (15 mg/kg) (Gilman et al., 1990), including restlessness, nervousness, and irritability, and progressing to delirium, emesis, neuromuscular tremors, and convulsions. Other symptoms included tachycardia and increased respiration.

ABSORPTION, DISTRIBUTION, AND METABOLISM

Caffeine is rapidly and completely absorbed in humans, with 99 percent being absorbed within 45 minutes of ingestion (Bonati et al., 1982; Liguori et al., 1997). When it is consumed in beverages (most commonly coffee, tea, or soft drinks) caffeine is absorbed rapidly from the gastrointestinal tract and distributed throughout body water. More rapid absorption can be achieved by chewing caffeine-containing gum or other preparations that allow absorption through the oral mucosa.

Peak plasma concentrations occur between 15 and 120 minutes after oral ingestion. This wide variation in time may be due to variation in gastric emptying time and the presence of other dietary constituents, such as fiber (Arnaud, 1987). Once caffeine is absorbed, there appears to be no hepatic first-pass effect

(i.e., the liver does not appear to remove caffeine as it passes from the gut to the general circulation), as evidenced by the similarity in plasma concentration curves that follow its administration by either the oral or the intravenous route (Arnaud, 1993). Caffeine binds reversibly to plasma proteins, and protein-bound caffeine accounts for about 10 to 30 percent of the total plasma pool. The distribution volume within the body is 0.7 L/kg, a value suggesting that it is hydrophilic and distributes freely into the intracellular tissue water (Arnaud, 1987, 1993). However, caffeine is also sufficiently lipophilic to pass through all biological membranes and readily crosses the blood–brain barrier. Its elimination is by first-order kinetics and is adequately described by a one-compartment open model system (Bonati et al., 1982). In a study of adult men, a dose of 4 mg/kg (280 mg/70 kg human, or about 2–3 cups of coffee) had a caffeine half-life of 2.5–4.5 hours, and was not affected by age (Arnaud, 1988).

Because caffeine is readily reabsorbed by the renal tubules, once it is filtered by the glomeruli only a small percentage is excreted unchanged in the urine. Its limited appearance in urine indicates that caffeine metabolism is the rate-limiting factor in its plasma clearance (Arnaud, 1993). Caffeine metabolism occurs primarily in the liver, catalyzed by hepatic microsomal enzyme systems (Grant et al., 1987). In healthy humans, repeated caffeine ingestion does not alter its absorption or metabolism (George et al., 1986). It is metabolized in the liver to dimethylxanthines, uric acids, di- and trimethylallantoin, and uracil derivatives. In humans 3-ethyl demethylation to paraxanthine is the primary route of metabolism (Arnaud, 1987). This first metabolic step accounts for approximately 75–80 percent of caffeine metabolism and involves cytochrome P4501A2 (Arnaud, 1993). Paraxanthine is the dominant metabolite in humans, rising in plasma to concentrations 10 times those of theophylline or theobromine. Caffeine is cleared more quickly than paraxanthine, so 8 to 10 hours after caffeine ingestion, paraxanthine levels exceed caffeine levels in plasma (Arnaud, 1993).

The fact that the human body converts 70–80 percent of caffeine into paraxanthine with no apparent toxic effects following caffeine doses of 300–500 mg/day suggests that paraxanthine's toxicological potency is low. Formation of paraxanthine and its excretion in the urine appears to be the major pathway for caffeine metabolism (Stavric, 1988).

Hetzler et al. (1990) demonstrated that lipolytic effects of caffeine may be due to the action of paraxanthine rather than caffeine itself. Increasing concentration of plasma-free fatty acids following intravenous administration of caffeine was negatively correlated to plasma caffeine concentrations, and highly positively correlated to plasma paraxanthine concentrations. Paraxanthine has been found to be an equipotent adenosine antagonist to caffeine in vitro. Benowitz et al. (1995) demonstrated that both caffeine and paraxanthine significantly increased diastolic blood pressure, plasma concentrations of epinephrine, and free fatty acids. Plasma levels of caffeine peaked 75 minutes after oral dosing of caffeine, while plasma levels of paraxanthine peaked at 300 minutes after

an oral dose of paraxanthine. At doses of 4 mg/kg BW, caffeine and paraxanthine were equipotent. At doses of 2 mg/kg BW, however, caffeine was more potent. Benowitz and colleagues (1995) concluded that after a single dose of caffeine, paraxanthine concentrations are relatively low and probably do not contribute much to the effect of caffeine. However, with long-term exposure to caffeine there is a substantial accumulation of paraxanthine, and thus paraxanthine almost certainly contributes to the pharmacologic activity of caffeine. It would be reasonable to expect then, that with long-term caffeine exposure, paraxanthine would also contribute to development of tolerance to caffeine and withdrawal symptoms.

There is likely to be considerable individual variation in the extent of conversion of caffeine to paraxanthine, and because paraxanthine has pharmacologic activity, the extent of conversion would be a factor in determining individual differences in response to caffeine.

FACTORS AFFECTING CAFFEINE METABOLISM

Caffeine metabolism is increased by smoking, an effect mediated by an acceleration in its demethylation (it also increases xanthine oxidase activity) (Parsons and Neims, 1978). Smoking cessation returns caffeine clearance rates to nonsmoking values (Murphy et al., 1988). A number of studies with rodents have demonstrated an additive effect of caffeine and nicotine on both schedule-controlled behavior and locomotor activity (Lee et al., 1987; Sansone et al., 1994; White, 1988). However, data in humans are scarce. Kerr et al. (1991) found both caffeine and nicotine facilitated memory and motor function in a variety of psychomotor tasks. Though there were differences across tasks, combining caffeine and nicotine did not appear to produce a greater effect than either drug alone. Conversely, nicotine did not decrease the effectiveness of caffeine.

The effects of caffeine on women have been examined in the context of its effects on menstrual function, interactions with oral contraceptives, pregnancy and fetal health, and postmenopausal health. Earlier studies suggested that elimination of caffeine may vary across the menstrual cycle, with elimination being about 25 percent longer in the luteal phase (Balogh et al., 1987). More recent studies, however, indicate no significant effects on caffeine pharmacokinetics across phases of the menstrual cycle in healthy, nonsmoking women who are not using oral contraceptives (Kamimori et al., 1999). Decreased paraxanthine or caffeine metabolic rates in healthy postmenopausal women on estrogen replacement therapy suggest that exogenous estrogen in older women may inhibit caffeine metabolism through the P450 isozyme CYP1A2, an isozyme common to both estrogen and caffeine metabolism (Pollock et al., 1999). Additionally, it is known that oral contraceptive use can double caffeine half-life (Abernethy and Todd, 1985; Patwardhan et al., 1980). The effects of newer oral contraceptives on caffeine half-life have not been studied.

PHYSIOLOGICAL EFFECTS

Caffeine administration affects the functioning of the cardiovascular, respiratory, renal, and nervous systems. Proposed mechanisms of action differ for different physiological effects. Caffeine action is thought to be mediated via several mechanisms: the antagonism of adenosine receptors, the inhibition of phosphodiesterase, the release of calcium from intracellular stores, and antagonism of benzodiazepine receptors (Myers et al., 1999).

Caffeine and Adenosine Receptors

The ability of caffeine to inhibit adenosine receptors appears to be highly important in its effects on behavior and cognitive function. This ability results from the competitive binding of caffeine and paraxanthine to adenosine receptors and is of importance in contributing to CNS effects, especially those involving the neuromodulatory effects of adenosine. Due to the blocking of adenosine inhibitory effects through its receptors, caffeine indirectly affects the release of norepinephrine, dopamine, acetylcholine, serotonin, glutamate, gamma-aminobutyric acid (GABA), and perhaps neuropeptides (Daly et al., 1999).

There are two main classes of adenosine receptor: A_1 and A_2; caffeine and paraxanthine are nonselective antagonists at both, although they are not especially potent antagonists. The caffeine concentrations attained in vivo that cause mild CNS stimulation (5–10 μM) and that are associated with antiasthmatic effects (50 μM), are in the range associated with adenosine receptor blockade (as quantitated by in vitro receptor binding assays) (Daly, 1993).

Caffeine and Phosphodiesterase

Caffeine increases intracellular concentrations of cyclic adenosine monophosphate (cAMP) by inhibiting phosphodiesterase enzymes in skeletal muscle and adipose tissues. These actions promote lipolysis via the activation of hormone-sensitive lipases with the release of free fatty acids and glycerol. The increased availability of these fuels in skeletal muscle acts to spare the consumption of muscle glycogen. Increased cAMP could also lead to an increase in blood catecholamines. However, caffeine is a fairly weak inhibitor of phosphodiesterase enzymes, and the in vivo concentrations at which behavioral effects occur are probably too low to be associated with meaningful phosphodiesterase inhibition (Burg and Werner, 1975; Daly, 1993).

In contrast, phosphodiesterase inhibition may account for caffeine's (and theophylline's) cardiostimulatory and antiasthmatic actions, since nonxanthine phosphodiesterases are cardiac stimulants (Schmitz et al., 1989) and are also effective as bronchiolar and tracheal relaxants. Indeed, in the latter case, the po-

tency correlates with phosphodiesterase inhibition, not with affinity for adenosine receptors (Brackett et al., 1990; Persson et al., 1982; Polson et al., 1985).

Caffeine and Calcium Mobilization

The earliest proposed mechanism of action for caffeine involved the mobilization of intracellular calcium. Certain actions of caffeine in skeletal muscle appear to involve ionic calcium (Ca^{++}). Caffeine in high concentrations (1–10 mM) was found to interfere with the uptake and storage of calcium in the sarcoplasmic reticulum of striated muscle and to increase the translocation of Ca^{++} through the plasma membrane (Nehlig et al., 1992). Caffeine may also increase myofilamental sensitivity to Ca^{++} through its binding to ryanodine receptors in calcium channels of muscle and brain (McPherson et al., 1991).

Although caffeine has been shown to release calcium from intracellular storage pools (sarcoplasmic reticulum) in skeletal and cardiac muscle, the threshold concentration required in vitro to observe this effect (250 μM) is substantially higher than the concentrations required in vivo for cardiac stimulation (50 μM). Hence, this subcellular action of caffeine is probably physiologically irrelevant (though it conceivably could be relevant at toxic concentrations of caffeine) (Daly, 1993).

Caffeine and Benzodiazepine Receptors

Caffeine modifies or antagonizes the effects of benzodiazepines on behavior in both animals and humans (de Angelis et al., 1982; ME Mattila et al., 1992; MJ Mattila et al., 1992). The mechanism for this antagonism was proposed to be the blocking of benzodiazepine receptors by caffeine. Caffeine does have weak antagonistic properties at these receptors. However, this mechanism requires very high concentrations of caffeine (Nehlig et al., 1987; Weir and Hruska, 1983). More recent evidence (Lopez et al., 1989; Nehlig et al., 1992) suggests that the interaction between caffeine and benzodiazepines is mediated through caffeine's effects on adenosine receptors. There is some evidence that caffeine may also be a histamine receptor antagonist (Acquaviva et al., 1986).

General Effects of Caffeine on Physiological Functions

The effects of caffeine on sodium–potassium–adenosine triphosphate pump activity lead to a decrease in plasma potassium concentrations, and affect the depolarization–repolarization process during exercise with potential effects on fine motor coordination.

The effects of caffeine on the heart are primarily stimulatory and are accompanied by increased coronary blood flow. These effects are thought to be mediated not by an action on adenosine receptors (Collis et al., 1984), but in-

stead via phosphodiesterase inhibition. In the lungs caffeine can cause smooth muscle relaxation and bronchial dilatation, possibly accounting for its antiasthmatic effects. However, the relative roles of adenosine receptors and phosphodiesterase as mechanisms of caffeine's antiasthmatic actions remain unresolved (Brackett and Daly, 1991; Ghai et al., 1987; Persson et al., 1982).

The effects of caffeine on the kidney—diuresis, increased blood flow, and rennin secretion—appear to be due to an action of caffeine at adenosine receptors (Spielman and Arend, 1991). Caffeine's behavioral effects appear to be mediated both through adenosine receptors and phosphodiesterase effects and can readily be seen on neurochemically specific neurons. Caffeine's stimulatory action on dopamine, norepinephrine, serotonin, acetylcholine, glutamate, and GABA neurons is hypothesized to result from its ability to block the action of adenosine, which typically inhibits neuronal function. Phosphodiesterase inhibition by xanthines may also account for some stimulatory effects.

Interactions with other nutrients and drugs also characterize certain effects attributed to caffeine. Such interactions include those associated with aspirin, alcohol, nicotine, cocaine, certain other botanicals, and other narcotics (Callahan et al., 1982; Falk and Lau, 1991; Kuribara and Tadokoro, 1992; Parsons and Neims, 1978; White, 1999).

The repeated administration of caffeine does not change its pharmacokinetics, but in many cases development of tolerance does occur. Tolerance is not observed for all effects of the drug, such as fat cell lipolysis (Holtzman et al., 1991), but is seen for certain behavioral actions, such as some of its stimulant properties (increase in locomotor activity in rats) (Finn and Holtzman, 1986). Following the cessation of caffeine use, withdrawal-like symptoms are sometimes seen in humans, such as headache, irritability, nervousness, and a reduction in energy (Griffiths et al., 1986, 1990). The physiological bases for these symptoms are not known. Although the development of withdrawal symptoms might indicate an addictive property, caffeine does not have a convincing profile as an addictive drug.

SUMMARY

Caffeine is rapidly and completely absorbed within an hour following ingestion. It is distributed throughout body water and readily crosses cell membranes including the brain. Its primary mechanisms for stimulatory activity appear to be the blocking of adenosine receptors and inhibition of phosphodiesterases. Caffeine is metabolized and excreted in humans primarily as paraxanthine, which also has pharmacologic activity. With repeated caffeine dosing, paraxanthine may contribute to development of tolerance and withdrawal symptoms. Caffeine clearance rates are affected by both environmental and physiological factors, such as use of oral contraceptives, smoking, and pregnancy. Tolerance to some of caffeine's physiological affects develops with continued use.

3

Efficacy of Caffeine

Caffeine has been shown clinically to induce a variety of positive effects that have contributed to its extensive use worldwide. Caffeine use has been associated with increased alertness and enhanced physical performance, and as a countermeasure to the effects of sleep deprivation. Extensive research has been done on each of these effects of caffeine. A brief summary of research findings on the efficacy of caffeine is presented here.

PHYSICAL PERFORMANCE

Caffeine has been proposed as an ergogenic aid in physical performance. Its use is associated with a reproducible increase in endurance time in activities of moderate intensity and long duration. Caffeine consumed both at rest and during exercise increases a variety of physiological processes (heart rate, respiratory rate, blood pressure), probably through the secretion of epinephrine. Recent research indicates that caffeine may also act by altering pain perception since it has been reported to increase plasma β-endorphins during endurance exercise (Laurent et al., 2000). Typically, the magnitude of the exercise response far exceeds and masks the resting effects of caffeine intake. However, if the intensity of the exercise is low and the caffeine dose is high, the effect of the caffeine may be obvious even during exercise. Caffeine also shifts cellular metabolism, possibly through antagonism of adenosine receptors (Graham et al., 1994). Specifically, caffeine increases lipolysis via activation of hormone-sensitive lipase, decreases glycogenolysis via direct inhibition of glycogen phosphorylase, and

increases blood glucose and oxygen consumption (Spriet, 1999). Earlier work indicated this increase in lipolysis may actually be stimulated by the caffeine metabolite, paraxanthine, rather than by caffeine itself (Hetzler et al., 1990). Energy derived from fat during exercise is increased with caffeine ingestion, while the energy derived from carbohydrate is somewhat reduced at the same intensity of exercise (Sasaki et al., 1987). Glycogen utilization is, at least initially, depressed (Erickson et al., 1987; Essig et al., 1980; Spriet et al., 1992). Blood lactate, which usually increases in exercise above 70–75 percent of VO_{2max}, is not affected by caffeine at rest, and may (Flinn et al., 1990; McNaughton, 1986) or may not (Dodd et al., 1991; Gastin et al., 1990) be affected by caffeine during exercise, depending on the intensity of the exercise and the level of caffeine ingested.

Although in today's military there is an increasing reliance on sophisticated computer-controlled systems, special operations and infantry missions will always rely on the physical fitness of the soldier. These operations consist of either prolonged endurance or brief, high-intensity activity. The efficacy of caffeine in promoting physical performance is different for these two kinds of activity.

Four separate reviews (Dodd et al., 1993; Graham et al., 1994; Spriet, 1995; Tarnopolsky, 1994) have concluded consistently that caffeine enhances endurance performance in a variety of activities (i.e., running, cross-country skiing, cycling), with doses from 2 to 9 mg/kg, in naive and habituated, trained and untrained test subjects. The performance effects are seen at intakes that result in urinary caffeine levels below the legal limits stipulated by the International Olympic Committee and are more pronounced in well-trained athletes (Spriet, 1999).

These same reviews concluded that there was little effect of caffeine on activities requiring high power outputs over a short time, such as lifting, carrying, and sprinting. Such activities utilize primarily anaerobic generation of adenosine triphosphate, a process that is probably not affected by caffeine. In contrast, other studies have shown slightly increased power output due to caffeine intake (Anselme et al., 1992; Collomp et al., 1992; Wiles et al., 1992), or increased time to exhaustion in brief (2-minute) supramaximal exercise (Jackman et al., 1996). This suggests a possible direct effect of caffeine on muscle tissue (Green et al., 1990; Lopes et al., 1983; Tarnopolsky et al., 1992).

Response to caffeine ingestion may vary among studies as a consequence of the caffeine habits of participants. As mentioned elsewhere in this report, chronic use of caffeine results in habituation to some of its effects, possibly by up-regulation of adenosine receptors. The epinephrine response to circulating caffeine or methylxanthine by-products may be attenuated as a result (Tarnopolsky et al., 1989; van Soeren et al., 1993). If the epinephrine response is required for the performance-enhancing effects of caffeine to be realized, habitual users may require a higher dose of caffeine to garner the positive results (Spriet et al., 1992). The dose of caffeine required for significant improvements in physical performance ranges from 3 to 9 mg/kg (Graham and Spriet, 1995). It should be

noted, as well, that exercise has been shown to counteract the anxiety that may accompany high doses of caffeine. Youngstedt et al. (1998) showed that after ingestion of 800 mg of caffeine, cycling for 60 minutes at 60 percent of VO_{2max} significantly reduced anxiety compared with consumption of this amount of caffeine while at rest.

Carbohydrate–Caffeine Mixtures

The most important theoretical mechanism of action of caffeine in the context of physical performance of the whole organism is a shift in the primary fuel used for exercise. In adipocytes, caffeine promotes lipolysis by increasing cyclic adenosine monophosphate levels, which in turn increase stimulation of hormone-sensitive lipase. The resulting increase in circulating free fatty acids hypothetically spares muscle glycogen. An independent effect of caffeine on muscle glycogenolysis has also been postulated (as discussed in previous section). In addition, carbohydrate has been shown to enhance performance during continuous exercise lasting at least 50–60 minutes (Armstrong and Maresh, 1996). The hypothesis has been put forward that incorporating the lipolytic qualities of caffeine with the carbohydrate utilization-promoting qualities of carbohydrate ingestion might augment the performance effects of both, suggesting that caffeine delivered in a carbohydrate-containing medium may further enhance performance. The following three studies have tested the efficacy of such a mixture.

Wemple et al. (1997), using a carbohydrate and electrolyte drink (3 mL/kg) with and without caffeine (60 mg per dose), evaluated time to exhaustion at 85 percent VO_{2max} after 3 hours of continuous cycling exercise in six trained subjects. Cycling performance was not affected by including caffeine in the carbohydrate-containing fluid. However, caffeine intake in this experiment was extremely low.

Kovacs et al. (1998) added different doses of caffeine (2–4.5 mg/kg) to a carbohydrate–electrolyte solution and examined the effects on substrate metabolism and endurance performance time in 15 trained subjects during a 1-hour time trial. The addition of caffeine to the carbohydrate–electrolyte drink resulted in a significant improvement in the performance times as compared to placebo or carbohydrate–electrolyte drink alone, with a maximum effect at an intake of about 3 mg of caffeine per kilogram. There was no apparent change in metabolic fuel used during the cycling exercise, thus ruling out fuel shifts as the mechanism by which caffeine augmented the carbohydrate effect. No caffeine-only treatment was included in this experiment, leaving the question open as to how much of the effect was due to caffeine alone and how much to the interaction of caffeine and carbohydrate.

Sasaki and colleagues (1987) looked at the effect of placebo, sucrose, caffeine (approximately 6 mg/kg of body weight), and a sucrose plus caffeine mixture on time to fatigue in five trained males running at 80 percent VO_{2max}. There was no additive effect of caffeine on time to exhaustion when it was given

with sucrose, although the mean distance covered was greater in the two trials where the subjects consumed sucrose compared to placebo. Caffeine alone resulted in a distance intermediate between the two sucrose trials, but it was not significantly different from either. Caffeine alone was associated with an increase in energy derived from fat, whereas sucrose alone was associated with an increased utilization of carbohydrate. Sucrose in combination with caffeine maintained the higher carbohydrate utilization equivalent to sucrose alone. The small number of subjects in this experiment makes it difficult to project these findings to all other populations, including military personnel.

Other Effects on Physical Performance

It has been postulated that caffeine might impinge on physical performance via changes in body temperature and fluid balance. Caffeine apparently has no effect on rectal temperature, plasma volume change, or sweat rate during endurance exercise in warm (25–29°C) environments (Falk et al., 1990; Gordon et al., 1982). No similar studies have been conducted in hotter conditions; however, if an effect is not seen at 25–29°C, it is unlikely that there would be a differential response due to caffeine at temperatures greater than 29°C. Further, a study by Cohen et al. (1996) on performance in a hot and humid environment showed no effect of consuming 5 or 9 mg of caffeine per kilogram on time to exhaustion, body temperature, or blood levels of glucose and lactate during multiple 21-km runs in trained men and women.

High-altitude exposure may augment the positive effects of caffeine on endurance performance. Exercise performance is dramatically reduced by altitude exposure, and maximal effort may be diminished by as much as 25 percent. Submaximal performance may be improved with acclimatization, but maximal effort does not normally recover (IOM, 1996). However, Fulco et al. (1994) showed that ingestion of caffeine (4 mg/kg) could increase the time to exhaustion in eight trained men riding a cycle ergometer at 80 percent of high-altitude VO_{2max} (65 percent of sea-level VO_{2max}) at 4,300 m, but not at sea level. This positive effect was present after 1 hour of altitude exposure (54 percent increase in time to exhaustion with caffeine ingestion 1 hour before exercise) and tended to remain after 2 weeks of acclimatization (24 percent increase). Because Fulco et al. did not find any differences in substrate metabolism between the two conditions, they hypothesized that the mechanism of improvement involved an increase in residual lung capacity (tidal volume) or an improvement in muscle strength. Similarly, Berglund and Hemmingsson (1982) showed that caffeine significantly decreased the race time (by 101 seconds after one lap, 152 seconds after two) of trained cross-country skiers in a 21-km race at 2,900 m. No change in race time occurred in a test at an altitude of 300 m.

A combination of caffeine and ephedrine enhances running performance (Bell and Jacobs, 1999), but also raises metabolic heat production and thus poses

a theoretical risk of hyperthermia during exercise–heat stress. However, during 2 hours of brisk treadmill walking in a 40°C hot, dry environment, Bell et al. (1999) observed that this increased metabolic heat production was offset by increased heat dissipation and that the internal body temperature change was no greater than during a control trial. However, recent information on adverse cardiovascular and central nervous system events resulting from the use of ephedra-containing supplements (Haller and Benowitz, 2000) makes the use of a caffeine–ephedra combination less than desirable. Although hyperthermia is more likely when prolonged, strenuous exercise and intense environmental stress are concurrent, the effects of caffeine in this situation have not been examined.

COGNITIVE FUNCTION AND ALERTNESS

Both common experience and the results of scientific investigations support the belief that caffeine enhances performance on a variety of cognitive tasks. However, a review of the experimental literature reveals inconsistencies in the amount of caffeine that is required to produce positive effects on cognitive behavior. These discrepant findings can be explained by differences among experiments in a number of variables including whether or not subjects were tested following a period in which they had abstained from using caffeine, the tasks used to assess cognitive behavior, the age and gender of the subjects, the subjects' history of caffeine use, and whether the subjects were rested or sleep deprived.

There has been some debate whether caffeine enhances cognitive performance or simply restores degraded performance following caffeine withdrawal in rested individuals. James (1994, 1995, 1998) argued that the majority of studies reporting the effects of caffeine in rested subjects studied moderate caffeine consumers (200–300 mg/d) who were required to abstain from caffeine for some period of time prior to cognitive testing (2–24 hr). Abstinence for regular caffeine users could have resulted in symptoms of withdrawal which include headaches, fatigue, and irritability (Griffiths and Mumford, 1995; Griffiths et al., 1990). James (1994, 1995, 1998), hypothesized that comparisons between caffeine and placebo conditions in experiments assessing the effects of caffeine on cognitive behavior could represent a reversal of deteriorated performance. This may be due to caffeine withdrawal in the placebo condition compared to baseline performance in the presence of caffeine.

A clearer picture of caffeine's effects on cognitive function and behavior has begun to emerge, however. Caffeine can enhance performance on some types of cognitive tasks, and some aspects of mood in rested individuals independent of its ability to reverse symptoms of withdrawal and regardless of the background consumption of caffeine. Warburton (1995) demonstrated that caffeine administered in doses of 0, 75, and 150 mg to adult male, nonsmoking, regular caffeine users, without abstinence from caffeine prior to treatment, improved attention, problem solving, and delayed recall and significantly improved

mood ratings. Rogers et al. (1995), using caffeine doses of 0, 70, and 250 mg/day in caffeine users (> 200 mg/d) and nonusers (< 15 mg/d), demonstrated that although caffeine withdrawal had a negative effect on mood, it did not appear to affect psychomotor performance. Jarvis (1993) reported results of a large survey study on coffee and tea consumption showing a highly significant dose–response relationship between habitual caffeine intake and psychomotor performance (simple reaction time, choice reaction time, incidental verbal memory, and visuo-spatial reasoning). This report also clearly demonstrates that tolerance to the performance-enhancing effects of caffeine, if it occurs at all, is incomplete with the result that higher daily caffeine consumers tend to perform better than do low consumers (Jarvis, 1993).

Using objective measures of alertness (multiple sleep latency test, visual and auditory vigilance tasks), Zwyghuizen-Doorenbos et al. (1990) demonstrated in rested, moderate (< 250 mg/d) caffeine users that caffeine administered in 250-mg doses twice a day compared to placebo improved daytime alertness and reaction time on auditory vigilance tasks. Kenemans and Lorist (1995), using male and female undergraduate students with an average coffee consumption of 5.9 cups/day, demonstrated that caffeine given in a single dose of 3 mg/kg body weight (≈ 250 mg/day) increased cortical activation, increased sensitivity (rate at which information on stimuli is accumulated), and increased both speed and accuracy of target selection.

Amendola et al. (1998) reported caffeine at doses of 0, 64, 128, and 256 mg/day enhanced accuracy and reduced reaction time on auditory and visual vigilance tasks in a dose-related manner. Moreover, caffeine significantly increased self-reports of vigor and decreased reports of fatigue, depression, and hostility on the Profile of Moods Scale (POMS). Self-assessments of energy levels were also improved by caffeine (Lieberman et al., 1987; Sicard et al., 1996). However, caffeine did not improve long-term memory (list learning), false alarms in an auditory vigilance task, commission of errors in a four-choice reaction time, or motor coordination. In a simulated military situation involving a tedious task that required sustained attention for proficient performance (i.e., sentry duty), caffeine eliminated the vigilance decrement that occurred with increasing time on duty, reduced subjective reports of tiredness, and did not impair rifle firing accuracy (Johnson, 1999). Additionally, in this situation, caffeine increased the number of correct target identifications in both males and females. However, the reason for this differed with gender. With prolonged sentry duty and no caffeine, men were more likely to fire at friendly targets and women were less likely to fire at foes. Caffeine returned both of these deficits to baseline levels (Johnson, 1999).

Thus, caffeine's effects on cognitive function and mood can be detected in rested individuals, both users and nonusers of caffeine, using a variety of standardized tests. Only certain behavioral functions appear to be susceptible to the influence of moderate doses of caffeine (32–256 mg). In particular, it appears

that in well-rested individuals, low and moderate doses of caffeine preferentially affect functions related to vigilance (i.e., the ability of the individual to maintain alertness and appropriate responsiveness to the external environment for sustained periods of time), but have limited effects on memory and problem-solving abilities. At high doses caffeine can interfere with performance of tasks requiring fine motor control (Durlach, 1998; Rogers and Dernoncourt, 1998).

The effects of caffeine on cognitive behavior vary according to dose, the subject's experience with caffeine, and gender. In general, low to intermediate doses (100–600 mg) of caffeine are associated with increased alertness, energy, and concentration, while higher doses can lead to anxiety, restlessness, insomnia, and tachycardia (Heishman and Henningfield, 1992, 1994). Individuals who do not consume caffeine on a regular basis appear to be more susceptible to the negative consequences of caffeine than regular consumers. With respect to gender, because of their smaller lean body mass, women may be more affected by a given dose of caffeine than men.

A number of studies have reported on the effect of age on physiological and cognitive responses to caffeine. Arciero et al. (1995) reported that caffeine ingestion (5 mg/kg fat-free mass) increased free fatty acids and tended to increase rate of appearance of fatty acids in younger men (19–26 years old), but not in older men (65–80 years old); while norepinephrine kinetics and fat oxidation were not affected by caffeine in either age group. Arciero et al. (1998) reported on effects of caffeine ingestion (5 mg/kg fat-free mass) on blood pressure, heart rate, norepinephrine kinetics, and behavioral mood in younger and older men. Resting baseline blood pressure was significantly lower for younger men than for older men. Following caffeine ingestion, blood pressure increased significantly above baseline for older men whereas it remained statistically unchanged in younger men. Heart rates in both groups were unaffected by caffeine ingestion. Norepinephrine kinetics (appearance and clearance rates) were not affected by caffeine in either group, although older men had higher norepinephrine concentrations with caffeine. Older men reported declines in feelings of tension and anger following caffeine ingestion, while younger men reported increased feelings of anger.

Rees et al. (1999) examined the interaction of caffeine and age and found that 250 mg of caffeine significantly decreased reaction times in both 20- to 25-year-olds and 50- to 65-year-olds with no effect on word recall. In contrast, Hogervorst et al. (1998) evaluated the effects of 225 mg of caffeine on memory and memory-related processes in three age groups: young (20–34 y), middle-aged (46–54 y), and older (66–74 y). Short-term memory was negatively affected by caffeine in the young group, positively affected in the middle-aged group, and had no effect in the older group. Jarvis (1993), in a large survey study on coffee and tea consumption, found that when results for reaction time tests were categorized by age group (16–34 y, 35–54 y, 55+ y), caffeine intake had a greater performance-enhancing effect for older people (35–54 y, 55+ y)

than younger people (16–34 y). The author hypothesized that this greater sensitivity to caffeine in older adults might be due to the fact that older people tend to operate further below their ceiling than do the young. Alternatively, since the survey only measured coffee and tea consumption, the caffeine intake in the young group was more likely to be underestimated due to much heavier cola and soft drink use in this age group (Jarvis, 1993). Amendola et al. (1998), using subjects in two age groups (18–30 y and > 60 y), tested oral caffeine doses of 0, 64, 128, and 256 mg and found a dose-dependent improvement in mood and performance on the modified Wilkinson Auditory Vigilance Task that was not affected by age.

Thus, it would appear that caffeine effects on performance of vigilance types of tasks is independent of age, while caffeine effects on memory-related tasks may be age-dependent.

COMPENSATION OF SLEEP DEPRIVATION IMPAIRMENTS

Effects of Sleep Deprivation on Cognitive Behavior

Military personnel face many situations in which extended wakefulness may be required, including sentry duty, deployment-related activities, air transportation during emergencies, submarine duty, and combat. As part of their duties in these situations, individuals may have to perform complex cognitive tasks. The performance of these tasks is compromised during periods of extended wakefulness. Sleep deprivation leads to a sequence of impairments in cognitive functioning. These impairments include decreases in alertness, decrements in mental performance, reductions in self-reports of vigor, increases in sleepiness and fatigue, and increases in response reaction time (Kautz, 1999; Newhouse et al., 1989; Penetar et al., 1993, 1994; Wyatt, 1999).

A variety of instruments have been used to quantify the effects of sleep deprivation on behavior in controlled-experimental as well as simulated real-world situations. Alertness has been assessed using objective measures such as ambulatory vigilance monitors, visual and auditory vigilance tasks, and subjective measures such as self-reports and questionnaires. Studies using these measures have found that sleep deprivation impairs performance on vigilance tasks and decreases self-reports of alertness (Bonnet and Arand, 1994a,b; Bonnet et al., 1995; Caldwell et al., 1995; Penetar et al., 1993). A number of mental tasks, such as a serial add–subtract test, logical reasoning, mental rotation, perceptual cueing, and memory tests have been used to assess the effects of sleep deprivation on higher cognitive processes. Using these tasks, mental performance deteriorates as a function of sleep deprivation (Bonnet, 1999; Caldwell et al., 1995; Kautz, 1999; Newhouse et al., 1989; Penetar et al., 1993; Smith, 1999; Stickgold, 1999). Of particular significance, sleep deprivation leads to impairments in

performance on cognitive tasks that would be encountered in military situations, such as piloting helicopters, fixed-winged aircraft, submarines, or advance warning aircraft; monitoring sonar or radar screens; and sentry duty. Sleep deprivation also affects mood as measured by standard scales such as the POMS and visual analogue scales. More specifically, as subjects become incrasingly sleep-deprived, increases in fatigue, tension, and depression and decreases in vigor are reported (Bonnet, 1999; Caldwell et al., 1995; Kautz, 1999; Newhouse et al., 1989; Penetar et al., 1993; Smith, 1999; Stickgold, 1999). Sleepiness, as assessed by objective measures including latency to sleep, eyelid movements, electroencephalograms, and muscle tone, and subjective measures such as self-report sleepiness scales, increases directly as a function of the amount of sleep deprivation incurred.

Recent advances in the understanding of sleep mechanisms have identified adenosine as a moderator of the sleep-inducing effects of prolonged wakefulness. Studies have shown that extracellular concentrations of adenosine in the cholinergic regions of the basal forebrain increased progressively during prolonged wakefulness and declined slowly during recovery sleep (Porkka-Heiskanen, 1999; Porkka-Heiskanen et al., 1997). Caffeine, as a known antagonist of adenosine, could thus be expected to promote wakefulness by preventing neuronal uptake of the sleep-promoting adenosine.

Two recently identified neuropeptides (orexins A and B, or hypocretins) are produced exclusively by a well-defined group of neurons in the lateral hypothalamus. These unique orexin peptides act directly at axon terminals to stimulate the release of the major inhibitory neurotransmitter, gamma-amino benzoic acid, and the major excitatory neurotransmitter, glutamate. Together, these two neurotransmitters are responsible for almost all fast synaptic activity in the hypothalamus.

Chemelli and colleagues (1999) reported the development of a strain of orexin knockout mice that developed symptoms virtually identical to narcolepsy in humans. To further evaluate the role of orexin in stimulating wakefulness, the antinarcoleptic drug, modafinil (see Chapter 6) or placebo was administered to normal mice. Modafinil strongly activated the orexin neurons in the lateral hypothalamus. No research has yet been reported that examines the effect of caffeine or paraxanthine on orexin neurons.

Restoration of Sleep Deprivation-Induced Cognitive Deficits with Sleep

All of the above-listed decrements in cognitive behavior can best be reversed by reconstituting sleep. There is a dose effect for the restorative effects of sleep duration on cognitive performance (Bonnet, 1999; Bonnet and Arand, 1994b; Bonnet et al., 1995). Any amount of sleep from as little as a 15-minute nap can restore some degree of function, although the longer the sleep episode, the greater the amount of cognitive function restored (Bonnet et al., 1995). Since

the drive for sleep is governed by both a homeostatic and a circadian drive, which are interactive (Wyatt, 1999), these factors must be taken into consideration in determining the timing of naps and their effectiveness in reconstituting mental functioning. Naps are effective both prior to (prophylactic naps) and during (restorative naps) a period of sleep deprivation (Bonnet, 1999; Bonnet and Arand, 1994a; Bonnet et al., 1995).

However, in an earlier, well-designed study, Dinges et al. (1987) examined the effects of temporal placement of naps for alertness during a 56-hour period of sleep deprivation. A 2-hour nap was preceded by either 6, 18, 30, 42, or 54 hours of wakefulness. Naps were placed 12 hours apart near the circadian peak or circadian trough. Performance was measured by a visual reaction time test, and mood was assessed using the Stanford Sleepiness Scale (SSS). Results indicated that a nap at any time during the period of sleep deprivation improved reaction time performance but not SSS ratings. The earlier naps (6 and 18 hours into the wakefulness period) yielded better, and longer-lasting reaction time performance improvements which could be detected more than 24 hours after the nap, despite the fact that these naps were comprised of lighter sleep than later naps. Bonnet (1999) also found that quality of sleep differs between prophylactic naps and naps taken during sleep deprivation. Prophylactic naps are associated with longer sleep latencies and less deep sleep than post-deprivation recovery sleep. Dinges et al. (1987) also found circadian placement of naps had no effect on any parameter measured, and concluded that napping prior to a night of sleep loss is more important in meeting subsequent performance demands than is circadian placement of the nap. Napping appears to prevent sleepiness more readily than it permits recovery from sleepiness. In addition, a negative side effect of sleep during a period of sleep deprivation (restorative sleep) is sleep inertia, a short period of mental confusion upon awakening from such naps that can last as long as 30 minutes (Dinges, 1989; Stamph, 1989).

Restoration of Sleep Deprivation-Induced Cognitive Deficits with Caffeine

When sleep is not an option, caffeine can help to alleviate decrements in cognitive functioning resulting from shift work (Walsh et al., 1990, 1995), performance during circadian troughs (Gander et al., 1998; Reyner and Horne, 2000), restricted or disrupted sleep (Belland and Bissell, 1994; Rosenthal et al., 1991), and complete sleep deprivation (Bonnet, 1999; Jarvis, 1993; Johnson, 1999; Kautz, 1999; Lieberman, 1999; Lorist et al., 1994a,b; Smith and Rubin, 1999). The effectiveness of caffeine in reversing sleep deprivation-induced decrements in performance varies among subjects, and its ability to restore mental performance is influenced by a number of factors. These include prior caffeine exposure, dosage schedule, formulation of caffeine, metabolic factors, concurrent drug use, degree of sleep deprivation, and time of day of dose administration

(Kaplan et al., 1997; Kuznicki and Turner, 1986; Linde, 1995; Lorist et al., 1994a,b). From the limited data available, gender does not appear to play a role in the effects of caffeine on mental abilities. However, this variable and other potential factors, such as P450 enzyme polymorphism, age, body weight, stress hormonal and other endocrine responses, concurrent illness, and drug interactions (Kamimori et al., 1999), which might potentially contribute to intra- or intersubject variability to the effects of caffeine, should be assessed further.

In sleep-deprived subjects, judicious use of caffeine can restore alertness, performance on mental tasks, and positive mood states. For example, Smith and Rubin (1999) found that caffeine had a similar profile to amphetamine and phentermine in that it reversed the sleep deprivation-induced increased response time and number of errors on a visual vigilance task, as well as the sleep deprivation-induced decrements in a running memory test. Similarly, Bonnet and Arand (1994b) observed that caffeine increased alertness and performance on a visual vigilance task, mental arithmetic tests, and logical reasoning in sleep-deprived subjects. A number of researchers have shown that caffeine is also effective in delaying sleep onset in sleep-deprived subjects (Bonnet, 1999; Kautz, 1999; Penetar, 1999; Smith, 1999). With respect to mood, caffeine administration in sleep-deprived subjects decreased reports of confusion and fatigue and increased reports of vigor, but had no effect on reports of tension, anger, and depression using the POMS (Kautz, 1999). Using visual analog scales, caffeine intake led to reports of decreased sleepiness and increased alertness, ability to concentrate, confidence, talkativeness, energy levels, anxiety, jitteriness, and nervousness (Kautz, 1999). One study suggested that some of the effects of caffeine were associated with increased measures of hypothalamic–pituitary–adrenal axis activity (plasma cortisol levels). However, further studies utilizing more extensive sampling are needed to confirm this effect.

Research suggests that doses of caffeine between 150 and 600 mg are effective in alleviating sleep deprivation-induced decrements in cognitive performance (Kelley et al., 1996; Penetar et al., 1993). Immediately following administration, doses in the range of 150 mg were just as effective as 300 or 600 mg in improving mental function in sleep-deprived subjects. However, the lower dose (150 mg) did not sustain performance on complex mental operations for as long as the higher doses (300 or 600 mg) (Kautz, 1999). Penetar et al. (1993) administered caffeine at levels of 0, 150, 300, and 600 mg following 49 hours of sleep deprivation and found a dose-related improvement in both subjective and objective measures of alertness and improvements in mood. Kelley et al. (1996) evaluated repeated doses of caffeine during 64 hours of sleep deprivation and measured effects on recovery sleep. Treatments were placebo, 300 mg of caffeine every 6 hours, or 400 mg of caffeine every 24 hours starting the evening of the first day of sleep deprivation. Subjects given the 300 mg every 6 hours developed a steady-state concentration of salivary caffeine by the third dose, while those receiving the 400 mg every 24 hours had salivary caffeine concentrations

that peaked and then declined to near placebo level by 18 hours after administration. Caffeine had no effect on recovery sleep with respect to sleep latency, total sleep time, or rapid eye movement sleep. There was actually a nonsignificant increase in slow wave sleep with caffeine compared to placebo.

In comparison to 20 mg of amphetamine however, caffeine's effects are modest. Newhouse et al. (1989) found that 20 mg of amphetamine effectively restored alertness to almost 100 percent of rested values for 2 hours and remained significantly better than placebo for 7 hours after administration. In the Penetar et al. (1993) study caffeine restored alertness to approximately 50 percent of that seen in the rested condition with effects declining after 4.5 hours, although subjective measures of sleepiness following caffeine administration were restored to rested levels for 2 to 12 hours.

Restoration of Sleep Deprivation-Induced Cognitive Deficits with a Combination of Caffeine and Naps

Bonnet and Arand (1994a) compared the effectiveness of a 4-hour prophylactic nap alone to a 4-hour prophylactic nap followed by 200 mg of caffeine during the sleep deprivation period. Results showed that subjects given a combination of a 4-hour prophylactic nap prior to 24 hours of sleep deprivation and 200 mg of caffeine administered at 0130 and 0730 (normal circadian trough) during the sleep deprivation period maintained alertness and performance at levels equal to or better than those demonstrated prior to sleep deprivation, and was significantly better than the 4-hour prophylactic nap alone. In a subsequent study, Bonnet et al. (1995) evaluated differing lengths of prophylactic naps and differing doses of caffeine on performance during sustained operations and found that an 8-hour nap prior to the period of sleep deprivation was most effective in maintaining performance during the first 24 hours without sleep, and that repeated doses of caffeine at 150 or 300 mg every 6 hours were more effective than a single dose of 400 mg. However, neither nap nor caffeine conditions could maintain performance near rested levels beyond 24 hours.

SUMMARY

Caffeine can significantly improve physical performance of an endurance nature. It is unclear at this time whether this is a result of increased production of free fatty acids to spare glycogen or an increase in release of endorphins that permits athletes to exercise longer by altering pain perception. Caffeine may be particularly beneficial in enhancing performance at high altitudes, with or without acclimation. The role of caffeine–carbohydrate combinations in enhancing physical performance still needs to be clarified.

Evidence is presented that caffeine can enhance certain types of cognitive performance, most notably vigilance and reaction times, in rested individuals

regardless of whether or not they are regular caffeine users. The response to caffeine in caffeine users has been shown to be over and above any alleviation of withdrawal symptoms.

Sleep is the most effective means of reconstituting the decrements in cognitive functioning brought on by sleep deprivation. Thus, in situations where it is feasible, sleep should be promoted. When naps are not an option, caffeine alone could be used to partially alleviate sleep deprivation-induced impairments in cognitive behavior. Combining naps with judicious caffeine use may be the best remedy for sleep deprivation-induced decrements in cognitive function in military situations where adequate sleep cannot be obtained.

The doses of caffeine most likely to be effective without causing undesirable mood effects are within the range of 100 to 600 mg.

4

Safety of Caffeine Usage

People around the world have been consuming caffeine for more than 1,000 years, but in the United States for at least the last 100 years its use has been surrounded by controversy related to potential negative health and behavioral effects, starting with federal seizure of a shipment of Coca-Cola syrup in October 1909. Among the charges used to support the legality of this seizure was that caffeine was an "added" and "poisonous and deleterious substance".

In 1959 caffeine was listed in the *Code of Federal Regulations* (21 CFR 182.1180—formerly 21 CFR 121.101) as generally recognized as safe (GRAS) when used in cola-type beverages with a tolerance set at 0.02 percent. The tolerance was based on industry practice at that time. The minor food use of caffeine in baked goods, frozen dairy desserts, and so forth is based on independent GRAS determinations.

In 1978 the Select Committee on GRAS Substances of the Federation of American Societies for Experimental Biology completed an evaluation of the safety of caffeine. That committee's conclusions stated that "while no evidence in the available information on caffeine demonstrates a hazard to the public when it is used in cola-type beverages at levels that are now current and in the manner now practiced, uncertainties exist requiring that additional studies should be conducted". The major concern raised in that report was the potential behavioral effect of caffeine, especially in young children.

In 1980 the Food and Drug Administration (FDA) proposed to delete the use of caffeine as an added food ingredient from the GRAS list, to declare that no prior sanction exists for the food use of caffeine, and to list caffeine as a food

additive on an interim basis pending the conduct of additional safety studies. The agency identified several safety issues of concern with regard to caffeine, namely potentially fetotoxic and teratogenic properties, potential behavioral effects, and potential carcinogenicity (FDA, 1980b).

In 1987 the FDA published a proposed rule on the use of caffeine in nonalcoholic carbonated beverages. Based on comments submitted to the agency in response to the proposal published in the *Federal Register* of October 1980, the FDA proposed to codify a prior sanction for the use of added caffeine in nonalcoholic carbonated beverages. Thus, the agency was proposing to use a provision of the Food, Drug and Cosmetic Act that exempts from the definition of a food additive any substance used in accordance with an approval granted prior to the Food Additives Amendment of 1958. The FDA concluded that existing data did not demonstrate that a level of 0.02 percent caffeine added to nonalcoholic, carbonated beverages presented any risk to humans. The agency also received several comments regarding the use of caffeine in other foods, but because these comments did not assert that such uses were sanctioned previously, these uses were not addressed in the proposal (FDA, 1987).

In 1992 FDA's Center for Food Safety and Applied Nutrition (CFSAN) carried out a review of scientific articles published from 1986 to 1991 that had bearing on the potential health effects of caffeine. The new information reviewed included animal and clinical studies on developmental, reproductive, behavioral, carcinogenic, cardiovascular, and other effects. Based on this review, CFSAN found that there was no evidence to show a human health hazard arising from the consumption of caffeine through use of cola beverages at 100 mg/person/day or less. The exposures to caffeine from the intake of cola beverages at the ninetieth percentile for children (aged 3 to 5 years) and over a lifetime are estimated by CFSAN to be 57 and 98 mg/person/day, respectively. These daily intakes are within the safe limit set by the prior sanction in 1959. Currently, caffeine is recognized by FDA as a substance that is a food additive with a provisional listing status.

Despite this extensive scrutiny there continues to be controversy surrounding the effects of caffeine on long-term health. The list of diseases in which caffeine has been implicated has changed over the years. Convincing research evidence has removed several diseases from consideration, including various cancers and benign breast disease. Extensive research also has evaluated the impact of caffeine consumption on the incidence of cardiovascular disease, reproduction and pregnancy outcomes, osteoporosis, and fluid homeostasis.

With respect to the actions of caffeine on the central nervous system, it has been shown that ingestion of very high doses of caffeine can produce undesirable effects on mental function such as fatigue, nervousness, and feelings of anger or depression. Additionally, caffeine use has been associated with physical dependence, which may be reflected in performance decrements during withdrawal under some circumstances.

CAFFEINE AND CARDIOVASCULAR DISEASE RISK

For more than 30 years caffeine has been of interest in the etiology of heart disease particularly because it may be associated with alterations in blood lipids and blood pressure, arrhythmias, and other adverse cardiac functions. While earlier studies suggested an effect of caffeine on blood lipids, Sedor et al. (1991) found no influence of coffee on serum lipoproteins in women with normal cholesterol levels. These different results may be accounted for by the finding that the method of preparing coffee could influence the relationship between caffeine and blood lipids. Only one fraction of boiled coffee was found to significantly increase blood cholesterol and low-density cholesterol in a dose-dependent manner (Pirich et al., 1993). In 1,074 adults studied in the United Kingdom, coffee consumption was not found to have a significant effect on total or high-density lipoprotein cholesterol (Lancaster et al., 1994). Similarly, Lewis et al. (1993) found no consistent associations between caffeine-containing beverages and serum lipoproteins in 5,115 healthy black and white, men and women aged 18–30 years. In contrast, in a 17-month follow-up of 2,109 healthy nonsmokers, Wei et al. (1995) found that total serum cholesterol increased by about 2 mg/dL for each subsequent increase in cups of regular coffee per day. Furthermore, a dose–response in serum lipoproteins was found among those who increased consumption, continued the same dose, or decreased consumption of regular coffee. This association was not observed with the consumption of decaffeinated coffee, regular or decaffeinated tea, or caffeine-containing colas. However, in a double-blind, randomized trial of 69 young healthy subjects whose habitual coffee consumption was 5.9 cups (140 mL) of filtered regular coffee per day, abstinence from caffeine resulted in no effect on serum lipids (Bak and Grobbee, 1991). Urgert and Katan (1997), in an extensive review, found effects of coffee brewing techniques on serum concentrations of total and low-density lipoprotein cholesterol. The compounds responsible for this effect are the diterpene lipids cafestol and kahweol, which make up about 1 percent (wt:wt) of coffee beans. These diterpenes are extracted by hot water but are retained by paper filters, thus explaining why filtered coffee has no effect on serum cholesterol, while boiled coffees such as Scandinavian cafetiere and Turkish coffees do.

Several approaches have been utilized to investigate the possible relationship between caffeine intake and blood pressure. Results summarized in recent reviews by Myers (in press) and Green and Suls (1996) suggested that caffeine-naive individuals may experience a small increase in blood pressure after acute dosing with caffeine. During chronic administration of caffeine, tolerance appears to develop, and chronic long-lasting changes in blood pressure are usually not seen in individuals who routinely consume caffeine. Coffee consumption was shown to have no significant effects on blood pressure in the 1,074 adults studied by Lancaster et al. (1994). Similarly, in the Coronary Artery Risk Development in Young Adults study of 5,115 black and white men and women aged 18–30

years, no consistent association was found between consumption of caffeine-containing beverages and blood pressure (Lewis et al., 1993). The 6-year data from the Multiple Risk Factor Intervention Trial showed a significant, independent, inverse relation between caffeine intake and both systolic and diastolic blood pressure (Stamler et al., 1997). In contrast, a recent report of a meta-analysis of 11 controlled trials showed an independent, positive relationship between cups of coffee consumed (median dose = 5 cups/day) and subsequent change in systolic blood pressure (Jee et al., 1999). Another recent review critically examined 30 years of controlled clinical and epidemiological studies on the blood pressure effects of coffee and caffeine (Nurminen et al., 1999). The authors concluded that the acute pressor effects of caffeine are well documented, but that at present there is no clear epidemiological evidence that caffeine consumption is causally related to hypertension. They also concluded, however, that high caffeine intake may be an additional risk factor for hypertension at the individual level due to long-lasting stress or a genetic susceptibility to hypertension.

In general, controlled clinical attempts to demonstrate the effects of caffeine on increasing heart rate or inducing arrhythmias have been unsuccessful (Myers, in press). Chelsky et al. (1990) reported that in patients with clinical ventricular arrhythmias, ingestion of 275 mg of caffeine did not significantly alter the inducibility or severity of arrhythmias. Newby et al. (1996) conducted a randomized, double-blind, 6-week intervention trial using dietary caffeine restriction, caffeinated coffee, and decaffeinated coffee in 13 patients with symptomatic frequent idiopathic ventricular premature beats. Results showed no significant changes in palpitation scores of premature beat frequencies during the intervention weeks and no significant correlation between these variables and serum caffeine concentrations.

A possible association between coffee and risk of coronary heart disease has been examined in case-controlled as well as longitudinal cohort studies. Case-control studies have produced variable results (Myers, in press). However, a meta-analysis of 11 prospective, longitudinal cohort studies showed no increased risk of coronary heart disease associated with consumption of up to 6 cups of coffee per day (Myers and Basinski, 1992). Based on a meta-analysis of 8 case-control studies and 15 cohort studies, Kawachi et al. (1994) reported a pooled case-control odds ratio of 1.63 (95 percent confidence interval [CI], 1.50–1.78) for the effect on coronary heart disease of drinking 5 cups of coffee per day versus none. However, the odds ratio from the 15 cohort studies was not statistically significant. A study of 10,359 men and women in the Scottish Heart Health Study showed a significantly higher prevalence of coronary heart disease in subjects who were nonusers of coffee than in those who drank varying amounts of coffee (Brown et al., 1993). A more recent follow-up of subjects in the Scottish Heart Health Study showed that for many conventional risk factors, coffee had a weak but beneficial gradient with increasing consumption (Woodward and Tunstall-Pedoe, 1999). A 10-year follow-up of North American

women participating in a large prospective cohort study showed no evidence for any positive association between coffee consumption and risk of subsequent coronary heart disease (Willett et al., 1996). For women initially drinking 6 cups or more of caffeine-containing coffee per day, the relative risk was 0.95 (95 percent CI, 0.73–1.26) compared to women who did not consume regular coffee.

Despite numerous studies attempting to show a relationship between caffeine and serum lipoproteins, blood pressure, cardiac arrhythmias, and risk of coronary heart disease, results have failed to show a consistent adverse effect of ingestion of moderate amounts of caffeine. Thus, increased risk of cardiovascular problems resulting from the use of caffeine supplements by the military would, in most cases, not appear to be a major concern.

One potential risk should be noted, however. A number of studies have demonstrated that caffeine consumption produces a transient elevation in blood pressure and that this occurs regardless of whether the individual is or is not a habitual user of caffeine (James, 1990; Lane et al., 1990, 1998). Caffeine consumption has also been demonstrated to potentiate the effects of acute exercise and mental stress in increasing blood pressure (Höfer and Bättig, 1993; Lane et al., 1990; Myers et al., 1989). This effect of caffeine is more pronounced in those with high stress reactivity (i.e., high levels of anxiety) and those who are borderline hypertensive or are hypertensive (James, 1990; Lane et al., 1998; Lovallo et al., 1991; Sung et al., 1995). Lovallo et al. (1996) demonstrated that in borderline hypertensive men, the use of caffeine in situations of behavioral stress may elevate blood pressure to a clinically meaningful degree and that these types of blood pressure rises in hypertensives would be large enough to transiently reduce the therapeutic effects of antihypertensive medication. However, earlier work by Greenberg and Shapiro (1987) compared two levels of caffeine in males with or without a family history of hypertension and found systolic blood pressure levels were significantly greater in individuals with a family history of hypertension across all conditions, but not specifically in response to caffeine. Wise et al. (1996) examined the effects of placebo or 6 mg of caffeine per kg lean body mass on calcium metabolism in normotensive and hypertensive individuals. Urinary excretion of calcium over 72 hours following caffeine/placebo dosing was not different with respect to caffeine treatment, or between hypertensive and normotensive subjects. Both Eggertsen et al. (1993) and MacDonald et al. (1991) reported 24-hour ambulatory blood pressures were not different between decaffeinated and caffeinated coffee in treated hypertensives.

Since military scenarios in which the use of caffeine supplements might be desirable would frequently occur when personnel are also under acute mental and/or physical stress, this could be a concern to those personnel with family histories of hypertension.

CAFFEINE EFFECTS ON REPRODUCTION

Caffeine consumption has been suggested as the cause of numerous negative reproductive outcomes, from shortened menstrual cycles to reduced conception, delayed implantation, spontaneous abortions, premature birth, low infant birthweight, and congenital malformations. As with most other aspects of caffeine consumption, there is a paucity of reliable data concerning the metabolic effects of caffeine on reproductive processes. As a general conclusion, no adverse effect on reproduction (e.g., conception, pregnancy, lactation) has been linked consistently to caffeine consumption (Christian and Brent, 2001; Leviton, 1998). Similarly, the effects of small amounts of caffeine on infants and children seem to be modest and typically innocuous (Castellanos and Rapoport, in press). Nevertheless, physicians conventionally recommend that caffeine intake be limited in pregnant women and nursing mothers. This position is also taken by the FDA (Williams, 1999). Such recommendations are in keeping with pharmacological data showing that caffeine is distributed throughout body water, crosses the placenta to enter the fetus, and is secreted in milk.

A number of reviews have examined the association between caffeine consumption and fertility, as well as its effects during pregnancy on risk of premature births, spontaneous abortions, and fetal problems including low birthweight and congenital malformations. In an epidemiological study of 403 healthy premenopausal women, heavy caffeine consumption (more than 300 mg of caffeine per day) was associated with a shortened menstrual cycle, but not with anovulation or short luteal or long follicular phase (Fenster et al., 1999). The conflicting results and methodological inadequacies of some studies surveying the association in humans between caffeine intake and effects on fertility, birthweight, premature births, or congenital malformation (when malformations of all organs is used as the outcome measure) suggest that caffeine has no consistent effect on these outcomes in humans (Leviton, 1993, 1998) despite the findings in animal studies.

Extremely high doses of caffeine in pregnant rats (well outside the range of normal human consumption) are associated with teratogenicity and fetal and maternal loss (Christian and Brent, 2001; Leviton 1993, 1998; Purves and Sullivan, 1993). Similar teratogenic effects have not been confirmed in humans, and the relevance of the route of administration (intraperitoneally) in animal studies is dubious. More recent animal studies, using lower doses of caffeine, have indicated that preconceptual exposure of rats to caffeine reduced fertility due to effects on implantation rather than fertilization rate and was associated with lower birthweight and lower neonatal and prepubertal growth rates (Pollard et al., 1999). Other studies by these same authors have indicated that in rats, caffeine administration during pregnancy is also associated with increased fetal mortality, impaired sexual differentiation, and reduced maturation of neuronal mechanisms controlling respiration and parturition.

Recent reviews of human studies suggest that some of the initial reported associations between caffeine and reduced fertility, teratogenicity, and other fetal and maternal effects in humans may be explained by confounders such as associated cigarette smoking, reporting inaccuracies, and other methodological errors (Christian and Brent, 2001; Leviton, 1998). A prospective study of 210 women consuming moderate and high levels of caffeine showed no association between birthweight of offspring and caffeine consumption (Caan et al., 1998). In contrast, a population-based study of 7,855 live births showed a small but significant increase in the odds ratio for low birthweight and preterm delivery in mothers consuming both caffeinated and decaffeinated coffee, compared to those consuming neither (Eskenazi et al., 1999). Cigarette smoking was controlled in this study; however the authors could not rule out reporting confounders for caffeine consumption. This would seem to imply that some compound in coffee other than caffeine is the potential cause. A recent meta-analysis of studies encompassing 42,988 pregnancies indicated that there was a small but statistically significant increase in risk of spontaneous abortion and low-birthweight babies in pregnant women consuming more than 150 mg of caffeine per day; however, contributing factors such as maternal age, smoking, ethanol use, or other confounders could not be excluded (Fernandes et al., 1998). A recent population-based, case-control study that controlled for confounders showed no effect of caffeine on low birthweight, preterm births, or intrauterine growth retardation (Santos et al., 1998).

Early spontaneous abortions in caffeine-consuming women have been reported in some studies but not others (Dlugosz and Bracken, 1992). Theoretically, early spontaneous abortions could be related to a caffeine-induced depressed production of placental hormones and a vulnerable implantation. On the other hand, pregnancy slows caffeine metabolism and clearance, especially in the last trimester.

One approach to obtaining an objective assessment of caffeine intake and exposure is to use biomarkers such as serum paraxanthine levels. A recent, well-controlled study of 487 women with spontaneous abortions and 2,087 normal controls, in which caffeine exposure was quantitated objectively by serum paraxanthine levels, showed that the mean serum paraxanthine concentration was significantly higher in women who had spontaneous abortions than in controls (752 versus 583 ng/mL). However, the odds ratio for spontaneous abortion was not significantly increased except in subjects with extremely high paraxanthine levels (> 1,845 ng/mL). The authors concluded that moderate consumption of caffeine was not likely to increase the risk of spontaneous abortion (Klebanoff et al., 1999).

Taken together, these studies suggest that the effects of caffeine on pregnancy and fetal health vary according to the route of exposure and dosing schedule in animals, and according to caffeine dose and levels of exposure in both animals and humans. In humans, confounders that may account for many of

the observed effects of caffeine in pregnancy include concurrent cigarette smoking, maternal age, ethanol consumption, and inaccuracies in reporting of caffeine consumption.

Early reports of delayed conception in women who chronically consume as little as 100 mg of caffeine per day have been confirmed in some but not all subsequent studies. However, this relationship is often confounded by coexisting cigarette smoking, which does lead to subfecundity. Based on the available evidence, some physicians recommend that caffeine consumption be avoided entirely by women who wish to become pregnant (Jensen et al., 1998; Stanton and Gray, 1995).

In the 1970s caffeine consumption was linked to benign lumps and fibrocystic disease of the breasts. However, extensive subsequent research has failed to establish a causal relationship between caffeine use and either benign or malignant diseases of the breasts. Wolfrom and Welsh (1990) concluded that the scientific literature to that point demonstrated no consistent role of methylxanthines in the etiology of fibrocystic breast disease and no consistent beneficial effect on the disease of reducing or eliminating methylxanthine consumption.

Possible effects on caffeine metabolism that are caused by hormonal changes during the menstrual cycle are unclear and poorly studied. Elimination of caffeine from the diet has been recommended to lessen premenstrual symptomology (Rossignol, 1985), but the evidence for such an effect remains inconclusive.

CAFFEINE EFFECTS ON BONE MINERAL DENSITY

Caffeine consumption has been proposed as a risk factor for osteoporosis. One of the first papers indicating a deleterious effect of caffeine came from the laboratory of Heaney and Recker (1982). Metabolic studies conducted in a large number of middle-aged women showed that caffeine intake contributed to a negative calcium balance. However, the overall loss amounted to less than 5 mg of calcium per cup of coffee. This original observation stimulated several observational studies that examined the possible relationship between caffeine consumption, calcium intake, and various indices of bone health. In the large number of studies that have since been conducted, there appears to be no consistent trend linking caffeine consumption and negative effects on bone mineral density or incidence of fracture. A moderate increase in hip fracture risk was seen in subjects in the Framingham Study who consumed more than 2 cups of coffee or 4 cups of tea per day (Kiel et al., 1990). In a prospective study of a large number of women aged 34–59 years, a positive relation was observed between caffeine intake and risk of hip but not forearm fracture (Hernandez-Avila et al., 1991). In contrast to these findings, more recent studies have failed to show a detrimental effect of caffeine on total bone mineral gain in three groups of teenage women with mean daily intakes of caffeine ranging from 14 to 77 mg (Lloyd et al., 1998), or in college-age women with a mean caffeine intake of 103 mg/day

(Packard and Recker, 1996). No effect of caffeine on hip fracture rates was found in women with coffee intakes of 5 cups or more per day (Tavani et al., 1995). No effect was observed on bone loss in postmenopausal women whose habitual dietary caffeine intake ranged from 0 to 1,400 mg/day (Lloyd et al., 1997) or on bone mineral density in older men (Glynn et al., 1995). Although early experimental studies also indicated a significant effect on acute calcium diuresis (Massey and Hollingbery, 1988; Massey et al., 1989), subsequent work indicated that this acute phase of excretion was compensated by a later decrease in excretion of calcium in the urine (Kynast-Gales and Massey, 1994). Moreover, in contrast to initial studies, later studies found either no significant effect of caffeine on calcium balance (Barger-Lux et al., 1990) or negative balance only in subjects consuming less than about 660 mg of calcium per day, or half of the currently recommended intake of calcium. After a comprehensive evaluation of currently available data, Heaney (in press) concluded that any deleterious effect of caffeine on calcium balance could be offset by only 1 or 2 tablespoons of milk added to coffee and that the real issue of concern is low calcium intake rather than high caffeine intake.

CAFFEINE EFFECTS ON FLUID HOMEOSTASIS

Wemple et al. (1997) found that the consumption of approximately 2,500 mL of a carbohydrate–electrolyte beverage containing caffeine led to a greater mean 3-hour urine output than the carbohydrate–electrolyte drink alone in a resting condition (1,843 mL with caffeine versus 1,411 mL without caffeine). During exercise, however, the difference between treatments was not significant (398 mL in 5.75 hours with caffeine and 490 mL in 5.75 hours without caffeine with 2,200 mL of fluid consumed during exercise). It should be noted that the caffeine dose in this experiment was extremely low (approximately 1 mg/kg) and was not sufficient to produce a positive effect on cycling performance. The fact that urine volume was affected by this dose could be of significance in military situations where significantly higher caffeine doses may be used. Caffeine ingestion is of particular concern in situations where water balance is already in jeopardy, such as in hot or high-altitude environments.

Although moderate- to high-dose caffeine consumption (e.g., 600–900 mg) may increase fluid and electrolyte losses in urine, a normal diet will replace these losses in most military scenarios (Maughan and Leiper, 1994).

Nussberger et al. (1990) administered an oral dose of 250 mg of caffeine to eight healthy subjects and found an increase in diuresis, and increased sodium, potassium, and osmol excretion within 1 hour post-treatment. However, aldosterone and vasopressin concentrations remained unchanged. Neuhauser-Berthold et al. (1997), in a controlled experiment with 12 healthy volunteers, administered enough coffee to provide 642 mg of caffeine in a single day, and monitored fluid homeostasis in comparison with a group with an equal amount of

fluid consumption from mineral water only. Subjects given the caffeine had a highly significant increase in 24-hour urine output of 753 ± 532 ml, a corresponding negative fluid balance, and a corresponding decrease in body weight of 0.7 kg. Total body water as measured by bioelectrical impedance decreased by 2.7 percent, and sodium and potassium excretion increased by 66 and 28 percent, respectively. Caffeine use during prolonged operations in hot environments increases the risk of dehydration because such operations involve large sweat losses and/or inadequate fluid and electrolyte intake. The scientific literature indicates that a total body water deficit may (Gonzalez-Alonso et al., 1992; Maughan and Leiper, 1994; Neuhauser-Berthold et al., 1997) or may not (Brouns et al., 1998; Massey and Wise, 1984) occur. The deficit depends on the amount of caffeine consumed, the individual's history of acute and chronic caffeine use, and the total solute load of the beverage plus accompanying meals (Brouns et al., 1998; Wemple et al., 1997).

Finally, a recent study by Kiyohara et al. (1999), in an attempt to determine if serum uric acid concentration could be used as an indicator of increased urination, examined 2,240 Japanese men. They found that men consuming less than 1 cup of coffee per day had a mean serum uric acid concentration of 60 mg/L, while those consuming 5 or more cups of coffee per day had a mean concentration of 56 mg/L.

DETRIMENTAL EFFECTS OF HIGH DOSES OF CAFFEINE

High doses of caffeine can be acutely toxic. Ingestion of caffeine in doses up to 10 g has caused convulsions and vomiting with complete recovery in 6 hours (Dreisbach, 1974). The fatal acute oral dose of caffeine in humans is estimated to be 10–14 g (150–200 mg/kg) (Hodgman, 1998), but numerous factors can alter an individual's sensitivity to caffeine (e.g., smoking, age, prior caffeine status, pregnancy status, concurrent drug use) and thus alter the toxic dose. Doses of 1,000 mg (approximately 15 mg/kg body weight) have generated detrimental side effects, with early symptoms being insomnia, restlessness, and agitation. These symptoms may progress to mild delirium, emesis, and convulsions. Other symptoms can include tachycardia, asystole, and rapid respiration (Kamimori et al., 1999).

One potential risk of high doses of caffeine that needs further substantiation is dose-related decrements in mental functioning (Kaplan et al., 1997; Kuznicki and Turner, 1986; Lieberman, 1999). A number of researchers have found that high doses of caffeine can adversely affect mental performance. Kaplan and colleagues (1997) reported that although a relatively low dose of caffeine (250 mg) produced favorable subjective effects (e.g., elation, pleasantness) and enhanced performance on cognitive tasks in healthy volunteers, higher doses (500 mg) led to less favorable subjective reports (e.g., tension, nervousness, anxiety, restlessness) and less

improvement in cognitive performance than placebo. Negative effects may be more pronounced in nonusers than in regular users of caffeine (Kuznicki and Turner, 1986). Excessive intake of caffeine (caffeinism) may be mistaken for anxiety disorder (Benowitz, 1990). Caffeine has been shown to produce anxiety or panic attacks in individuals with agoraphobia or panic disorders, but not in healthy controls (Boulenger et al., 1984; Charney et al., 1985).

Foreman et al. (1989) examined the effects of 0, 125, and 250 mg of caffeine on performance of a numerical version of the Stroop test, which requires sustained vigilance and intense cognitive effort as well as fast responses. Subjects receiving 250 mg of caffeine had significantly slower response times. Streufert et al. (1997) investigated the impact of 400 mg of caffeine in excess of normal consumption by persons who were already moderate to heavy caffeine consumers (400–1,000 mg/day) on performance of complex managerial tasks. Increased caffeine consumption in these individuals had mixed results. Speed of response to incoming information was faster with added caffeine; however, the managers' capacity to utilize opportunity decreased. The authors postulated that increased response speed in association with decreased effectiveness in immediate recall (Warburton, 1995) may have had unfavorable effects on a performance that requires bringing together events and actions that occur across a time dimension.

Effects of Caffeine in the Context of Stress

Among the preexisting variables that may contribute to variations in caffeine sensitivity are baseline levels of stress exposure. Stress may include physical stress (exercise—see Chapter 3), physiological stress (heat stress—see Chapter 3, infection, sleep deprivation), or psychological stress. Stress exposures in the military may be acute or chronic (IOM, 1999). After stress exposure, stress-responsive neurohormonal and neurotransmitter systems are activated, with associated release of the stress hormones corticotropin-releasing hormone, adrenocorticotropic hormone, and cortisol, and the adrenergic neurotransmitters (epinephrine, norepinephrine), which all interact with caffeine. Caffeine also alters the degree of responsiveness of these stress response systems to stressful stimuli (Iancu et al., 1996). For example, caffeine has been shown to increase plasma norepinephrine, to potentiate epinephrine and cortisol stress reactivity to acute psychosocial stress (Lane et al., 1990), and to increase plasma cortisol levels in response to exam stress in medical students (Pincomb et al., 1987). Caffeine also alters measures of autonomic nervous system function, including heart rate, skin conductance, and electrodermal activity (Zahn and Rapoport, 1987). The degree of responsiveness in these studies varied according to previous caffeine consumption (habitual users versus nonusers).

Risks of Caffeine in Combination with Ephedrine and Other Stimulants

The risks of additive effects of caffeine on cardiovascular function in the context of self-dosing with supplement preparations such as ephedrine or yohimbine should be considered when evaluating additional dosing of caffeine. Waluga et al. (1998) found that administration of a combination of caffeine and ephedrine slightly increased systolic blood pressure during exercise, and addition of yohimbine to this combination increased diastolic pressure and heart rate during rest and increased cardiac work load during exercise. Caffeine and ephedrine have also been found to significantly increase heart rate during exercise (Bell and Jacobs, 1999; Bell et al., 1999) and may also transiently increase metabolic heat production (Horton and Geissler, 1996). A recent FDA-requested review related a number of reported adverse cardiovascular and central nervous system events to the use of ephedra-containing supplements (Haller and Benowitz, 2000). White (1999) recently reviewed interactions of caffeine with nicotine, benzodiazepines, and alcohol on behavior.

Physical Dependence and Withdrawal

The use of caffeine by humans is generally not associated with abuse or addiction (Dews et al., in press). Tolerance to some of the physiological effects of caffeine develops when caffeine-containing beverages are consumed regularly. Withdrawal symptoms often occur with the abrupt removal of caffeine from the diet. The frequency of occurrence of withdrawal, as reported in survey studies and clinical trials, varies anywhere from 4 to 100 percent (Goldstein et al., 1965; Griffiths and Woodson, 1988; Griffiths et al., 1986; Naismith et al., 1970; Robertson et al., 1981; Weber et al., 1993). The symptoms of cessation, when they do occur, are not long-lasting.

The signs and symptoms of withdrawal vary widely and can range from mild to severe, following withdrawal from both low and high doses of caffeine (Silverman et al., 1992). These include headaches, drowsiness, irritability, fatigue, low vigor, and flu-like symptoms including myalgia, nausea, and vomiting.

Caffeine acts as a vasoconstrictor of the cerebral arteries, reducing regional blood flow (Cameron et al., 1990; Mathew et al., 1983), including blood flow velocity in the medullar–cerebral artery (Perod et al., 2000). Caffeine withdrawal is associated with electroencephalogram changes (Reeves et al., 1995) and also causes changes in cerebral blood flow leading to vasodilation in high caffeine users that is thought to be associated with a throbbing, vascular-type headache, one of the most commonly observed caffeine withdrawal symptoms (Couturier et al., 1997; Lader, 1999; Mathew and Wilson, 1985).

This withdrawal phenomenon could lead to decrements in performance during military operations and thus should be avoided. Consuming low doses of

caffeine (25–50 mg) or slowly tapering the dose of caffeine can prevent withdrawal symptoms (Griffiths and Mumford, 1995).

SUMMARY

Caffeine is approved as a food additive with provisional status by the FDA, thus indicating that the agency concludes there is no evidence of a human health hazard arising from consumption of caffeine added to foods and cola beverages. However, controversy continues with respect to caffeine's role in cardiovascular disease, negative reproductive outcomes, physical dependency and withdrawal, and excessive intake. The preponderance of evidence indicates that the use of caffeine by the military would not place personnel at increased risk of cardiovascular disease. Evidence on the risk of large doses of caffeine for individuals who are hypertensive or borderline hypertensive is inconclusive. For women there may be a small increase in risk of spontaneous abortion in the first trimester of pregnancy. The effects of caffeine on calcium metabolism may be of some concern only for those with very low calcium intakes (less than 50 percent of the current recommended intake). Caffeine can significantly increase 24-hour urine output, and may or may not alter total body water. Therefore, if caffeine supplements are used, emphasis should be placed on adequate fluid consumption, particularly in hot or high-altitude environments.

High doses of caffeine can have a negative effect on mood and cognitive performance, and thus the maximum content of caffeine in the delivery form of choice should not exceed 600 mg. In addition, caffeine potentiates the effects of physical, physiological, and psychological stress. Military personnel who are habitual caffeine consumers should not be denied access to caffeine in order to maximize effects of a caffeine supplement.

5

Doses and Delivery Mechanisms

Numerous studies exist in the scientific literature evaluating the safety and efficacy of caffeine. These studies have used a wide array of caffeine dosages and delivery mechanisms. This chapter briefly reviews that information (see Chapters 3 and 4 for detailed reviews) and provides recommendations on the doses and forms of delivery most appropriate for military purposes.

OPTIMUM CAFFEINE DOSAGE

The effective doses of caffeine vary from individual to individual, depending on a variety of factors including time of day, usual caffeine intake, whether the individual is rested or fatigued, whether they smoke, or whether they use oral contraceptives. Similarly, the response to sleep deprivation also varies between individuals. Caffeine doses experimentally evaluated for their effects on both physical and cognitive performance have ranged from as little as 32 mg of caffeine (Lieberman et al., 1987) to as much as 1,400 mg (Streufert et al., 1997).

Physical Performance

The levels of caffeine that have consistently enhanced endurance performance, as discussed in Chapter 3, range from about 200 to 600 mg. Pasman et al. (1995) evaluated the effects of 0, 5, 9, and 13 mg of caffeine per kg of body weight on endurance performance as measured using a cycle ergometer. These doses were equivalent to approximately 360, 648, and 936 mg of total caffeine.

Caffeine significantly increased time to exhaustion compared to the placebo, and there were no differences between levels of caffeine, thus the 360 mg dose (5 mg/kg) was as effective as the higher doses.

A series of extensive reviews (Dodd et al., 1993; Graham et al., 1994; Spriet, 1995; Tarnopolsky, 1994) of the scientific literature have consistently concluded that caffeine enhances endurance performance in a variety of activities with doses from 2 to 9 mg mg/kg of body weight (approximately 150–650 mg) However, the mechanism by which caffeine improves endurance exercise performance is unclear, and has variously been attributed to increased lipolysis, decreased glycogenolysis, increased secretion of β-endorphins, and decreased plasma potassium concentrations.

Hogervorst and colleagues (1999) examined the effects of 0, 150, 225, and 320 mg of caffeine, administered in a carbohydrate–electrolyte solution, on cognitive performance of endurance-trained athletes before and after strenuous physical exercise. Prior to exercise, 150 mg of caffeine significantly improved delayed memory recall. Exercise alone improved selective attention and both simple and complex motor functions. Immediately following exercise, 225 mg of caffeine significantly improved signal detection efficiency and reaction time.

Cognitive Performance

Numerous studies of the effects of different caffeine dosages on various aspects of cognitive performance have been conducted in both civilian and military settings. For example, Dimpfel et al. (1993) measured the effects of placebo, 200, and 400 mg of caffeine on human electroencephalogram (EEG) patterns at rest and during mental concentration tests. In addition to the finding that the effects of caffeine can be quantified with EEG spectral densities, they also found that subjects achieved the best results on concentration tests when given 200 mg of caffeine. This included both the number of problems solved per unit time and the percentage of correct solutions. Results of treatment with 400 mg of caffeine tended to be below those of the placebo condition. Foreman et al. (1989) compared the effects of placebo, 125, and 250 mg of caffeine on cognitive performance using memory tests and the Stroop test. They found no effect of caffeine on performance in either test, but there was a trend toward fewer words recalled in the short-term memory test with 250 mg of caffeine. However, Lieberman et al. (1987) found improved performance on four-choice reaction time tests and the Wilkinson vigilance test at all levels of caffeine evaluated (0, 32, 64, 128, and 256 mg) with no effect on self-rated feelings of tension or anxiety.

Warburton (1995) examined the effects of 0, 75, and 150 mg of caffeine on attentional, verbal memory, nonverbal working memory, and problem-solving speed and accuracy in 18 men who were regular coffee drinkers (no more than 3 cups/day). Caffeine improved speed and accuracy on attentional tests (visual information processing) in a dose-dependent manner. Similar to the data of

Foreman et al. (1989), there was no effect of caffeine on immediate verbal recall; however there was a dose-related effect of caffeine on delayed verbal recall. Caffeine also significantly improved the accuracy, but not the speed, of problem solving. Rogers et al. (1995) found significant improvement in reaction time with 70 mg of caffeine compared to placebo. Similarly, Lorist and Snel (1997) found that caffeine at 3 mg/kg (210 mg for a 70 kg person) given to habitual users improved reaction time and decreased false alarm rates in selective attention tasks. Streufert et al. (1997) evaluated the effects of 400 mg of caffeine added to regular caffeine consumption in moderate to heavy caffeine users (400–1,000 mg/day) and found faster responses to incoming information.

In sleep-deprived individuals, similar to those engaging in sustained operations, caffeine at levels of approximately 100–600 mg appears to improve performance (e.g., vigilance, mood, higher cognitive functions) with few acute adverse behavioral effects; some of the positive effects may persist for 8–10 hours (Gander et al., 1998; Kuznicki and Turner, 1986; Lieberman, 1999; Mitchell and Redman, 1992; Reyner and Horne, 2000; Rogers et al., 1995; Smith, 1999; Walsh et al., 1990, 1995). Even individuals who do not normally consume caffeine appear to obtain these caffeine-related positive effects.

An earlier report to the military concerning use of caffeine as a performance enhancer (IOM, 1994) indicated that two of the primary issues still needing resolution in providing caffeine to military personnel were the appropriate carrier to provide the supplement and the amount required to achieve the desired benefit in personnel both habituated and nonhabituated to caffeine. The data reviewed in this report indicate that caffeine will improve cognitive performance regardless of habituation status and thus there is no need to have different dose levels. Caffeine doses between 100 and 600 mg that can be self-selected would be adequate for all personnel.

CAFFEINE DELIVERY MECHANISMS

Doses of caffeine could be delivered to military personnel during sustained operations in a variety of ways (e.g., tablet or capsule form, beverage, food, or gum). Each of these forms has advantages and disadvantages. For example, caffeine provided in pill or capsule form may not be as readily absorbed as caffeine in a food or beverage.

Brachtel and Richter (1992), in a letter to the editor of the *Journal of Hepatology*, described a study in which they compared the bioavailability of a base dose of 366 mg of caffeine from intravenous infusion, an oral dose in aqueous solution, and an oral dose as an uncoated tablet. Using the area under the curve of serum concentration over time, the bioavailability of caffeine in tablet form was found to be 80 ± 16 percent, significantly lower than the 100 percent bioavailability for the intravenous and oral aqueous solution methods of

delivery. Liguori et al. (1997) compared absorption and subjective effects of 400 mg of caffeine administered in coffee, cola, and capsule form. Using salivary caffeine levels as an indicator, they found that peak increase in saliva levels was similar for coffee and cola, and somewhat lower from capsules. The time to peak saliva levels of caffeine was also similar for coffee and cola (42 and 39 minutes, respectively), but was slower for the capsule (67 minutes).

Because caffeine is commonly consumed in the military (Lieberman, 1999) and most individuals are familiar with its effects, a clearly labeled caffeine product that permits self-dosing to obtain effective dose levels would appear to be appropriate. Such a self-dose might be provided in increments similar to those within the experience of most caffeine users (e.g., 100 mg). For example, a food/energy bar containing a total of 600 mg of added caffeine could be scored in 6 segments of 100 mg each, pills could be provided in doses of 100 mg each, or a pack of chewing gum could contain 100 mg/piece of gum. Caffeine (600 mg) in a beverage would make individual dose control more difficult unless supplied in dehydrated packets of beverage mix containing 100 mg of caffeine per packet to be reconstituted using an individually selected number of packets.

Labeling would permit the few individuals who might experience adverse effects from use of caffeine, or whose religious beliefs precluded its use, to avoid it. The advantage of food or beverage delivery of caffeine is that it permits simultaneous provision of nutrients (e.g., water), consumption of which may otherwise be inadequate under the stress of sustained operations. Food or beverage delivery also provides the ability to include substances that may potentiate the effects of caffeine (e.g., sugar). Since caffeine is a diuretic, beverages may have a particular advantage in situations in which dehydration is likely. However, adherence to appropriate behavioral directives (e.g., adequate consumption of food and beverages) can reduce this risk.

Sustained operations vary in their operational constraints. In aviation missions, for example, low weight and compactness of the caffeine delivery mechanism (e.g., pills, gum) may have advantages over beverages and food bars, yet beverages and bars have the advantage of providing additional fluid and nutrients. Beverages have some advantages in certain situations. For example, dehydration at altitude is often a problem, and the beverage delivery system lessens this hazard; however, under these conditions thirst may not be sufficient to ensure that an effective caffeine dose is consumed (IOM, 1996). In addition, use of a caffeinated beverage, while light in weight if dehydrated, would require time and a water source for mixing, thus making it a less viable alternative than gum or a food bar. Food/energy bars have the advantage over beverages in their ability to deliver a wider variety of other needed nutrients at equal weight; this is an important consideration in many missions. Pills and gums are both very light in weight and small in size, so they can easily be carried in pockets; gum has the advantage of stimulating salivation and enhancing the speed of absorption. It may be necessary to consider at least two caffeine delivery systems: food bars

and gum. Both can be manufactured to provide multiple doses in a single package so that the individual can easily customize his or her optimal effective dose. There is some advantage in having caffeine increments constant at 100 mg (e.g., the score on the bar or the contents of one stick of gum should deliver the same dose) regardless of the delivery mechanism, so that the various forms are more or less interchangeable for self-dosing purposes.

Often sustained operations missions must be altered with little advance notice. In the committee's judgment, it is important that the caffeine delivered be absorbed and metabolized rapidly so that the beneficial effects on performance are present within an hour after administration. Moreover, the dose should not be released over a long time interval because beneficial effects may be delayed and changes in mission cannot easily be accommodated. Information presented in the previous chapters suggests that repeated caffeine dosing during sleep deprivation does not interfere with recovery sleep, suggesting little benefit other than convenience to sustained-release preparations over large single doses (Prusaczyk, 1999). More frequent dosing with rapidly absorbed and metabolized forms of caffeine therefore appears to offer advantages over sustained-release preparations.

SUMMARY

Caffeine has been consistently found to enhance physical endurance performance when administered in amounts ranging from 150 to 650 mg. Similar amounts have also been found to enhance cognitive performance. Caffeine may be administered in a variety of ways, including as a pill or capsule, in a food bar, in a beverage, and in chewing gum. Delivery of caffeine in a food bar or as chewing gum appears to be most advantageous.

6

Special Considerations

In light of wide-ranging individual differences in caffeine sensitivity, dosing for optimal efficacy and minimal side effects should take into account qualitative conditions based on trait and state variations in individual sensitivity to caffeine. Trait variations are those based on the individual's genetic makeup and include such factors as stress reactivity, rate of metabolism of caffeine to paraxanthine, and kidney clearance rates. State variability factors include preexisting caffeine intake from other sources (beverages, foods, supplements), other stimulant drug intake (ephedrine, over-the-counter, or prescription drugs), other drug or hormone use (e.g., oral contraceptives), smoking, stress (heat stress, exercise, other stress), sleep deprivation status, and relevant health conditions (e.g., hypertension, anxiety disorder).

Ideally, a composite quantitative dosing scale (composite caffeine intake index) could be developed using parametric analysis to take into account differences in state and trait variations in individual sensitivity to caffeine. Such a scale could be applied to determine more quantitatively the amount of caffeine that should be administered to individuals in specific contexts for improvements in cognitive function with minimal side effects based on individual differences in preexisting trait sensitivity as well as state variability factors.

If the military adopts the use of caffeine to preserve cognitive performance and vigilance in special and sustained operations, consideration should be given to soldiers' information needs as well as to potential safety issues and ethical concerns. An order to use caffeine may be appropriate to ensure the safety of personnel and the success of a military operation. The committee endorses the

concept that the command structure at the lowest level should have the authority to require the consumption of caffeine if, in their judgment, the welfare of the war fighters or the success of a mission would otherwise be at risk. Nevertheless, the dosage of caffeine should include some element of individual choice. That is, the individual should be permitted to control the intake of caffeine much like the civilian population does when consuming coffee or caffeine-containing soft drinks, on the basis of perceived need to sustain performance.

EDUCATION AND TRAINING ISSUES

An education or information component should be a crucial part of any program to provide military personnel with caffeine in order to facilitate an informed decision process. This information component should include potential benefits, methods of implementation including the timing and the dose that may be effective, potential dangers of misuse, physiological symptoms of excessive intake, and the potential dependence (and subsequent withdrawal symptoms) associated with continued moderate to high levels of use. This information component should also include opportunities for consumption of products that contain added caffeine in a controlled situation so that each individual will be aware of how a product impacts his or her own performance and mood prior to use in an operational situation.

Training of command personnel is also essential to assist them in making decisions about when the products are appropriate to use, directions for their use, and any potential adverse effects resulting from misuse.

LABELING

Any nontraditional product that is used as a vehicle for providing caffeine to military personnel (particularly if it could possibly reach civilian hands) should be prominently labeled, including on the principal display panel, that the product contains added caffeine and is intended for use only during sustained or special operations. The label should also contain the amount of caffeine per recommended serving (e.g., stick of gum) and the total amount per package or container. Appropriate doses should be clearly labeled and the product (e.g., chewing gum, food/energy bar, or tablet) should be scored or metered to facilitate obtaining a certain dose (e.g., 100 mg).

The Food and Drug Administration (FDA) has determined that there is no evidence to show a human health hazard arising from the use of caffeine and has approved its use as a food additive with a provisional listing status. Thus the committee believes that a warning statement is not necessary and could lead to unnecessary concern on the part of military personnel instructed to use the product. However, prominent information statements on the misuse of the product

are needed. For example, it is recommended that the label display instructions for use that provide the maximum single dose and the frequency of dosing.

ETHICAL CONSIDERATIONS

The committee has deliberated the potential ethical issues associated with the use of caffeine or other stimulants in military operations. In the committee's judgment, it is unethical to coerce any individual to consume caffeine, if for religious or health reasons that individual does not wish to consume stimulants. The committee is aware that it is the military custom for the individual and his or her superior to discuss concerns of this nature and secure consultation of the unit's surgeon if a waiver is necessary.

There are also ethical concerns when caffeine, which is a legal and acceptable substance in American society, is denied in the course of a military operation simply because of a logistical decision. In such situations heavy users are likely to undergo withdrawal symptoms and may put themselves and other war fighters at increased risk due to fatigue, sleepiness, loss of attentiveness, and other withdrawal symptoms.

Although clinical studies do not provide evidence of acute side effects from caffeine consumption in the range under consideration, there seems to be an abundance of anecdotal information that some individuals have significant discomfort when consuming levels of caffeine equivalent to that found in 1 cup of coffee. It is recommended that research be done with volunteers who have been so identified to determine if there is a small segment of the population that may have an increased sensitivity to caffeine.

ALTERNATIVES TO CAFFEINE FOR MAINTENANCE OF COGNITIVE PERFORMANCE

There are a number of possible alternatives to caffeine for maintaining cognitive performance during sustained operations. These include naps and the use of various prescription drugs, which are discussed below.

Naps

Decrements in cognitive behavior due to sleep deprivation can best be reversed by providing sleep. There is a dose effect for the restorative effects of sleep on cognitive performance (Bonnet, 1999; Bonnet and Arand, 1994a; Bonnet et al., 1995). Any amount of sleep from as little as a 15-minute nap can restore some degree of function, although the longer the sleep episode, the greater the amount of cognitive function restored (Bonnet et al., 1995). Since the drive for sleep is governed both by a homeostatic drive and a circadian drive, which are interactive (Wyatt, 1999), these factors must be taken into consideration in

determining the timing of naps and their effectiveness in reconstituting mental functioning. Naps are effective both prior to (prophylactic naps) and during (restorative naps) a period of sleep deprivation (Bonnet, 1999; Bonnet and Arand, 1994b; Bonnet et al., 1995). However, the quality of sleep differs between prophylactic naps and naps taken during sleep deprivation. Despite the fact that prophylactic naps are associated with longer sleep latencies and less deep sleep than post-deprivation recovery sleep (Bonnet, 1999), studies by Dinges et al. (1987) demonstrated that prophylactic naps were more beneficial than restorative naps. They concluded that napping prior to a period of extended wakefulness was more important than circadian placement of the nap. A negative side effect of naps during a period of sleep deprivation (restorative naps) is sleep inertia, a short period of mental confusion upon awakening which can last as long as 30 minutes.

A combination of caffeine and naps is the most effective nonprescription drug alternative to caffeine administration alone when a normal sleep regimen is not possible. In a series of studies (Bonnet 1993; Bonnet et al., 1995; Horne and Reyner, 1996) the combination of a short nap and caffeine significantly decreased driving impairment, subjective sleepiness, and drowsiness as measured by electroencephalogram (EEG) activity. The combination of a nap and caffeine also increased alertness during long periods of sleep deprivation compared to either caffeine or naps alone. Thus wherever possible commanders should incorporate strategies that can provide short naps with the use of caffeine for maintaining vigilance, alertness, and other physiological and cognitive functions that are needed for sustained operations (SUSOPS). Placement of the nap as early as possible in the sleep deprivation period, followed by caffeine administration during circadian troughs, would be most effective (Bonnet et al., 1995).

A number of potential alternatives to caffeine (other than sleep) for maintenance of cognitive performance were examined. The use of stimulants, other than caffeine, most frequently referred to in the scientific literature were the drugs pemoline, modafinil, and dextroamphetamine. Although methylphenidate, a drug very similar to amphetamine, is mentioned below, no studies were found in which it was used to enhance or maintain cognitive performance in normal, healthy individuals.

Pemoline

Pemoline is a central nervous system (CNS) stimulant structurally dissimilar from the amphetamines and methylphenidate. It is an oxizolidine compound with poor aqueous and lipid solubility. It is absorbed slowly from the gastrointestinal tract and, in adults, reaches peak plasma concentrations within 2 to 4 hours of administration. The half-life is about 11 to 12 hours, and more than 90 percent of an oral dose is excreted in the urine, with 40 to 50 percent excreted as unchanged drug (Anonymous, 1992b; Sallee et al., 1992; Vermeulen et al.,

1979). Pemoline has a pharmacological activity similar to other CNS stimulants but with minimal sympathomimetic effects. Although the exact mechanism of action is not known, pemoline may act through dopaminergic mechanisms (Molina and Orsinger, 1990; Nicholson and Pascoe, 1989).

Until around 1990 the primary indication for this drug was as a treatment for attention deficit disorder in children and adolescents. However, due to the considerable individual variation in onset and duration of action, its use was secondary to that of methylphenidate and *d*-amphetamine. Subsequently, it has also been evaluated for the treatment of narcolepsy and excessive daytime sleepiness (EDS).

Since 1990 pemoline has been investigated, primarily by the British Royal Air Force, as a means of maintaining cognitive function during intensive and sustained military operations. Effects of pemoline on cognitive performance during 64 hours of sleep deprivation (Babkoff et al., 1992; Gomez et al., 1993) varied depending on the administration protocol. With single-dose administration, pemoline improved performance on Matrix Pattern Recognition and a tapping test, but had no effect on the speed of reaction. However, on a maintenance protocol (pemoline administered every 12 hours during 64 hours of sleep deprivation), there was less effect on accuracy but a significant improvement in speed of response. Naitoh et al. (1992) compared the effects of prophylactic naps, no naps, pemoline (37.5 mg every 12 hours for a total dose of 200 mg), and placebo during a continuous 64-hour work period. Changes in performance were measured with a four-choice serial reaction time test. Significant benefits in counteracting fatigue and sleep loss were found both for naps and for pemoline, with pemoline showing only a minimal loss in reaction time compared to the no-nap and placebo groups.

Nicholson and Turner (1998) evaluated the effects of pemoline at doses of 10, 20, 30, and 40 mg on subjective alertness of volunteers during a 12-hour overnight work period, using a battery of cognitive function tests. A 6-hour prework sleep period and a 4-hour recovery sleep period were monitored. Pemoline significantly improved subject alertness and performance on all tasks except mental arithmetic and first basic reaction time compared with placebo. The first effects of pemoline were observed 4.5 hours after ingestion for the highest dose on digit symbol substitution and sustained attention reaction time. Positive effects of the lower doses were not seen until 6 hours postingestion, and maximal effects of the drug occurred 9 hours post-ingestion. Both the 30- and 40-mg doses impaired recovery sleep.

Pemoline appears to have limited abuse or dependence potential. In animal studies, pemoline was not self-administered, either in naive or in cocaine-dependent animals (Langer et al., 1986). However, an earlier study by Nicholson et al. (1980) found that 60 or 100 mg of pemoline significantly reduced sleep duration and percentage of rapid eye movement (REM) sleep, and increased the delay to the first REM period. Both doses shortened and fragmented sleep.

Adverse effects that have been most frequently reported from the use of pemoline include hepatic dysfunction and dyskinetic movements of tongue, lips, face, and extremities.

The onset of beneficial cognitive effects from pemoline is quite slow, ranging from 6 to 9 hours postingestion for 10-, 20-, and 30-mg doses. Higher doses have a more rapid onset but also interfere with recovery sleep. Plasma clearance rates are also relatively slow, with a half-life of 11–12 hours. In addition, it is recommended that liver function tests be conducted prior to the use of pemoline because of potential side effects of the drug. Results published to date on aspects of cognitive performance improved by pemoline are confusing.

Modafinil

Modafinil is a benzhydrylsulfinylacetamide derivative first synthesized and produced in France in 1986 as a treatment for narcolepsy that would achieve alertness effects equivalent to those of amphetamine but without impairment of sleep. In the United States modafinil was designated as an orphan drug (for the treatment of EDS in patients with narcolepsy) in 1993. It was approved by FDA for this purpose in 1998, and is classified as a Schedule IV controlled substance (FDA, 1999).

Absorption of modafinil occurs at a rate similar to that of pemoline, with peak plasma concentrations occurring 2–4 hours after oral administration and increasing linearly with doses from 200 to 600 mg. The extent of modafinil absorption was not significantly affected by the presence of food, but the rate of absorption was slightly reduced in fed versus fasted subjects (Moachon et al., 1996). Modafinil has low aqueous solubility and approximately 60 percent is bound to serum proteins, primarily albumin. It is metabolized extensively by the liver to inactive metabolites, modafinil acid (primary form), and modafinil sulfone. The plasma half-life of modafinil after 7 days of dosing was 9–14 hours. Modafinil is excreted in the urine primarily as modafinil acid; only about 10 percent of the dose is excreted unchanged (Moachon et al., 1996).

The mechanism of action of modafinil has not been clearly established, but evidence suggests that it may indirectly increase wakefulness, at least in part, by decreasing gamma-aminobutyric acid-mediated neurotransmission (McClellan and Spencer, 1998), or increasing the secretion of the neuropeptide orexin (Chemelli et al., 1999). Modafinil induces wakefulness and increases locomotor activity in a variety of animal species without causing stereotyped behaviors (Hermant et al., 1991; Lin et al., 1992; Nicolaidis and De Saint Hilaire, 1993). Evidence suggests that the site of action of modafinil differs from that of amphetamines and methylphenidate (Lin et al., 1996), and studies in rats have indicated a potential mechanism of action for modafinil through the stimulation of excitatory amino acids in the cerebral cortex (Piérard et al., 1995). Edgar and Seidel (1997) compared the effects of equivalent doses of modafinil and amphetamine on

wakefulness and motor activity in rats and found that modafinil was equal to amphetamine in potently promoting wake time, but did not increase locomotor activity or produce rebound hypersomnolence. The authors concluded that the specificity of modafinil's wake-promoting effects further differentiated it from classical psychomotor stimulants such as amphetamine and methylphenidate.

In a study with cocaine stimulus-trained monkeys and rats, Gold and Balster (1996) evaluated the abuse potential of modafinil, using amphetamine and ephedrine as positive controls. In both rats and monkeys, modafinil did exhibit reinforcing and discriminative stimulus effects, but only at the highest doses tested (0.3 mg/kg). Modafinil was over 200 times less potent than amphetamine and was also less potent than ephedrine.

Broughton and coworkers (1997) compared the effectiveness of placebo to 200 or 400 mg of modafinil in 75 patients meeting international diagnostic criteria for narcolepsy. Compared to placebo, modafinil significantly increased mean sleep latency, with no significant difference between the two doses. Modafinil also reduced the number of daytime sleep episodes and periods of severe sleepiness without interfering with nocturnal sleep initiation, maintenance, or architecture. There were also no changes in blood pressure or heart rate in either normotensive or hypertensive patients. The 400-mg dose of modafinil was associated with increased reports of nausea and nervousness compared to placebo or the 200-mg dose.

A number of studies have examined the effects of modafinil on cognitive performance and sleep recovery in sleep-deprived, but otherwise healthy, volunteers. Modafinil (300 mg) was as effective as 20 mg of dextroamphetamine in maintaining both subjective estimates of mood and fatigue and objective measures of reaction time, logical reasoning, and short-term memory when administered three times during 64 hours of sleep deprivation (Pigeau et al., 1995). Effects on recovery sleep were also monitored in this study, and results indicated that the effects of amphetamine on recovery sleep were similar to those reported previously, with increased sleep latency, decreased total sleep time, decreased REM sleep, and reduced sleep efficiency. Results from the modafinil group exhibited decreased time in bed and sleep period time, with fewer sleep disturbances during the first night of recovery sleep compared to the amphetamine group. There was no effect of modafinil on REM sleep during the first night of recovery sleep, and the second night of recovery sleep did not differ from placebo. Thus, modafinil allowed sleep to occur, displayed sleep patterns close to placebo, and decreased the need for a long recovery sleep to compensate for total sleep deprivation (Buguet et al., 1995). Stivalet et al. (1998) compared the effects of 300 mg of modafinil every 24 hours to placebo in healthy individuals during 60 hours of sleep deprivation. This experiment used the visual search paradigm for assessing speed and accuracy in target detection. Rapid search rates remained unchanged for placebo and modafinil; however, slow search rates increased linearly in the placebo condition with increasing time

without sleep, but remained the same as rested controls with modafinil. The number of errors also increased with placebo but remained the same for modafinil. Batejat and Lagarde (1999) examined the effects of 200 mg of modafinil in conjunction with naps on performance during two 27-hour periods of sleep deprivation. The effects of modafinil on cognitive performance during sleep deprivation suggested the compound may act at two levels. First, modafinil maintains an efficient level of CNS general activation close to awakening, and second, it seems also to have a more specific action on neurophysiological mechanisms underlying short-term memory. As in previous studies, modafinil did not prevent sleep if sleep opportunities were available.

Caldwell and coworkers (1999) recently reported on the use of modafinil in a helicopter simulator study with pilots exposed to two 40-hour periods of sleep deprivation. Three 200-mg doses of modafinil or placebo were administered during the 40-hour period. Modafinil significantly attenuated the effects of sleep deprivation on four of six flight maneuvers: straight and levels, straight descent, left standard-rate turns, and left descending turns, maintaining them at baseline levels. Modafinil also reduced the amount of slow-wave EEG activity (indicative of reduced CNS activation), lessened self-reported problems with mood and alertness, and curtailed the performance decrements that were found with placebo. According to these researchers, the most noticeable benefit of the drug was seen when the combined impact of sleep loss and circadian trough was most severe. As in other studies, no disruptions in recovery sleep architecture were observed.

Compared with other well-known stimulatory substances such as caffeine and amphetamine, modafinil appears to have the advantage of combining wakening and stimulating properties, with an appreciable absence of unwanted side effects.

Modafinil does appear to have some abuse potential at high doses, but it was 200 times less potent than amphetamines in this regard. Modafinil has been used as long as 3 years in the treatment of narcolepsy without signs of drug dependence. No toxic effects of high levels of modafinil have been observed in animals.

A dose of 200 mg appears to be quite effective as a single dose for short periods (24 hours) of no sleep or repeated at 12-hour intervals during long periods of sleep deprivation. Maximum effectiveness occurs about 4 hours postingestion and is most beneficial when modafinil is administered during circadian troughs.

Amphetamine

Amphetamines are sympathomimetic amines with CNS stimulant activity. CNS effects are mediated by release of norepinephrine from central noradrenergic neurons. At higher doses, dopamine may be released in the mesolimbic system. Peripheral alpha- and beta-adrenergic activity includes elevation of systolic and diastolic blood pressures and weak bronchodilator and respiratory stimulant activity.

Following oral administration, amphetamines are completely absorbed within 3 hours and widely distributed throughout the body with high concentrations in the brain. Peak plasma levels may occur within 2–3 hours, and maximum effects are reported in the second hour (Angrist et al., 1987).

Amphetamine is metabolized in the liver by aromatic hydroxylation, N-dealkylation, and deamination. Urinary excretion of the unchanged drug is pH-dependent. Urinary acidification to pH below 5.6 yields a plasma half-life of 7–8 hours; alkalinization increases half-life (18–34 hours). For every one unit increase in urinary pH, there is an average 7-hour increase in plasma half-life (Anonymous, 1992a). Although the elimination half-life of amphetamines normally exceeds 10 hours, its behavioral and subjective effects show a clear decline after 4 hours (Angrist et al., 1987).

Studies of the effects of amphetamines on human performance began over 60 years ago. Reviews of the effects of amphetamines on daytime alertness of well-rested subjects show conflicting results. Overall, well-rested individuals appear to benefit little from amphetamines (Spiegel, 1979).

In situations of reduced alertness (e.g., sleep deprivation, night work, sustained-attention tasks), amphetamines have proven to be potent in reversing or preventing performance decrements (Akerstedt and Ficca, 1997). Newhouse et al. (1989) evaluated the effects of placebo, 5, 10, or 20 mg of *d*-amphetamine during 60 hours of sleep deprivation. The drug was administered 48 hours into the deprivation period, after which sleep latency, behavioral parameters, and cognitive performance were measured. The 20-mg dose returned sleep latency to baseline for 7 hours postadministration. It also improved accuracy on attentional arithmetic tests and improved performance on verbal reasoning tasks. The lower doses were less effective.

In extensive helicopter simulator and in-flight testing, 10 mg of *d*-amphetamine was observed by investigators (Caldwell and Caldwell, 1997; Caldwell et al., 1995, 2000) to "improve subjective feelings of fatigue, confusion, and depression while increasing feelings of vigor" compared to placebo during 40 hours of sleep deprivation. Amphetamine improved performance of flight maneuvers both in a flight simulator and in actual test flights. Performance was most noticeably improved in the early morning hours following 24 hours of sleep deprivation. Both EEG data and mood ratings showed that alertness was significantly maintained with amphetamine. In a comprehensive follow-up study, Caldwell et al. (2000) administered 10 mg *d*-amphetamine or placebo three times on each of two sleep-deprivation days (a total of 64 hours of sleep deprivation). Amphetamine sustained flight performance, physiological arousal, and mood throughout the 64-hour period. There were no clinically significant side effects attributable to amphetamine. Some of the aviators complained of palpitations and "jitteriness" with amphetamine, but this did not detract from their flight performance.

In another study of 64 hours of sleep deprivation with 40 subjects in a continuous work environment, 20 mg of *d*-amphetamine, administered on three occasions during the period, significantly improved objective measures of reaction time, logical reasoning, and short-term memory (Pigeau et al., 1995).

All of these studies have demonstrated serious impacts of amphetamine on recovery sleep. At least two uninterrupted 8-hour nights of recovery sleep were needed (Buguet et al., 1995; Caldwell et al., 2000). Amphetamine reduces the amount of REM sleep and reduces sleep efficiency.

Because of the powerful effects of low doses of amphetamine against sleep loss, the military has had considerable interest in the use of amphetamines in sustained operations.

This interest is not new, however. Stimulants were used by both British and German aviators during World War II. Senechal (1988) reported on the use of amphetamine by British Royal Air Force pilots in connection with the Libyan air strike. Emonson and Vanderbeek (1995) reported on the incidence and effectiveness of amphetamine use by U.S. Air Force pilots during operations Desert Shield and Desert Storm. Both the U.S. Air Force (2001) and the U.S. Navy (2000) currently have official memoranda and protocols in place that provide detailed guidance on the use of amphetamines by aviators during continuous operations.

Amphetamine is a controlled substance and thus requires an individual medical evaluation to determine risk factors and health status before a prescription can be issued. Nevertheless, it is possible with appropriate supervision and control that amphetamine could show promise of providing benefits to individuals with unique skills whose performance is critical to the safety of military personnel and complex military hardware.

Amphetamines are very effective in maintaining alertness, cognitive performance, and mood during extended periods of sleep deprivation. These effects can be achieved at low doses (5–10 mg) where adverse side effects are minimal or nonexistent. The military has considerable experience in the use of this stimulant in combat operations, and both the Navy and the Air Force have protocols for use already in place. To date, this use has been restricted to aviators during sustained flight operations.

Amphetamine has a pronounced detrimental effect on recovery sleep that can last two or more nights. In contrast to caffeine in food, beverages, chewing gum, and pill or tablet form, most military personnel have little experience with amphetamine pill self-dosing and the hazards and adverse effects of self-dosing might therefore be expected to be greater. For all the armed services it would be preferable if maintenance of cognitive performance can be achieved without such substances. The potential for abuse of amphetamines is considerable, and appropriately monitoring its dispensing and use may add unnecessary burdens to personnel in the intense and demanding tasks that are directly involved in guaranteeing the success of SUSOPS. Although amphetamine (10 or 20 mg) was more effective in reversing the negative effects of sleep deprivation on alertness

than caffeine at doses of 300 mg, it had deleterious effects on recovery sleep, which may also be important in the ultimate success of demanding and constantly changing SUSOPS (Bray et al., 1999). Amphetamine should not be considered a substitute for sleep, and more nights of recovery sleep are needed after its administration (Caldwell, 1999). Therefore, considerable caution is warranted, and use of this stimulant should be restricted to only those extreme circumstances when such measures are considered essential to the success of highly sensitive operations.

The use of modafinil in place of amphetamines under these special circumstances should be explored thoroughly. Research to date indicates that the potential for abuse of modafinil is considerably less than for amphetamines (about 200 times less), and modafinil does not affect initiation of recovery sleep. The committee recommends that modafinil receive more evaluation in simulated military operations before operational testing of the drug.

Prescription drugs in the United States can be prescribed for off-label uses by physicians. In the case of modafinil, which is approved as a wakefulness-promoting drug, its use in healthy individuals as opposed to narcoleptics would be an off-label use. However, there is ample precedence for off-label use of drugs by military medical personnel, as in the limited prescription of dextroamphetamine to pilots on long-range missions and the use of pyridostigmine bromide as a pretreatment to protect troops from the harmful effects of nerve agents during the Gulf War.

SUMMARY

Any program designed to provide caffeine to military personnel should allow individual control of dosage and include an education and training component. Personnel should have the opportunity to experience the dose to be used in a nonoperational situation. Commanders should be advised of when caffeine use is appropriate as well as signs of adverse effects due to excessive dosing. Caffeine-supplemented products should be clearly labeled as such, along with instructions for use.

Ethical considerations would include the question of forcing caffeine use when an individual has strong religious or health reasons for not doing so. Conversely, denying access to caffeine for habituated users either for logistical reasons or in expectation of a subsequent need for a caffeine supplement could risk decrements in performance due to caffeine withdrawal.

Alternatives to caffeine for maintaining cognitive performance include use of naps or prescription drugs. The prescription drugs that have been evaluated for use in healthy adults undergoing sleep deprivation are pemoline, amphetamine, and modafinil. Of these three compounds, modafinil and amphetamine have been shown to be superior to pemoline. Modafinil is as effective as am-

phetamine, but does not interfere with recovery sleep and has significantly less potential for abuse.

7

Response to Military Questions, Conclusions, and Recommendations

This chapter presents the committee's responses to the specific questions posed by the military by briefly reviewing the pertinent information provided in the earlier chapters. It then presents the committee's conclusions and recommendations.

1. Efficacy: Does the Committee on Military Nutrition Research stand by its earlier recommendation that there are sufficient data to recommend a caffeine product to enhance performance? What are the specific indications for use and contraindications for use?

Caffeine has been shown to induce a variety of positive effects that have contributed to its extensive use worldwide. Caffeine use has been associated with enhanced physical performance and increased alertness, and as a countermeasure to the effects of sleep deprivation. Extensive research has been done on each of these caffeine effects.

Caffeine use is associated with a reproducible increase in endurance time in physical activities of moderate intensity and long duration. Caffeine enhances endurance performance in a variety of activities (i.e., running, cross-country skiing, cycling), with doses in the range of 2 to 9 mg/kg (approximately 150–650 mg in a 70–72 kg individual), in both naive and habituated, trained and untrained, test subjects. High-altitude exposure may augment the positive effects of caffeine on endurance performance. Exercise performance is dramatically reduced by altitude exposure, and maximal effort may be diminished by as much as 25 percent. Ingestion of caffeine (4 mg/kg) increased the time to exhaustion

in eight trained men riding a cycle ergometer at 4,300 m, but not at sea level. This positive effect was present after 1 hour of altitude exposure and tended to remain even after 2 weeks of acclimatization.

There is some debate about whether caffeine enhances cognitive performance or simply restores degraded psychomotor performance in rested individuals. The majority of studies that have examined the effects of caffeine in rested subjects studied moderate caffeine consumers (200–300 mg of caffeine per day) who were required to abstain from caffeine for some period of time prior to cognitive testing. Some researchers have speculated that for regular caffeine users, this abstinence could have resulted in some degree of withdrawal. Thus, beneficial effects on cognitive behavior may represent remediation of deteriorated performance during caffeine withdrawal back to baseline performance in the presence of caffeine rather than a net enhancement of performance.

A number of studies have demonstrated that caffeine enhances cognitive performance independent of its ability to reverse symptoms of withdrawal (see Chapter 3). Caffeine can enhance performance on some types of cognitive tasks and some aspects of mood in nonsleep-deprived individuals. Caffeine enhanced accuracy and reduced reaction time on auditory and visual vigilance tasks in a dose-related manner. Moreover, caffeine significantly increased self-reports of vigor and decreased reports of fatigue, depression, and hostility on the Profile of Moods Scale. Self-assessments of energy levels were also improved by caffeine. In a simulated military situation involving a tedious task that required sustained attention for proficient performance (i.e., sentry duty), caffeine eliminated the vigilance decrement that occurred with increasing time on duty, reduced subjective reports of tiredness, and did not impair rifle firing accuracy. Additionally, in this situation, caffeine increased the number of correct target identifications in both males and females.

Military personnel face many situations in which extended wakefulness may be required including sentry duty, deployment-related activities, air transportation during emergencies, radar and sonar monitoring, submarine duty, and combat. As part of their duties in these situations, individuals are required to perform complex cognitive tasks. The performance of these tasks is compromised during periods of extended wakefulness.

A variety of instruments have been used to quantify the effects of sleep deprivation on behavior. Alertness has been assessed using objective measures such as ambulatory vigilance monitors, visual and auditory vigilance tasks, and subjective measures such as self-reports and questionnaires. Studies using these measures have found that sleep deprivation impairs performance on vigilance tasks and decreases self-reports of alertness. A number of mental tasks, including mental arithmetic tasks, such as a serial add–subtract test, logical reasoning, mental rotation, perceptual cueing, and memory tests have been used to assess the effects of sleep deprivation on higher cognitive processes. Using these tests, mental performance deteriorates as a function of length of sleep deprivation.

All of the above-listed decrements in cognitive behavior can best be reversed by sleep. Any amount of sleep from as little as a 15-minute nap can restore some degree of function, although the longer the sleep episode, the greater the amount of cognitive function restored. Naps are effective both prior to (prophylactic naps) and during (restorative naps) a period of sleep deprivation. The only negative side effect of sleep in this context is sleep inertia, a period of mental confusion upon awakening from such naps that may last up to 30 minutes.

In sleep-deprived subjects, judicious use of caffeine can restore alertness, performance on mental tasks, and positive mood states. Caffeine reversed the sleep deprivation-induced increased response time, and increased alertness and performance on a visual vigilance task, mental arithmetic tests, and logical reasoning in sleep-deprived subjects. Caffeine is also effective in delaying sleep onset in sleep-deprived subjects. Using visual analog scales, caffeine intake led to reports of decreased sleepiness and increased alertness, ability to concentrate, confidence, talkativeness, energy levels, anxiety, jitteriness, and nervousness.

Conclusions

Caffeine at levels ranging from 200 to 600 mg/d enhances endurance performance in a variety of activities. Limited research has shown caffeine to be especially useful in restoring decrements in physical performance that occur at high altitudes. Food and fluid intake have to be monitored carefully when caffeine is used for this purpose, particularly at high altitudes and in hot environments. The documented declines in food intake during special operations would be of particular concern if food is the delivery vehicle chosen for administering caffeine.

Although there is considerable variation in doses tested and subject responses to the effects of caffeine on cognitive function, overall research shows that caffeine in the range of 100 to 600 mg is effective in increasing speed of reaction time without affecting accuracy and in improving performance on visual and audio vigilance tasks. A number of studies have also reported improved performance on long-term memory recall, but not short-term word recall. These enhancing effects of caffeine on cognitive performance are most pronounced when functions are impaired or suboptimal (e.g., as a result of sleep deprivation).

Recommendations

Caffeine in doses of 100 to 600 mg may be used to maintain cognitive performance, particularly in situations of sleep deprivation. Specifically, it can be used in maintaining speed of reactions and visual and auditory vigilance, which in military operations could be a life or death situation.

A similar dose range (200–600 mg) is also effective in enhancing physical endurance and may be especially useful in returning some of the physical function lost at high altitude. However, if caffeine is used either at high altitudes or

in extremely hot environments, fluid and food intake of personnel should be monitored to ensure adequate intake.

2. Safety: What are the medical risks to individuals associated with ready availability of caffeine, including acute health risks, long-term health risks, potential interaction with other drugs or factors specific to military operations, and potential problems of habituation of use?

The effect of caffeine on various aspects of health has been and continues to be an active area of scientific research, in spite of the fact that caffeine has been used for more than 1,000 years without apparent ill effects. Over the past 100 years, the list of diseases in which caffeine has been implicated has changed. Convincing research evidence has removed several diseases from consideration, including various cancers, hypercholesteremia, and benign breast disease. Extensive research also has evaluated the impact of caffeine consumption on the incidence of hypertension, cardiovascular disease, reproduction and pregnancy outcome, osteoporosis, and fluid homeostasis. It has been shown that ingestion of very high doses of caffeine can produce undesirable effects on mental function. Additionally, caffeine use has been associated with physical dependence, which may be reflected in performance decrements during withdrawal under some circumstances.

Hypertension

Results summarized in recent reviews by Myers (in press) and Green and Suls (1996) suggest that caffeine-naive individuals may experience a small increase in blood pressure after acute dosing with caffeine. During chronic administration of caffeine, tolerance appears to develop, and chronic, long-lasting changes in blood pressure are usually not seen in individuals who consume caffeine routinely. A recent critical review of 30 years of controlled clinical and epidemiological studies on the blood pressure effects of coffee and caffeine (Nurminen et al., 1999) concluded that the acute pressor effects of caffeine are well documented, but that at present there is no clear epidemiological evidence that caffeine consumption is causally related to hypertension. They also concluded, however, that high caffeine intake may be an additional risk factor for hypertension at the individual level due to long-lasting stress or to a genetic susceptibility to hypertension.

Caffeine consumption has also been demonstrated to potentiate the effects of acute exercise and mental stress in increasing blood pressure. This effect of caffeine is more pronounced in those with high stress reactivity (i.e., high levels of anxiety) and those who are borderline hypertensive or are hypertensive.

Cardiovascular Disease

In spite of numerous studies (including controlled clinical trials) attempting to show a relationship between caffeine and serum lipoproteins, blood pressure, cardiac arrhythmias, and risk of coronary heart disease, results have failed to show a consistent adverse effect of ingestion of moderate amounts of caffeine. Whereas case-control studies have produced variable results, a meta-analysis of 11 prospective, longitudinal cohort studies showed no increased risk of coronary heart disease associated with consumption of up to 6 cups of coffee per day. Thus, increased risk of cardiovascular problems resulting from the use of caffeine supplements by the military would not appear to be of major concern in most cases.

Reproduction

Caffeine consumption has also been suggested as the cause of numerous negative reproductive outcomes, from shortened menstrual cycles to reduced conception, delayed implantation, spontaneous abortion, premature birth, low birthweight, and congenital malformations. As with most other aspects of caffeine consumption, there is a paucity of reliable data concerning the effects of caffeine on reproductive processes.

More recent reviews of human studies suggest that some of the initial reported associations between caffeine and reduced fertility, teratogenicity, and other fetal and maternal effects in humans may be explained by confounders such as associated cigarette smoking, alcohol consumption, reporting inaccuracies, and other methodological errors.

A recent well-controlled study of 487 women with spontaneous abortions and 2,087 normal controls, in which caffeine exposure was quantitated objectively by serum paraxanthine levels, showed that the mean serum paraxanthine concentration was significantly higher in women who had spontaneous abortions than in controls (752 versus 583 ng/mL). However, the odds ratio for spontaneous abortion was not significantly increased except in subjects with extremely high paraxanthine levels (> 1,845 ng/mL). These authors concluded that moderate consumption of caffeine was not likely to increase the risk of spontaneous abortion.

Osteoporosis

Caffeine consumption has been proposed as a risk factor for osteoporosis. The original observations stimulated several epidemiological studies that examined the possible relationship among caffeine consumption, calcium intake, and various indices of bone health. There appears to be no consistent trend linking caffeine consumption and negative effects on bone mineral density or incidence of fracture.

Although early experimental studies also indicated a significant effect on acute calcium diuresis, subsequent work indicated that this acute phase of excretion was accompanied by a later decrease in excretion of calcium in the urine. Moreover, later studies found either no significant effect of caffeine on calcium balance or negative balance only in subjects consuming less than about 660 mg of calcium per day, or half of the currently recommended intake of calcium.

Fluid Homeostasis

Consumption of 2,500 mL of a carbohydrate–electrolyte solution containing approximately 1 mg of caffeine per kg body weight increased 3-hour urine output by over 400 mL as compared to the same amount of solution without caffeine (Wemple et al., 1997). While this level of caffeine was too low to produce a positive effect on cycling performance, the fact that urine volume was affected could be of significance in military situations where considerably higher caffeine doses may be used. An oral dose of 250 mg of caffeine increased diuresis, sodium, potassium, and osmol excretion within 1 hour post-treatment (Nussberger et al., 1990), while amounts of coffee sufficient to provide 642 mg of caffeine in a single day caused a highly significant increase in 24-hour urine output of 753 ± 532 mL compared to an identical amount of fluid provided as mineral water. Total body water as measured by bioelectrical impedance decreased 2.7 percent, and sodium and potassium excretion increased by 66 and 28 percent, respectively (Neuhauser-Berthold et al., 1997). The information to date is inconsistent, indicating that caffeine may or may not create a total body water deficit. The deficit may depend on the amount of caffeine consumed, the individual's history of caffeine use, and the total solute load of any accompanying food or beverage. However, the risk of water deficit may be increased when caffeine is used in situations already known to put personnel at risk of dehydration, such as in hot or desert environments (IOM, 1993), or in cold environments (IOM, 1996).

Behavioral Effects

One potential risk of high doses of caffeine, which needs further substantiation, is dose-related decrements in mental functioning. A number of researchers have found that high doses of caffeine can adversely affect mental performance. Although a relatively low dose of caffeine (250 mg) produced favorable subjective effects (e.g., elation, pleasantness) and enhanced performance on cognitive tasks in healthy volunteers, higher doses (500 mg) led to less favorable subjective reports (e.g., tension, nervousness, anxiety, restlessness) and less improvement in cognitive performance than placebo. Negative effects may be more pronounced in nonusers than in regular users of caffeine. Caffeine has been shown to produce anxiety or panic attacks in individuals with agoraphobia or panic disorders, but not in healthy controls. However, caffeine has been shown to potentiate hormonal responses to other stressors.

Physical Dependence and Withdrawal

The use of caffeine by humans is generally not associated with abuse or addiction. Tolerance develops to some of the physiological effects of caffeine when caffeine-containing beverages are consumed regularly; however, there have been no reports of tolerance for caffeine effects on cognitive performance. Withdrawal symptoms can occur with the abrupt removal of caffeine from the diet. The symptoms of cessation, when they do occur, are not long-lasting and are generally mild. These include headaches, drowsiness, irritability, fatigue, low vigor, and flu-like symptoms.

Caffeine acts as a vasoconstrictor of the cerebral arteries, reducing regional blood flow. Caffeine withdrawal also causes changes in cerebral blood flow, resulting in vasodilation in persons with high caffeine intake that is thought to be associated with a throbbing, vascular-type headache, one of the most commonly observed symptoms of caffeine withdrawal. This withdrawal phenomenon could conceivably lead to decrements in performance during military operations.

Caffeine and Stress

Among the variables that may contribute to differences in caffeine sensitivity are baseline levels of stress exposure and genetically mediated stress reactivity. Stress may include physical stress (e.g., exercise), physiological stress (e.g., heat stress, infection, sleep deprivation), or psychological stress. After stress exposures, stress-responsive neurohormonal and neurotransmitter systems are activated, with associated release of the stress hormones and the adrenergic neurotransmitters (epinephrine, norepinephrine, corteotrophin-releasing hormone, adrenocorticotropic hormone, and cortisol), which all interact with caffeine. Caffeine alters the degree of responsiveness of these systems to stressful stimuli. For example, caffeine has been shown to increase plasma norepinephrine and to potentiate epinephrine and cortisol stress-reactivity to acute psychosocial stress. The degree of responsiveness in these studies varied according to previous caffeine consumption (habitual users versus nonusers).

Conclusions

The acute pressor effects of caffeine are well documented, but at present there is no clear epidemiological evidence that caffeine consumption is causally related to hypertension. One potential risk should be noted, however. A number of studies have demonstrated that caffeine consumption produces a transient elevation in blood pressure and that this occurs regardless of whether or not the individual is a habitual user of caffeine. In borderline-hypertensive men, the use of caffeine in situations of behavioral stress may elevate blood pressure to a clinically meaningful degree; it has been hypothesized that these types of blood

pressure increases in hypertensives would be large enough to transiently reduce the therapeutic effects of antihypertensive medication. However, other studies have found no differences in the effect of caffeine in individuals with or without a family history of hypertension, and no difference in 24-hour ambulatory blood pressure in treated hypertensives between caffeinated and decaffeinated coffee. Thus, high caffeine intake may be an additional risk factor for hypertension at the individual level due to long-lasting stress or to a genetic susceptibility to hypertension. Since military scenarios in which the use of caffeine supplements might be desirable would frequently occur when personnel are also under acute mental and/or physical stress, this could be a concern to those personnel with family histories of hypertension.

In spite of numerous studies attempting to show a relationship between caffeine and cardiovascular health, results have failed to show a consistent adverse effect of ingestion of moderate amounts of caffeine. Increased risk of coronary heart disease resulting from the use of caffeine supplements by the military would not appear to be of major concern.

Results of studies of the effects of caffeine on reproduction have been very mixed, and many of those showing increased reproductive problems have been confounded with other life-style factors, particularly smoking. The most convincing evidence relates to caffeine and the increased risk of spontaneous abortion. However, since this requires caffeine consumption during the first trimester of pregnancy, it is likely to be a concern for sustained military operations only if female personnel are unaware of their pregnancy at the time of deployment.

The preponderance of research on caffeine and osteoporosis has found no relationship. Although caffeine can increase calcium diuresis, this is compensated by subsequent lower than normal calcium excretion. The use of caffeine in this case is less of a concern than is low calcium intake.

Caffeine functions as a diuretic, and this effect appears to increase with increasing caffeine level. Evidence is equivocal as to whether acute doses of caffeine cause a total body water deficit. The increased risk of dehydration may be of concern for military personnel in operational environments where dehydration may already be a concern, such as desert environments, or where thirst mechanisms are inadequate, such as in cold or high-altitude environments.

High doses of caffeine (> 600 mg) can cause decrements in cognitive function. Negative effects may be more pronounced in nonusers than in regular users of caffeine. Caffeine can also potentiate the effects of stress.

Recommendations

Use of caffeine under conditions of sustained operations (SUSOPS) would not appear to pose any acute or chronic health risks for military personnel.

Caffeine use in SUSOPS in hot environments, cold environments, or at high altitudes may increase the risk of dehydration, and fluid and food intake of personnel should be closely monitored in these situations.

Female military personnel should be advised of the potential for a slight increase in risk of spontaneous abortion in the first trimester of pregnancy.

3. Dose and Warning Labels: What dose level should be recommended to habituated caffeine users and to nonusers? What warnings should be provided on such a product in the context of ethical, religious, and potential caffeine habituation concerns?

The effective doses of caffeine will vary from individual to individual depending on a variety of factors including time of day, usual caffeine intake, and whether the individual is rested, smokes, or uses oral contraceptives. Doses evaluated experimentally for their effects on both physical and cognitive performance have ranged from as little as 32 mg of caffeine (Lieberman et al., 1987) to as much as 1,400 mg (Streufert et al., 1997).

The levels of caffeine that have consistently enhanced endurance performance, as discussed in Chapter 3, range from about 150 to 600 mg. Numerous studies of the effects of different caffeine dosages on various aspects of cognitive performance have been conducted in both civilian and military situations. Levels of caffeine in the range of 100 to 400 mg have consistently demonstrated reductions in reaction time and enhanced performance on vigilance tests, whereas levels of caffeine in excess of 600 mg have shown negative effects on mood and behavior (negative effects may be seen at lower levels in individuals nonhabituated to caffeine).

In sleep-deprived individuals (similar to those engaging in SUSOPS), levels in the range of 100 to 600 mg of caffeine appear to improve performance (e.g., vigilance, mood, higher cognitive functions) with few acute adverse behavioral effects; some of the positive effects persist for 8–10 hours. Even individuals who do not normally consume caffeine appear to obtain these caffeine-related positive effects.

Important ethical considerations include providing personnel with adequate information and training on the use of the product, providing the opportunity for personnel to test the product in a nonoperational situation, and use of a product that allows individual control of the dosage.

Regular moderate to heavy users of caffeine may experience headaches, fatigue, and drowsiness if denied access to caffeine.

Conclusions

A caffeine dose of 100–600 mg can be expected to improve vigilance and enhance cognitive performance regardless of an individual's normal caffeine status. A delivery mechanism that provides 100-mg dose increments could be

used to allow individuals of smaller body size, nonhabituated caffeine users, and those with heightened sensitivity to caffeine to individually control their dose.

In keeping with the rulings of the U.S. Food and Drug Administration (FDA) with respect to determination of the risks of caffeine as a food additive, no warning label is necessary for a military product designed for maintenance of cognitive performance during sustained operations. However, educational and training information is needed for military personnel prior to the use of such a product.

Recommendations

A caffeine delivery vehicle that provides caffeine in 100-mg increments with a total content not exceeding approximately 600 mg would appear to be the most appropriate dose for use in sustained military operations. Since there is little information regarding the extent to which tolerance to caffeine's cognitive effects in habitual users develops, no differential dosing is recommended for habitual compared to first-time caffeine users. For subsequent dosing, the dosing interval should be based on the considerations that too-frequent dosing might (1) produce a buildup of caffeine (or its primary metabolite, paraxanthine) levels sufficient to precipitate negative effects; and (2) inhibit sleep onset in some individuals at a time when sleep is desired. Since the half-life of caffeine in blood can vary from 1.5 to 9.5 hours, with an average half-life of 4 to 5 hours, a dosing interval of no less than 3 to 4 hours is recommended.

Any product that is used as a vehicle for providing caffeine to military personnel should be prominently labeled. The labeling should include a statement on the principal display panel that the product contains added caffeine and should only be used to maintain performance when involved in SUSOPS or sustained vigilance activities. The message on the principal display panel should direct the user to specific information elsewhere on the label that indicates the level of caffeine per unit of product and the total amount per package or container. An example is shown in Box 7-1. This content information is vital for the command structure to make decisions about directions for use and for individuals to adapt consumption to their individual needs.

An in-depth training program on the benefits, directions for use, and potential side effects or symptoms of excess intake of caffeine should be designed for command personnel. In addition, if caffeine is to be used to enhance performance, military personnel should be given adequate training to ensure the benefits of caffeine supplementation and avoid any potential side effects. Such training should include the use of caffeine during periods of sleep deprivation and possibly altered work–rest cycles. All personnel should test the effects of the recommended dose in a nonoperational situation prior to use in an operational situation.

BOX 7-1 Example of an information statement for a caffeine-containing supplement.

! IMPORTANT !
This product contains 100 mg of added caffeine per unit (e.g., 1 pill, 1 food bar segment, 1 stick of gum) for a total added caffeine content of 600 mg, and is designed for use in maintaining alertness during military operations.
Recommended initial dose is 200 mg.
Do not exceed 600 mg in a single dose.
Do not repeat dose sooner than 3–4 hours.

Military personnel who regularly consume caffeine-containing products should not be denied access to these products in anticipation of the use of a caffeine supplement. Symptoms of withdrawal such as fatigue, decreased alertness, and headaches could cause decrements in performance prior to the consumption of the caffeine supplement.

4. Alternatives: Are there practical alternatives to caffeine that would better serve the intended purpose of enhancing or maintaining performance in fatigued service members?

Sleep is the most effective means of reconstituting the decrements in cognitive functioning brought on by sleep deprivation. Thus, in situations where it is feasible, sleep should be promoted. There is a dose effect for the restorative effects of sleep duration on cognitive performance (Bonnet, 1999; Bonnet and Arand, 1994a,b; Bonnet et al., 1995; Dinges et al., 1987). Any amount of sleep from as little as a 15-minute nap can restore some degree of function, although the longer the sleep episode, the greater the amount of cognitive function restored (Bonnet et al., 1995). Dinges et al. (1987) demonstrated that prophylactic naps were better than restorative naps and were more important than circadian placement of naps.

Combination of Caffeine and Naps

The most effective nonprescription drug alternative to caffeine administration alone when a normal sleep regimen is not possible, is a combination of caffeine and naps. The combination of caffeine and naps increased alertness during long periods of sleep deprivation compared to either caffeine or naps alone. The committee recommends that wherever possible, commanders adopt strategies that incorporate the use of caffeine with short naps for maintaining vigilance, alertness, and other physiological and cognitive functions needed for SUSOPS. According to the literature reviewed in this report, naps taken early in

the period of extended wakefulness followed by caffeine taken around the time of circadian troughs would be most effective.

Amphetamine

In extensive simulator and in-flight testing, amphetamine was observed by investigators (Caldwell and Caldwell, 1997; Caldwell et al., 1995) to "improve subjective feelings of fatigue, confusion, and depression while increasing feelings of vigor". Amphetamine is, however, a controlled substance and thus requires an individual medical evaluation to determine risk factors and health status before a prescription can be issued. With appropriate supervision and control, the use of amphetamine has benefited individuals with unique skills whose performance was critical to the safety of personnel and complex military hardware. In contrast to caffeine in food, beverages, chewing gum, and pill or tablet form, there is little experience with amphetamine pill self-dosing for most military personnel, and the hazards and adverse effects of self-dosing might therefore be expected to be greater. It would be preferable if ergogenic effects can be achieved without such substances. The potential for abuse of amphetamines is considerable, and the appropriate monitoring of its dispensing and use may add unnecessary burdens to medical personnel in the intense and demanding tasks that are directly involved in guaranteeing the success of SUSOPS. Although amphetamine (20 mg) was effective in reversing the negative effects on alertness during sleep deprivation and these effects are greater than those of caffeine at 300 mg, it had deleterious effects on recovery sleep, which also may be important in the ultimate success of demanding and constantly changing SUSOPS (Bray et al., 1999). Amphetamine should not be considered as a substitute for sleep, and more nights of recovery sleep may be needed after its administration (Caldwell, 1999). Therefore, considerable caution is warranted, and use of this stimulant should be restricted to only the most extreme circumstances when such measures are considered essential to the success of highly sensitive operations. Clearly, more research should be done before consideration can be given to the routine use of amphetamine.

Modafinil

Modafinil is a new prescription drug approved by the FDA as a wakefulness-promoting substance. It has undergone evaluation as a treatment for excessive daytime sleepiness (EDS) in narcolepsy (Broughton et al., 1997). This drug appears to be useful in reducing EDS without effecting voluntary nap or nocturnal sleep initiation. These properties suggest that the stimulant may be useful in extending high levels of vigilance in SUSOPS. A number of studies have examined the effects of modafinil on cognitive performance and sleep recovery in sleep-deprived, but otherwise healthy, volunteers. Modafinil (300 mg) was as

effective as 20 mg of dextroamphetamine in maintaining both subjective estimates of mood and fatigue, and objective measures of cognitive performance when administered three times during 64 hours of sleep deprivation (Pigeau et al., 1995), with no effects on recovery sleep (Buguet et al., 1995). Another study compared the effects of 300 mg of modafinil every 24 hours to placebo in healthy individuals during 60 hours of sleep deprivation using the visual search paradigm for assessing speed and accuracy in target detection. Slow search rates and number of errors increased linearly in the placebo condition with increasing time without sleep, but remained the same as rested controls with modafinil (Stivalet et al., 1998). Batejat and Lagarde (1999) examined the effects of 200 mg of modafinil in conjunction with naps on performance during two 27-hour periods of sleep deprivation. Modafinil maintained an efficient level of central nervous system (CNS) general activation close to awakening. As in previous studies, modafinil did not prevent sleep if sleep opportunities were available.

Caldwell et al. (1999) recently reported on the use of modafinil in a helicopter simulator study with pilots exposed to two 40-hour periods of sleep deprivation. Three 200-mg doses of modafinil or placebo were administered during the 40-hour period. Modafinil significantly attenuated the effects of sleep deprivation on four of six flight maneuvers, reduced the amount of slow-wave electroencephalogram activity (indicative of reduced CNS activation), lessened self-reported problems with mood and alertness, and curtailed the performance decrements that were found with placebo. The most noticeable benefit of the drug was seen when the combined impact of sleep loss and circadian trough was most severe.

Conclusions

Providing the opportunity and environment for adequate sleep is the ideal but is obviously impractical for continuous military operations. Combining naps with judicious caffeine use may be the best remedy for sleep deprivation-induced decrements in cognitive function in military situations where adequate sleep cannot be obtained. When naps are not an option, caffeine alone could be used to mitigate sleep deprivation-induced impairments in cognitive behavior for up to 24 hours of sleep deprivation.

The Department of Defense (DOD) has precedents for the use of prescription drugs in healthy individuals, such as the use of amphetamines. Furthermore, in the United States, physicians are permitted to prescribe drugs for off-label use (i.e., for uses not included in the product's labeling).

The use of amphetamine is superior to caffeine in offsetting decrements in cognitive performance; however, the risks outweigh the benefits for most situations. It is a controlled substance, it has a high abuse potential, and it interferes with recovery sleep. In addition, it is assumed that the majority of combat personnel would not have had previous experience with the drug.

The drug modafanil, which was developed more than 10 years ago as a treatment for narcolepsy, shows considerable promise. It appears to be as effective as amphetamines in offsetting performance degradation, does not interfere with recovery sleep, is not an appetite suppressant, and appears to have a much lower abuse potential.

Recommendations

It is recommended that the military have in place a doctrine related to the importance of sleep prior to extended missions and the importance of naps whenever possible during operations. Naps would be most effective when taken early in the period of sleep deprivation. Of the psychostimulant compounds, caffeine would be the compound of choice, since many personnel would have personal experience with the compound, it is not a restricted substance, it does not interfere with recovery sleep following periods of sleep deprivation, and it is generally considered to have very low abuse potential.

The DOD should continue to research the drug modafinil to further explore its potential for sustaining cognitive performance during military operations. Research published to date indicates that it may prove far superior to caffeine in maintaining cognitive performance over extended periods of sleep deprivation, without the adverse side effects and abuse potential of amphetamines.

5. Formulation: (a) Does the inclusion of other components (e.g., glucose) improve the beneficial effects of caffeine in sustained operations, as previously suggested by the committee? (b) Is there a better approach to caffeine delivery than the nutrient bar currently produced for the military?

The evidence is unclear on the utility of adding glucose or other carbohydrates to caffeine to further enhance physical performance. Caffeine enhances the availability of free fatty acids and decreases glycogenolysis, whereas carbohydrate as glucose increases the availability, and presumably the use, of this substrate. Some researchers have proposed that caffeine be delivered in a carbohydrate-containing medium to further enhance performance. However, most studies to date have been flawed in some way and reported variable results. Additional well-designed research is still necessary regarding the combined effects of caffeine and carbohydrate on physical performance. No studies were found in the published literature that examined the effects of caffeine combined with other nutrients on cognitive performance, and this may be an area for further research.

There may be nutritional reasons (e.g., provision of food energy, nutrients, or fluid) for including caffeine in a food form. A chocolate bar has been formulated by the military and found to be acceptable in preference studies. The bars have also been used as delivery vehicles for other food constituents such as

tyrosine, creatine, and antioxidant nutrients that might enhance performance under certain circumstances.

The committee considered various approaches to caffeine delivery for SUSOPS, including the food/energy bar and other alternatives such as caffeinated chewing gum, tablets (both sustained release and regular), and beverages. In assessing the alternatives, the pros and cons of each of these vehicles were considered. Their major characteristics are summarized in Table 7-1. There is good evidence that caffeine is absorbed rapidly and completely from the gut when supplied in a liquid form, with virtually all (99 percent) of the administered dose absorbed in about 45 minutes (Blanchard and Sawers, 1983; Chvasta and Cooke, 1971). However, the committee is unaware of evidence on the absorption of caffeine from a food matrix, as in solid foods such as bars. Theoretically, absorption should be slower than it is from liquids because gastric emptying time might be slower. Lipid solubility and possibly caffeine absorption in the stomach may influence these processes as well. Studies of the rapidity of absorption and action of caffeine are needed for such bars if they are to be considered as a caffeine delivery vehicle.

A caffeine delivery vehicle that is most appropriate in one setting may not be so in another, as presented in Table 7-1. Caffeine in a food matrix may be advantageous when it is important to deliver nutrients, fluid, or other food constituents simultaneously, but the satiating effects of the food may somewhat limit consumption, especially if high intakes are required to obtain a sufficient dose. Chewing gums are more appropriate if rapid absorption and action are needed, and would facilitate tailoring of individual doses. Caffeine in a fluid or gel matrix may be more appropriate when dehydration is an issue.

Various characteristics of the individual also alter the effects of a given dose of caffeine. These involve both individual factors (e.g., age, sex, smoker versus nonsmoker) and states that can vary greatly from one situation to another (e.g., stress hormonal status, ingestion of certain drugs, illness, heat stress, sleep status); thus, the delivered dose may have different psychological and physiological effects at one time than it does at another.

Conclusions

A summary of the characteristics of different methods of caffeine delivery is presented in Table 7-1. Although evidence of a potentiating effect of carbohydrates on caffeine effectiveness is equivocal, there are other reasons to consider providing supplemental nutrients along with the caffeine. For example, inadequate food and fluid intake is a common problem during military operations. The use of a caffeinated chewing gum would appear to provide the most rapid absorption.

TABLE 7-1 Summary of Potential Caffeine Delivery Approaches

Vehicle	Dose of Caffeine	Other Components That Improve Beneficial Effects of Caffeine?	Weight or Volume
Food bar	600 mg caffeine, scored in 150-mg increments	Yes—sucrose and corn syrup are simple sugars that may enhance the positive effects of caffeine on certain aspects of physical performance (depending on the type of exercise being performed); also contains complex carbohydrate, fat, and protein	70 g
Food bar, modified dose	100 mg increments	Yes—sucrose and corn syrup are simple sugars that may enhance the positive effects of caffeine on certain aspects of physical performance (depending on the type of exercise being performed); also contains complex carbohydrate, fat, and protein	50 g
Caffeinated soft drinks	Caffeine content varies from 5 to 50 mg depending on type and source	Yes—Sucrose and corn syrup are present in regular brands, aspartame in diet brands	12 fluid oz (36 g)
Caffeinated chewing gum	100 mg/stick	Yes—sucrose, corn syrup; also contains small amounts of glycerin, lecithin, and aspartame	? 5 g
Caffeine pills	100 mg	No	—
Sustained release caffeine	200 or 300 mg	No	—

Possible Rapidity of Absorption	Likely Rapidity of Action	Abuse Potential	Comments
? Slower	? Slower	Low	Bioavailability uncertain; good vehicle for providing other nutrients or food constituents such as sugars if these prove to be useful; more bulky than chewing gum or pills; satiating effects possible if caffeine content is low
? Slower	? Slower	Low	Bioavailability uncertain; good vehicle for providing other nutrients or food constituents such as sugars; satiating effects possible if caffeine content low; more bulky than chewing gum or pills
Rapid (< 60 min)	Rapid	Low	Provide fluid; good vehicle for sugars if these prove useful; more bulky than chewing gum or pills; a dehydrated beverage powder requires water and time to mix
Most rapid	Most rapid	Low	Absorbed sublingually and rapidly; low bulk; no or little satiating effects likely
Rapid	Rapid	Low	Bioavailability somewhat lower than from caffeinated drinks; no satiating effects likely
Initial dose rapid, sustained release	Initially rapid followed by slower absorption	Low	Permits longer intervals between doses but less flexibility during suddenly altered operational situations

Environmental circumstances and individual characteristics may make one caffeine delivery vehicle appropriate in some circumstances and inappropriate in another.

Recommendations

If food/energy bars are used, they must be tested for the rapidity of caffeine absorption and action. Under certain circumstances such as heat stress or desert operations, chewing gums may offer practical operational advantages over a food/energy bar, but under other conditions, such as reconnaissance operations from a central point, the bar may be preferable. Thus, more than one delivery vehicle should be considered, provided complete data on absorption and rapidity of action are available. In terms of convenience, pills or capsules could be considered, however, the little data available suggest that the bioavailability of caffeine in this form is less than in oral liquids. In addition, the use of pills or capsules does not meet the Army's stated preference of providing performance aids in the context of a food or beverage.

ADDITIONAL RESEARCH RECOMMENDATIONS

In reviewing the caffeine literature for this report, it became clear that there are still gaps in the knowledge database concerning caffeine and other potential cognitive enhancers that may be of military relevance. These are:

- bioavailability of caffeine from substances other than liquids consumed orally;
- additional research on the effectiveness and optimal doses of the wakefulness-promoting drug, modafinil, in simulated combat environments;
- effectiveness of various combinations of naps and caffeine;
- effects of consumption of greater than recommended doses of caffeine on fluid homeostasis in different environments (e.g., consumption of the total 600 mg caffeine delivery product in a hot, cold, or high-altitude environment); and
- properly designed studies on the effects of caffeine combined with other nutrients (e.g., carbohydrate, fat) on physical and cognitive performance.

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Appendixes

A

Workshop Agenda and Abstracts

AGENDA

Caffeine Formulations for Sustainment of Mental Task Performance During Military Operations

Committee on Military Nutrition Research
February 2–3, 1999

Tuesday February 2, 1999

8:30 a.m. Welcome on Behalf of the Food and Nutrition Board
Dr. Allison A. Yates, Director, Food and Nutrition Board

8:40 Welcome on Behalf of the Committee on Military Nutrition
Dr. John Vanderveen, Chair, Committee on Military Nutrition Research

8:45 Opening Comments on Behalf of the Military
LTC Karl E. Friedl, U.S. Army Medical Research and Materiel Command, Fort Detrick, Frederick, MD

Part I. Effects on Mental and Physical Performance
Moderator: Dr. Robin Kanarek

9:00 General Overview of Military Interest and Research on Role of Caffeine in Physical and Cognitive Performance
COL David Penetar, U.S. Army Research Institute of Environmental Medicine, Natick, MA

9:35 Caffeine and Muscle Metabolism During Prolonged Exercise
Dr. Lawrence Spriet, University of Guelph, Ontario, Canada

10:10 Effect of Caffeine on Cognitive Function and Alertness
Dr. Harris Lieberman, U.S. Army Research Institute of Environmental Medicine, Natick, MA

10:45 BREAK

10:55 Caffeine and Sentry Duty Performance
Dr. Richard Johnson, U.S. Army Research Institute of Environmental Medicine, Natick, MA

11:30 Eyelid Movement as a Physiological Predictor of Cognitive Impairment During Sleep Deprivation
Dr. Robert Stickgold, Harvard Medical School, Boston, MA

12:05 p.m. Circadian and Sleep Homeostatic Modulation of Sleep and Performance
Dr. James Wyatt, Harvard Medical School and Brigham and Women's Hospital, Boston, MA

12:40 LUNCH

Moderator: Dr. Johanna Dwyer

1:45 Caffeine Effects During Sleep Deprivation and Recovery
Dr. Steven Smith, Pennington Biomedical Research Center, Louisiana State University, Baton Rouge, LA

2:20 Circadian and Homeostatic Interactions in Waking Neurobehavioral Functions During Partial and Total Sleep Deprivation: Effects of Caffeine
Dr. Hans Van Dongen, University of Pennsylvania School of Medicine, Philadelphia

2:55 Caffeine Research in the Navy
Dr. W.K. Prusaczyk, Naval Health Research Center, San Diego, CA

3:30 DISCUSSION

3:50 BREAK

Part II. Safety Issues of Caffeine Supplementation
Moderator: Dr. John Fernstrom

4:00 Caffeine as a Model Drug of Abuse
Dr. Steve Holtzman, Emory University School of Medicine, Atlanta, GA

4:35 Caffeine Physical Dependence and the Consequences of Caffeine Abstinence
Dr. Roland Griffiths, Johns Hopkins University School of Medicine, Baltimore, MD

5:10 Positive Effects of Caffeine or Negative Effects of Withdrawal
Dr. Andrew Smith, University of Bristol, United Kingdom

5:30 DISCUSSION

6:00 ADJOURN

Wednesday, February 3, 1999

Part III. Caffeine Dose and Formulations
Moderator: Dr. Gail Butterfield

9:00 a.m. Pharmacology of Caffeine
Dr. Gary Kamimori, Walter Reed Army Institute of Research, Washington, DC

9:35 Caffeine Usage on Submarines
Christine Schlichting, Naval Submarine Medical Research Laboratory, Groton, CT

10:10 Design of a Food Matrix for the Delivery of Performance-Enhancing Components
Dr. Jack Briggs, Natick Soldier Center, Natick, MA

10:45 BREAK

10:55 Caffeine and Carbohydrate Supplements for Physical Performance
Dr. John Ivy, University of Texas, Austin

11:30 DISCUSSION

12:00 noon LUNCH

Part IV. Alternatives to Caffeine for Mental and Physical Task Performance

Moderator: Dr. Esther Sternberg

1:15 p.m. Cognitive Performance Effects of Caffeine Versus Amphetamines Following Sleep Deprivation
CAPT Mary Kautz, Walter Reed Army Institute of Research, Silver Spring, MD

1:50 Use of Amphetamine to Counteract Sleep Deprivation in Aviators
Dr. John Caldwell, U.S. Army Aeromedical Research Laboratory, Fort Rucker, AL

2:25 Effect of Prophylactic Naps and Caffeine on Alertness During Sleep Loss and Nocturnal Work Periods
Dr. Michael Bonnet, Dayton Department of Veteran Affairs Medical Center, Dayton, OH

3:00 DISCUSSION

3:30 Summary and Closing Remarks
Dr. John Vanderveen, Chair, Committee on Military Nutrition Research

4:00 ADJOURN

Workshop Abstracts

The abstracts appear in the order in which they were presented during the workshop on "Caffeine Formulations for Sustainment of Mental Task Performance During Military Operations," which was held on February 2–3, 1999, in Washington, D.C.

GENERAL OVERVIEW OF MILITARY INTEREST AND RESEARCH ON ROLE OF CAFFEINE IN PHYSICAL AND COGNITIVE PERFORMANCE

David Penetar, Ph.D.
U.S. Army Research Institute of Environmental Medicine, Natick, MA

The military's interest in caffeine is manifold and revolves around some of caffeine's basic behavioral effects: those of enhancing alertness, improving cognitive performance, and increasing physical capabilities. The degree and extent to which caffeine is an effective agent for producing these changes, especially with regard to the stressful, severe, and at times life-threatening environments in which military personnel operate, is a complex area for psychopharmacological research. Modern warfare pushes the limits of human performance in many ways. Military operations can have severe disrupting effects on normal sleep patterns and contain periods of sustained high rates of work and carrying of heavy loads. This disrupted sleep coupled with heavy physical demands can affect critical decision making and other cognitive skills. Under certain circumstances, pharmacological interventions may be warranted to prevent cognitive decrements as well as, possibly, the enhancement of physical performance. Several avenues of research have been pursued. The most notable effects of restricted and fragmented sleep are on alertness, mood, and cognitive abilities. Caffeine and other stimulants have been studied in both laboratory and field settings. These studies explore the effective dose range and time course of action. The use of caffeine to enhance physical performance in extreme environments (e.g., high altitude) or under high workload is also an area of military interest. The question of enhancing cognitive performance beyond the normal well-rested state is not yet completely answered. Continued research will contribute to policies outlining the acceptability and usefulness of caffeine in military operations.

CAFFEINE AND MUSCLE METABOLISM DURING PROLONGED EXERCISE

Lawrence L. Spriet, Ph.D.
Human Biology and Nutritional Science, University of Guelph, Ontario, Canada

Caffeine is a dietary pharmacological agent that is routinely ingested by people worldwide. It rapidly appears in the blood following ingestion, is taken up by the tissues of the body, and therefore has the potential to significantly alter metabolism. Many athletes also routinely ingest caffeine and there has been considerable interest in the ability of caffeine to enhance performance during prolonged aerobic exercise (Spriet, 1995). Several, well-controlled studies have established that moderate doses of caffeine (3–6 mg/kg body mass, about 2–4, 8-oz cups of coffee) ingested 1 hour prior to exercise enhance endurance performance in the laboratory at intensities of 70–85 percent of maximal oxygen uptake VO_{2max} (Costill et al., 1978; Graham and Spriet, 1995; Ivy et al., 1979; Pasman et al., 1995). Moderate caffeine doses produce urinary caffeine levels well below the allowable limit set by sports governing bodies (12 μg/mL), meaning that athletes can legally enhance their performance in this manner. Higher doses of caffeine (9–13 mg/kg body mass) also produce increases in laboratory endurance performance but are often associated with "illegal" urinary caffeine levels (> 12 μg/mL) and a higher incidence of adverse side effects (Graham and Sphet, 1991; Pasman et al., 1995; Spriet et al., 1992). The performance results are specific to well-trained elite or recreational athletes. These studies also demonstrate a large variability between individuals in the metabolic and performance responses to caffeine. Lastly, it is not known if these findings improve performance in competitions because controlled caffeine field studies are lacking.

The precise mechanisms responsible for improved performance during prolonged exercise remain elusive. A central nervous system contribution to the improved performance is always a possibility when studying humans, since it is not possible to separate the "central" and "peripheral" (skeletal muscle) effects of caffeine. However, it does appear that metabolic mechanisms are part of the explanation for the improvement in endurance performance following caffeine ingestion (5–13 mg/kg), except at low caffeine doses (2–4 mg/kg) where this has not been fully examined. The decreased respiratory exchange ratio, increased concentration of plasma-free fatty acids (FFAs) at the onset of exercise, glycogen sparing in the initial 15 minutes of exercise, and increased intramuscular triacylglycerol use during the first 30 minutes of exercise suggest a greater role for fat metabolism early in exercise following caffeine ingestion (Chesley et al., 1998; Essig et al., 1980; Graham and Spriet, 1991; Ivy et al., 1979; Spriet et al., 1992).

It has been suggested that the increased fat oxidation and decreased glycogen use in muscle following caffeine ingestion could be explained by the classic glucose–fatty acid cycle. In this scheme, elevated FFA availability to the muscle produced increases in muscle citrate and acetyl-coenzyme A, that were believed to

inhibit the enzymes phosphofructokinase and pyruvate dehydrogenase. The subsequent decrease in glycolytic activity increased glucose 6-phosphate content, leading to inhibition of hexokinase and ultimately decreased muscle glucose uptake and oxidation. However, these mechanisms were not involved in the glycogen sparing during exercise at approximately 85 percent VO_{2max} with caffeine ingestion (Spriet et al., 1992). Instead, the mechanism for muscle glycogen sparing following caffeine ingestion appeared related to the regulation of glycogen phosphate activity via a more "defended" energy status of the cell. Subjects who spared muscle glycogen used less muscle phosphocreatine and had smaller increases in free adenosine 5'-monophosphate (AMP) and inorganic phosphate during exercise in the caffeine versus placebo trials (Chesley et al., 1998). The lower inorganic phosphate and AMP concentrations decreased the flux through glycogen phosphorylase and decreased glycogen use. There were no differences in these metabolites between trials in subjects who did not spare muscle glycogen. Presently, it is not clear how caffeine defends the energy state of the cell, but it may be related to an increased availability of fat and reducing equivalents (reduced nicotinamide–adenine dinucleotide) in the mitochondria at the onset of exercise.

Therefore, while it is clear that metabolic changes contribute to the ergogenic effect of caffeine during endurance exercise, aspects of the metabolic contribution have not been adequately examined in all situations. Measurements of muscle glycogen and triacylglycerol use and plasma FFA turnover are required to determine the magnitude of the metabolic link to improved performance at all caffeine doses and endurance exercise situations.

EFFECT OF CAFFEINE ON COGNITIVE FUNCTION AND ALERTNESS

Harris R. Lieberman, Ph.D.
U.S. Army Research Institute of Environmental Medicine, Natick, MA

Although the behavioral effects of caffeine have been a subject of scientific investigation for more than 100 years, it was not until recently that a clear picture of the substance's effects have started to emerge. Caffeine's effects on cognitive function and mood can be detected in rested and sleep-deprived volunteers using a variety of standardized tests. Only certain behavioral functions appear to be susceptible to the influence of moderate doses of caffeine. In particular, it appears that in well-rested volunteers, low and moderate doses of caffeine (32–256 mg) preferentially affect functions related to vigilance—the ability of individuals to maintain alertness and appropriate responsiveness to the external environment for sustained periods of time. Self-reported mood states that are related to vigilance, such as alertness, also are clearly improved by moderate doses of caffeine. Higher cognitive functions, such as memory and

visuospatial reasoning, do not appear to be affected in any substantial manner when the substance is administered in moderate doses to rested volunteers.

Among individuals who have been deprived of sleep, vigilance tests and mood questionnaires remain highly sensitive to the beneficial effects of caffeine. In addition, certain more complex cognitive functions also improve, although these effects may be secondary to improved vigilance. Recently we conducted a field study that demonstrated that even when volunteers are exposed to severe sleep deprivation in combination with mental, physical, and psychological stress, moderate doses of caffeine can partially restore vigilance and other key aspects of cognitive performance. This study may provide useful insight into the optimal dose of caffeine to employ under such circumstances.

Maintenance of vigilance is critical for a variety of military duties such as standing watch, sentry duty, communication monitoring, and operating vehicles, including aircraft and vessels. During military operations a single individual can be responsible for the safety of hundreds of individuals traveling in his or her vehicle or being protected by his or her weapons system. Therefore, lapses in vigilance can have devastating consequences. Even in well-rested individuals vigilance significantly deteriorates after brief periods of attempting to maintain optimal alertness during boring but critical activities. During wartime or other intense operations, sleep loss and environmental and psychological stress greatly reduce the ability of individuals to maintain even marginally adequate vigilance. Therefore, administration of caffeine in appropriate doses at the correct times may be an effective method for substantially improving key aspects of cognitive function in rested and sleep-deprived war fighters.

CAFFEINE AND SENTRY DUTY PERFORMANCE

Richard F. Johnson, Ph.D.
U.S. Army Research Institute of Environmental Medicine, Natick, MA

Proficient sentry duty performance requires both rifle marksmanship accuracy and sufficient alertness to detect the infrequent appearance of targets. At the U.S. Army Research Institute of Environmental Medicine, the Weaponeer M16 Rifle Marksmanship Simulator, a U.S. Army training device, has been adapted for assessing the components of sentry duty (target detection and rifle firing accuracy). Our research has shown that during 3 hours of baseline sentry duty, the soldier's speed of target detection becomes slower while rifle firing accuracy remains unimpaired.

In our first caffeine study with the sentry duty model, we tested the effects of the ingestion of 200 mg of caffeine on male soldiers' target detection speed and rifle firing accuracy. Target detection speed under the placebo condition deteriorated with time and was significantly slower after 60–90 minutes on the task. Under the caffeine condition, the impairment in target detection speed was

significantly attenuated. Regardless of drug condition, rifle firing accuracy showed no impairment during sentry duty.

Our second caffeine study was sponsored by the Defense Women's Health Research Program and focused on the sentry duty performance of both men and women. Both men's and women's target detection speeds deteriorated with time on sentry duty, and this performance decrement was eliminated by 200 mg of caffeine. While men's rifle-firing accuracy remained constant over time, women's rifle firing accuracy deteriorated after 90 minutes, regardless of drug condition.

Our third caffeine study, recently completed, was a replication and extension of the second and introduced the requirement to discriminate friendly from enemy targets. The decrement in both men's and women's target detection speed with time on sentry duty was again eliminated by 200 mg of caffeine. As in the second study, women's rifle firing accuracy was poorer than that of men's, but the relationship with time on the task was complex and did not clearly replicate the results of the second study. Compared to placebo, the number of correct target identifications (friend versus foe) was significantly improved by 200 mg of caffeine.

Conclusions

1. *Efficacy:* Without impairing rifle-firing accuracy, 200 mg of caffeine improves target detection speed and increases the likelihood of correct friend–foe target identifications during simulated sentry duty.
2. *Safety:* No adverse effects of caffeine were observed during these studies.
3. *Dose:* In sentry duty, effects of caffeine in doses other than that used in these studies (200 mg) is unknown.
4. *Alternatives:* Sentry duty of less than 60 minutes' duration does not lead to a decrement in performance and would not benefit from the prior ingestion of caffeine.
5. *Formulation:* We have tested caffeine only in the 200-mg tablet form.

EYELID MOVEMENT AS A PHYSIOLOGICAL PREDICTOR OF COGNITIVE IMPAIRMENT DURING SLEEP DEPRIVATION

Robert Stickgold, Ph.D.
Department of Psychiatry, Harvard Medical School, Boston, MA

Overall cognitive performance is modulated during sleep deprivation by both homeostatic and circadian factors. However, on a shorter time scale, performance decrements can be reversed by heightened interest and attention on the part of the subject. Within this context, it would be valuable to be able to easily monitor levels of functional arousal using physiological rather than behavioral measures. From a theoretical perspective, this would allow clarification of the role of atten-

tion and arousal in the maintenance of performance on specific tasks. From a more practical perspective, it could allow the ongoing monitoring and predicting of performance level before and during the execution of critical tasks.

We have developed a home-based sleep and vigilance monitor called the "Nightcap". The Nightcap uses a piezoelectric film to monitor both tonic and phasic muscle activity in the upper eyelid and has been used during waking and sleep, including periods of sleep deprivation. Activity is quantified as the number of 250-ms epochs/minute in which eyelid movement exceeds a threshold amount. This activity not only identifies sleep onset with high reliability but, in pilot studies, also correlates with levels of performance on a series of cognitive tests during periods of sleep deprivation.

In a pilot study, performance on a vigilance test administered repeatedly over 40 hours of sleep deprivation varied dramatically as a function of both homeostatic and circadian factors and correlated highly with eyelid activity recorded during the tests, measured both as reaction times (Pearson r-value = -0.82, df = 8, $p < 0.005$) and as error rates (Pearson r-value = -0.80, df = 8, $p < 0.005$). Overall, eyelid activity explained two-thirds of the variance in performance.

In contrast, performance on a mental rotation task was not diminished during sleep deprivation, with both reaction time and accuracy showing nonsignificant improvement with increased deprivation. Eyelid activity also showed no sleep deprivation effect during the mental rotation tests, despite the strong variations measured over the same 40 hours during the vigilance tests.

We conclude that the eyelid activity measured by the Nightcap reflects instantaneous arousal levels that correlate at the behavioral level with task performance on a range of cognitive tests. We believe that this eyelid activity reflects the modulatory activity of brainstem arousal systems that control both levels of behavioral arousal and the levator palpebrae muscle of the upper eyelid. As a result, the eyelid sensor permits both the monitoring of brainstem arousal systems and the prediction of behavioral outcomes.

CIRCADIAN AND SLEEP HOMEOSTATIC MODULATION OF SLEEP AND PERFORMANCE

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Two processes that contribute significantly to the modulation of sleep and waking neurobehavioral functioning are the sleep homeostat and the endogenous circadian pacemaker. Although the exact neurophysiological and neuropharmacological mechanisms remain to be conclusively delineated, the sleep homeostatic process can be found in impairments of neurobehavioral functioning with increasing durations of sustained wakefulness. Thus, minimal sleep homeostatic impairment of alertness and performance is seen during the first few hours of

wakefulness and increases thereafter. In its modulation of sleep continuity and structure, maximal sleep pressure is seen during the first third of a typical 8-hour sleep episode, with very low homeostatic drive for sleep in the final third.

In contrast, the endogenous circadian pacemaker, located in the suprachiasmatic nucleus of the hypothalamus, has a paradoxical phase relationship with the sleep homeostatic process. This relationship is beneficial, and in fact critical, in maintaining relatively stable alertness and performance across a typical, daytime, 16-hour wake episode. This is due to the higher homeostatic drive for sleep being offset by the maximal circadian drive for wakefulness in the latter half of the habitual waking day. Similarly, the low homeostatic drive for sleep seen during the latter part of the habitual sleep episode is offset by the circadian drive for sleep, which is itself maximal 1–2 hours prior to habitual wake time.

Under conditions of challenge to the sleep homeostatic system (e.g., sustained wakefulness of extended duty hours) and/or the circadian system (e.g., jet lag or night operations), impairments of neurobehavioral functioning become impressively evident. In our laboratory experiments with healthy normal volunteers, we have simulated exposure to rapid time-zone travel (bedtimes and wake times shifting) and extended duty hours (28.57-hour wake episodes and 14.28-hour sleep episodes) with a month-long protocol. Though blind to drug condition at this point, at the end of this study we hope to have information on the efficacy of low-dose, sustained caffeine administration as a countermeasure to deficits of neurobehavioral functioning encountered during this type of biological challenge.

Preliminary data are presented demonstrating the relative strength of homeostatic and circadian modulation of sleep propensity, sleep structure, and sleep consolidation seen under these conditions. Data are presented on the homeostatic and circadian modulation of several neurobehavioral measures, including reaction time and visual vigilance, short-term memory, cognitive throughput, and subjective alertness.

CAFFEINE EFFECTS DURING SLEEP DEPRIVATION AND RECOVERY

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Objective

The objective of this study was to determine the effectiveness of central nervous system-activating substances (*d*-amphetamine, caffeine, phentermine, tyrosine), compared to placebo, on the following parameters during sleep deprivation

and recovery: (1) sleep drive, (2) sleep quantity, (3) sleep quality, (4) mental and fine motor performance, and (5) biochemistry of the pituitary–adrenal axis.

Methods

To accomplish this task, we recruited 76 healthy males, ages 18–35, body mass index 20–27 kg/m^2, who participated in a parallel arm, randomized, double-blind, placebo-controlled study comparing tyrosine 150 mg/kg body weight (BW), phentermine 37.5 mg, *d*-amphetamine 20 mg, or caffeine 300 mg/70 kg BW, to placebo. We performed multiple polysomnography recordings, cognitive performance tests, and neuroendocrine assays before and during 40 hours of sleep deprivation and also during a recovery night.

Results

In sleep-deprived adults, we found a significant delay in time to sleep onset for amphetamine, compared to placebo. The amphetamine effect persisted longer than caffeine and phentermine. Caffeine significantly delays sleep onset compared to placebo, but not as greatly as amphetamine. Time to sleep onset latency was significantly greater for amphetamine and phentermine compared to placebo at recovery night. Caffeine did not appear to interfere with time to sleep onset during recovery. Amphetamine significantly decreased sleep quantity (decreased total sleep time, decreased sleep efficiency, increased sleep onset latency, increased wakefulness after sleep onset) whereas caffeine had an effect on sleep quantity during recovery similar to placebo. Amphetamine and phentermine significantly impaired sleep depth during recovery (decrease in percentage of rapid eye movement [REM], increased latency to REM). Amphetamine and phentermine produced significantly more awakenings than other agents tested. Caffeine had a profile similar to placebo (and tyrosine) in terms of sleep depth, architecture, and continuity during recovery. Amphetamine, caffeine, and phentermine significantly improved most target performance measures that show a performance decrement during sleep deprivation (logical reasoning, running memory, math processing, pursuit tracking, visual vigilance). Performance decrements during sleep deprivation were chiefly in response time and improvements were in response time, without sacrifices in accuracy.

Conclusions

The model demonstrated that caffeine is effective in reversing the negative effects on alertness during sleep deprivation. This effect was similar to phentermine, significantly better than placebo, but less than the observed effects of amphetamine. In contrast to amphetamine and phentermine, caffeine had no deleterious effects on recovery sleep. Caffeine, amphetamine, and phentermine

all had significant beneficial effects on performance indicators during sleep deprivation, especially with regard to response time. Caffeine is a candidate for policy implementation in conditions where sleep deprivation is inevitable. Furthermore, we suggest that future studies be conducted in situations that mimic military duty conditions in order to confirm these findings.

CIRCADIAN AND HOMEOSTATIC INTERACTIONS IN WAKING NEUROBEHAVIORAL FUNCTIONS DURING PARTIAL AND TOTAL SLEEP DEPRIVATION: EFFECTS OF CAFFEINE

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This ongoing double-blind, placebo-controlled, randomized trial of low-dose caffeine simulates the effects of sustained operations with and without sleep and caffeine, on a total of 56 male adults in the controlled environment of an isolated laboratory with light not brighter than 50 lux (range 25–45 lux). On 3 subsequent baseline days, subjects have 8 hours time for sleep (from 2330 until 0730 hours). During the next 3 days in the laboratory (i.e., 88 hours), they are either partially sleep-deprived (PSD) or totally sleep-deprived (TSD), In the PSD condition, 2-hour naps are taken every 12 hours, that is, from 1445 until 1645 hours and from 0245 until 0445 hours. During this 88-hour period of sleep deprivation, neurobehavioral tests are performed every 2 hours, waking electroencephalogram and sleep polysomnography with additional EEG and rectal temperature measurements are recorded continuously, and blood samples are taken every 90 minutes for the analysis of cortisol, melatonin, catecholamines, and plasma caffeine.

In this trial, there are 4 groups of 14 subjects each: TSD + sustained low-dose caffeine, TSD + placebo, PSD + sustained low-dose caffeine, and PSD + placebo. Starting at 0530 hours of sleep deprivation day one, subjects in the caffeine conditions receive a 0.3-mg/kg caffeine pill each hour, and the remaining subjects receive a placebo pill each hour (except when napping in the PSD condition). As of yet, the investigators are still blinded to conditions. Therefore, no results of the efficacy of caffeine intake on reducing neurobehavioral performance deficits in this protocol can be reported. In body temperature and plasma melatonin, however, a circadian phase delay is observed during sleep deprivation, regardless of deprivation condition (PSD or TSD). Clearly, since the investigators are still blinded, the involvement of caffeine in this phase drift cannot be determined, but the finding of Redman and Rajaratnam (1998) that caffeine induces a circadian phase advance in rats makes it unlikely that caffeine would cause the presently observed phase delay in humans.

CAFFEINE RESEARCH IN THE NAVY

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Navy policy precludes stocking or using amphetamines or sedatives to maintain or enhance performance. Due to their widespread acceptance and relative safety, caffeine and nicotine are frequently used to combat the effects of fatigue during sustained military operations. In fact, the Naval Aerospace and Operational Medical Institute has disseminated protocols for use of these products. With the Navy's current emphasis on a smoke- and nicotine-free fleet, the interest in caffeine is increasing. During Operation Southern Watch over Iraq, among carrier-based aircrew, caffeine was the preferred modality for maintenance of performance. Belland and Bissell (1993) reported that 63 percent of aircrew surveyed used some form of nonpharmaceutical stimulant. Of these, 75 percent used caffeine either as coffee (1–7 cups preflight) or caffeine tablets to maintain performance during the sustained operations.

Caffeine research in the Navy has, as do most areas of pharmacological performance enhancement research, two thrusts—physiological and psychological. In a study of the effects of caffeine on thermoregulatory responses, Ahlers et al. (1990) found that caffeine (3.5 mg/kg^{-1}) significantly attenuated rectal temperature afterdrop following cold water immersion. Subjects had an induced 0.5°C rectal temperature. At the nadir of afterdrop, subjects taking caffeine had a 0.3°C higher mean rectal temperature and returned to preafterdrop temperature sooner.

Caffeine has a purported ergogenic effect of sparing muscle glycogen. Prusaczyk et al. (1998) investigated the effect of caffeine on reducing muscle glycogen use following a carbohydrate loading protocol. In this double-blind, placebo-controlled study, it was found that caffeine did not alter the rate of glycogen use during prolonged exercise in carbohydrate-loaded subjects.

Studies of the psychological effects of caffeine ingestion have focused on the alleviation of fatigue and maintenance of mood during periods of sustained or continuous operations and during sleep deprivation. In 1995, Bonnet et al. reported the effects of prophylactic naps (0, 2, 4, or 8 hours) or caffeine (0, 150, 300, or 400 mg) on performance (logical reasoning, hand tremor, digit symbol substitution task) during 52 hours of sleep deprivation. The long nap was better than caffeine for maintaining performance, mood, and alertness. A repeated low dose of caffeine was better than no nap or large single doses of caffeine; however, neither nap nor caffeine could preserve performance at baseline levels beyond 24 hours.

Kelly et al. (1996, 1997) examined the effects of caffeine dosing (300 mg every 6 hours, 400 mg and placebo alternated every 6 hours, and placebo every 6 hours) during 64 hours of sleep deprivation on subsequent recovery sleep. Polysomnography revealed that caffeine affected sleep only during the first third of the first recovery night. Compared to baseline, caffeine-ingesting subjects

showed lighter Stage 2 sleep and decreased slow wave sleep. Caffeine may, in fact, make short sleeps deeper if not ingested close to sleep. The authors concluded that repeated caffeine dosing during deprivation appears not to interfere with recovery sleep following sleep deprivation.

CAFFEINE AS A MODEL DRUG OF ABUSE

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Low to moderate doses of caffeine produce many effects in humans and animals that resemble effects produced by low doses of nonxanthine psychomotor stimulants, such as amphetamine and cocaine. For example, they produce positive mood states and increases in wakefulness and motor activity. This has given rise to the inevitable question of whether or not caffeine has abuse liability. In fact, caffeine does have the principal features usually associated with a drug of abuse. These will be reviewed, drawing largely from studies in the preclinical literature and, where appropriate, will be compared to those of nonxanthine psychomotor stimulants.

Caffeine is reinforcing; humans and animals will work to get it, albeit not as hard as they will work to get other stimulant drugs. Caffeine is discriminable; humans and animals can recognize the fact that they have received caffeine. The discriminative effects of low to moderate doses of caffeine have commonalities with those of nonxanthine stimulants. Chronic administration of caffeine results in the development of insurmountable drug-specific tolerance to many effects, including psychomotor stimulation, as well as in physical dependence. The latter state is characterized by a subjective withdrawal syndrome in humans that includes headache, lethargy, and difficulty concentrating and by reduced activity in animals when caffeine administration is stopped.

The catecholamine neurotransmitters norepinephrine and dopamine have a prominent role in the autonomic and behavioral effects of nonxanthine stimulants. In animals, brain dopamine, in particular, has been implicated in the psychomotor stimulant effects of these drugs and in other actions relevant to potential for abuse, such as discriminative stimulus and reinforcing stimulus effects. Caffeine also enhances neurotransmission mediated by dopamine. However, in contrast to amphetamine and cocaine, which dramatically increase the concentration of dopamine in the synapse, the effects of caffeine on brain dopamine are more subtle and modest. The effects are secondary to the blockade of adenosine receptors by caffeine and are not associated with elevated concentrations of dopamine in the synapse.

Moderate to high doses of caffeine produce behavioral effects that are different from those produced by lower doses of caffeine and by nonxanthine psy-

chomotor stimulants. They often produce negative mood states in humans, with anxiety a prominent component, and appear to be aversive to animals. There is no evidence of tolerance to these effects. The neural mechanisms that underlie the high-dose effects of caffeine remain obscure.

It is evident that caffeine has most of the features of a drug of abuse. Nevertheless, the abuse liability of caffeine is negligible in comparison to that of many nonxanthine psychomotor stimulants. The reasons for this include the less intense psychomotor stimulant effects of low to moderate doses, the development of tolerance to those effects, and the often unpleasant and persistent effects of high doses that serve to limit drug intake by many individuals.

CAFFEINE PHYSICAL DEPENDENCE AND THE CONSEQUENCES OF CAFFEINE ABSTINENCE

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Physical dependence is manifested by time-limited biochemical, physiological, and behavioral disruptions (i.e., a withdrawal syndrome) upon termination of chronic or repeated drug administration. There have been more than 10 reports of caffeine withdrawal in laboratory animals, most of which have documented substantial behavioral disruptions following cessation of chronic caffeine dosing (e.g., 50–80 percent reductions in spontaneous locomotor activity; 20–50 percent reductions in operant responding). These studies have examined caffeine withdrawal in rats, cats, and monkeys.

Caffeine physical dependence has been clearly demonstrated in humans in approximately 60 case reports and human experimental studies. The most frequently reported withdrawal symptom is headache (also cerebral fullness), which is characterized as being gradual in development, diffuse, throbbing, and sometimes severe. Other symptoms, in roughly decreasing order of prominence, are drowsiness (e.g., increased sleepiness and yawning, decreased energy and alertness); increased work difficulty (decreased motivation for tasks or work, impaired concentration); decreased feelings of well-being or contentment (decreased self-confidence, increased irritability); decreased sociability, friendliness, or talkativeness; flu-like feelings (muscle aches or stiffness, hot or cold spells, heavy feelings in arms or legs, nausea); and blurred vision. In addition to these symptoms, composite scales of depression and anxiety may be elevated and psychomotor performance may be impaired. The occurrence of headache as a withdrawal symptom does not necessarily correlate with the occurrence of other symptoms (e.g., tiredness), suggesting that other signs and symptoms are not merely epiphenomena of headache.

The severity of caffeine withdrawal is an increasing function of caffeine maintenance dose. When symptoms of caffeine withdrawal occur, the severity can vary from mild to extreme. At its worst, caffeine withdrawal is incompatible with normal functioning and is sometimes totally incapacitating.

The incidence of caffeine withdrawal is an increasing function of caffeine maintenance dose. The best estimates of the incidence of caffeine withdrawal in the general population come from a survey study and an experimental study. A recent random-digit dial telephone survey in Vermont showed that among current users of caffeine who reported that they had abstained from caffeine for 24 hours or more, 27 percent reported withdrawal headaches when they abstained. The experimental study involved 62 individuals from the general community with a distribution of caffeine intake similar to the general population in the United States (mean caffeine intake of 235 mg). The study involved a double-blind, approximately 48-hour, caffeine abstinence trial under conditions that obscured the fact that the purpose of the study was to investigate caffeine. During caffeine withdrawal 52 percent reported moderate or severe headache and 8–11 percent showed abnormally high scores on standardized depression, anxiety, and fatigue scales. The incidence of headache observed from the survey and experimental study in the general population (27–52 percent) is in the range of that observed in several other recent studies conducted in special subject populations.

Although the incidence and severity of caffeine withdrawal are an increasing function of caffeine dose, two studies have shown that caffeine withdrawal can occur after relatively long-term administration of caffeine doses as low as 100 mg.

The caffeine withdrawal syndrome follows an orderly time course. Onset has usually been reported to occur 12–24 hours after terminating caffeine intake, although onset as late as 36 hours has been documented. Peak withdrawal intensity has generally been described as occurring 20–48 hours after abstinence. The duration of caffeine withdrawal has most often been described as ranging between 2 days and 1 week, although longer durations have been noted occasionally.

Physiological mechanisms underlying caffeine withdrawal remain uncertain, although some studies suggest that increased blood volume, possibly adenosine-mediated, may be involved with caffeine withdrawal headache.

Implications of Caffeine Physical Dependence for Performance Assessment

In assessing the effects of caffeine on performance, many previous studies have failed to differentiate between caffeine's restoring performance degraded by caffeine abstinence versus caffeine's enhancing performance. In examining such studies, attention should be given to the habitual daily caffeine dose consumed by subjects and the duration of caffeine abstinence immediately before testing. The effects of caffeine on performance may depend on a given individual's level of caffeine tolerance (decreased responsiveness to the drug due to

repeated past exposure) and physical dependence (behavioral disruptions upon termination of repeated drug administration).

POSITIVE EFFECTS OF CAFFEINE OR NEGATIVE EFFECTS OF CAFFEINE WITHDRAWAL

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This paper will consider the extent to which differences between caffeinated and decaffeinated conditions reflect the positive effects of caffeine or the negative effects of caffeine withdrawal. The background to this debate is presented and the relevant literature reviewed. It is concluded that the absence of strong negative effects of caffeine withdrawal on performance, and the demonstration of positive effects in nonconsumers, support the view that caffeine enhances performance and does not just remove impairments induced by withdrawal.

The second part of the paper will consider in detail the health consequences of withdrawal. Results on headaches and caffeine withdrawal will be discussed and it will be concluded that the increased incidence of headaches following caffeine withdrawal reflects factors such as expectancies and the ability to determine whether the caffeine has been withdrawn or not. This view will be contrasted with those suggesting a pharmacological addiction to caffeine.

Abstract

Previous research has shown that cessation of caffeine consumption may be associated with a distinct withdrawal syndrome, typified by an increase in headaches. Recent research suggests that low to moderate consumers of caffeine may report an increase in headaches if they perceive caffeine to have been withdrawn regardless of whether it has been or not. The present study provides additional support for the role of subjective perceptions in the caffeine withdrawal syndrome. Forty-four low-caffeine consumers recorded the incidence of headaches when drinking caffeinated or decaffeinated beverages. When caffeine was withdrawn the incidence of headaches increased, but this effect was significant only in those individuals who could discriminate whether they were consuming caffeinated or decaffeinated beverages. This result suggests a major role of subjective perceptions and expectancies in the caffeine withdrawal syndrome, a view that contrasts the notion that a significant proportion of caffeine consumers are physically dependent upon caffeine.

Introduction

A number of studies have demonstrated that cessation of caffeine consumption may result in a distinct withdrawal syndrome, typified by the occurrence of headaches (Dreisbach and Pfeiffer, 1943; Griffiths et al., 1990; Strain et al., 1994; van Dusseldorp and Katan, 1990). In the light of this evidence, caffeine withdrawal syndrome has been included in DSM-IV. These studies have tended to use individuals with histories of chronic high-dose caffeine consumption (≥ 500 mg) or else have increased the caffeine intake of participants to very high levels during the caffeinated condition of the experiment itself. Even with high-caffeine consumers the proportion of participants who report headaches during withdrawal has ranged from 25 to 100 percent. Similarly, those studies that have investigated withdrawal in low-dose consumers (< 200 mg) have found that headache reporting varies from 20 percent of the sample (Fennelly et al., 1991) to 50 percent (Silverman et al., 1992) or even 100 percent (Naismith et al., 1970)

Results from a recent study (Smith, 1996) suggest that low- to moderate-caffeine consumers may report an increase in headaches when they perceive caffeine to have been withdrawn regardless of whether it has been or not. The reporting of headache is seen, therefore, as a combination of an expectancy that caffeine withdrawal may increase headaches and the ability to discriminate whether caffeine has actually been withdrawn. This view is very different from previous assertions that a significant proportion of low- to moderate-caffeine consumers are physically dependent upon caffeine. Support for the role of subjective perceptions comes from our latest study of this issue, which is described below.

Method

Participants

Forty-three regular caffeinated tea and coffee consumers (22 females, 21 males, mean age 21.1 years, range 18–26 years) participated in a study examining the effects of caffeine withdrawal on reporting of headaches. Mean reported daily caffeine consumption from these sources was 175 mg (standard deviation = 91 mg; based on caffeine content of products provided by Debry [1994]). Each volunteer carried out a 2-day baseline period during which normal caffeine consumption was recorded using a diary, and headaches and other symptoms were measured. For all volunteers, either tea or coffee was the major source of caffeine. Following this the volunteers were given supplies of either caffeinated or decaffeinated tea and coffee and told to continue with their normal pattern of consumption but to use only the coffee and tea supplied. Volunteers were blind with regard to which days they were given decaffeinated products or caffeinated products. They were told to stop their normal consumption of other caffeinated products such as chocolates or soft drinks. Each volunteer carried out both caffeinated and

decaffeinated conditions for 2 days, the order of conditions being counterbalanced across participants. In addition to recording the presence or absence of headache and other symptoms, volunteers were asked whether they believed the beverages consumed that day to have been caffeinated or decaffeinated.

Results

The results showed that there was no significant difference in reporting headaches in the baseline (14.0 percent of sample reported a headache) and caffeinated drink conditions (18.6 percent). However, when caffeine was withdrawn, the frequency of headache increased to 39.5 percent (significantly greater than both baseline and caffeinated conditions, $p < 0.01$). Further analyses revealed that the effect of caffeine withdrawal depended on whether the participants were able to discriminate whether caffeine was present or not (22 participants correctly identified the two conditions). An analysis of variance showed that the condition x ability to discriminate caffeine was significant (F (2, 78) = 4.29, $p < 0.05$). For those who could tell whether caffeine was withdrawn or not, headache frequency increased from 7 percent in the baseline and 9.3 percent in the caffeinated condition to 48.8 percent in the decaffeinated condition. In contrast to this, caffeine withdrawal had little effect on headache reporting in those unable to tell the nature of the beverages (see Figure 1). Overall, the observed effect of caffeine withdrawal on headache frequency appeared to be due entirely to the reporting of headaches by those participants who were able to correctly identify whether caffeinated or decaffeinated drinks were consumed.

Discussion

Three possible explanations exist to explain the link between reporting of headaches and ability to discriminate whether or not the drinks were caffeinated. First, some individuals may develop headaches during caffeine withdrawal and use the increased symptoms to help identify the nature of the drinks. Alternatively, those individuals who could identify the nature of the drinks would then be influenced by the expectancy that caffeine withdrawal increases headache frequency. In contrast, those unable to discriminate between caffeinated and decaffeinated conditions would show no difference in headache frequency in these two conditions but should report an increase relative to baseline. This was found here. Finally, it is possible that both mechanisms may be involved in the overall pattern of results. In this context, one can view the expectancy effect as a factor that has inflated estimates of the number of people who are dependent on caffeine rather than being a total explanation for the caffeine withdrawal–headache association. Studies of headaches in patients withdrawn from caffeine prior to surgery suggest that headache frequency is around 25 percent. Given that

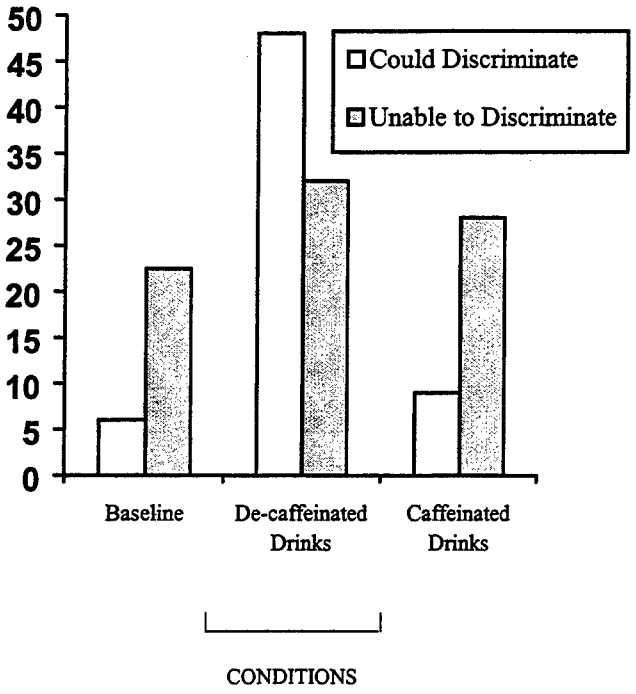


FIGURE 1 Percentage of volunteers reporting headaches in the various conditions (those who correctly identified the caffeine versus those who could not).

baseline headache rate in nonwithdrawn volunteers studied here was nearly 15 percent, one can see that we are clearly not looking at a large effect. Indeed, it may be that individuals who regularly get a lot of headaches do not show an increase when caffeine is withdrawn and are also poor at discriminating whether they have been consuming caffeinated beverages or not. Further research is required to resolve this issue.

Conclusion

In conclusion, the present study has demonstrated that the increased frequency of headaches during caffeine withdrawal reflects participants' detecting they are in that condition and reporting the symptoms they expect to be associated with it. Further research should address the direction of causality between perceptions of caffeine content and withdrawal symptoms. In addition, the ex-

tent to which similar effects are observed in those who consume higher doses of caffeine requires further investigation.

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PHARMACOLOGY OF CAFFEINE

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Caffeine is one of the most widely used drugs in the world. It is a naturally occurring stimulant that has a variety of unique characteristics. Although the pharmacokinetics and pharmacodynamics of caffeine have been the subject of thousands of studies over the past century, many of its characteristics (e.g., mechanisms of action, stimulant properties) are still unclear. The purpose of this presentation is to provide an overview of current knowledge pertaining to the pharmacokinetic characteristics, efficacy, safety, dynamic effects, and possible formulations for the delivery of caffeine. In addition, we review past and current caffeine research from the Department of Neurobiology and Behavior of the Walter Reed Army Institute of Research.

DESIGN OF A FOOD MATRIX FOR THE DELIVERY OF PERFORMANCE-ENHANCING COMPONENTS

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The utilization of performance-enhancing agents has a two-fold approach. First, the efficacy of the agent must be established using physiological and/or cognitive measurements. Second, a delivery system is necessary that ensures timely availability of the agent to the physiological point of need. There are several delivery systems currently available: transdermal, pills (including time release), inhalants, injections, and incorporation of the agent into food. The mode of delivery for the military is incorporation into common foods and restriction of any performance agent to that of a natural food constituent such as proteins, amino acids, antioxidants, and caffeine. There are several considerations when incorporating performance-enhancing agents into foods:

1. compatibility of the agent with the other food components,
2. shelf-life stability,
3. physiological uptake and delivery of the agent to the target organs, and
4. acceptance of the food item to ensure consumption of nutrients in the fortified item.

The military shelf-life requirements of 3 years at 80°F and 6 months at 100°F make this even more challenging than commercially developed products, which have a shorter shelf life.

This paper focuses on the development of a chocolate–caffeine food bar and placebo to be used in physiological performance testing. The bar was designed to deliver 6 mg of caffeine per kg weight of the subject (i.e., a 75-g bar for a 105-kg subject would contain 632 mg of caffeine, equivalent to 6 cups of coffee). In order to mask this level of caffeine, a chocolate mocha-flavored bar matrix was chosen. The bar weight was adjusted to maintain consistent dose weight for variable subject weights. Caffeine is a very bitter ingredient, which creates food technological challenges in developing an acceptable product, as well as a placebo that looks and tastes like the product. The bars were fed to military subjects prior to physical training. Caffeine uptake and distribution were monitored over a 2-hour period by analysis of caffeine in the subject's saliva.

CAFFEINE AND CARBOHYDRATE SUPPLEMENTS FOR PHYSICAL PERFORMANCE

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Both caffeine and carbohydrate supplementation have been found to have ergogenic effects on aerobic endurance and athletic performance. The means by which these supplements induce their ergogenic effects occur through different mechanisms of action and may be influenced by the type and intensity of exercise. There is ample evidence that caffeine improves aerobic endurance by increasing fat oxidation and sparing muscle. This is very beneficial for prolonged aerobic exercise in which muscle glycogen is a required fuel source. Caffeine also appears to function as a neurological stimulant and may improve aerobic endurance and exercise performance at high exercise intensities by reducing perception of effort and masking symptoms of fatigue. During prolonged low-intensity exercise, or prolonged exercise that varies from low to moderate intensity, carbohydrate supplementation improves aerobic endurance by increasing reliance on blood glucose and sparing muscle glycogen. When the exercise is moderately intense (65 to 75 percent, VO_{2max}), carbohydrate supplementation does not spare muscle glycogen but enhances aerobic endurance by preventing the onset of hypoglycemia and maintaining an adequate rate of carbohydrate

oxidation. Because the ergogenic effects of caffeine and carbohydrate supplementation occur through different mechanisms of action, it can be theorized that their effects on endurance performance would be additive. However, carbohydrate supplementation blunts the exercise-induced increase in lipolysis and inhibits fat oxidation. Therefore, the ergogenic effect of caffeine may actually be blunted, rather than enhanced, by the addition of carbohydrate to a caffeine supplement. Whether the combination of caffeine and carbohydrate supplements functions additively or antagonistically may depend on the type and intensity of exercise being performed and the timing of the supplementation. These conditions are discussed with regard to the ergogenic effects of each supplement.

COGNITIVE PERFORMANCE EFFECTS OF CAFFEINE VERSUS AMPHETAMINE FOLLOWING SLEEP DEPRIVATION

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With sustained military operations, round-the-clock work schedules often lead to sleep deprivation. It has been well documented that sleep deprivation impairs cognitive performance and alters mood, with a consequent increased threat to safety and productivity in a variety of industrial and military settings. Stimulants have long been used to reduce the effects of sleep loss and to counteract the sleepiness resulting from irregular work–rest hours. A number of studies in our laboratory at Walter Reed Army Institute of Research have examined the effects of stimulant administration following prolonged periods of wakefulness. Here, we present a comparison of the effects of caffeine and amphetamine in subjects who are tested through a total of 64 hours sleep deprivation. Performance, alertness, and mood measurements were taken throughout the study. At 48 hours of sleep deprivation, a dose of caffeine (150, 300, or 600 mg), amphetamine (5, 10, or 20 mg), or placebo was administered, and testing continued for at least 12 hours postdose. Both compounds, at the highest dose tested for each, produced comparable results in the following ways: cognitive performance improved and was sustained for 12 hours; measures of objective alertness improved; and there was an improvement in self-ratings of mood. There were also some adverse side effects, with amphetamine producing mild cardiovascular disturbances, disruptions in recovery sleep, and feelings of euphoria, while caffeine resulted in increased subjective reports of tremor and ratings of anxiety. Our recommendation is that given the universal availability and socially acceptable use of caffeine (with relatively few adverse side effects), it can be used only to "postpone" sleep up to 12 hours, not to replace it. Future studies in our laboratory will assess the synthetic compound modafinil, currently indicated for improving alertness in narcoleptics, and compare modafinil to

caffeine and amphetamine in our standard paradigm of measuring cognitive performance, alertness, and mood.

USE OF AMPHETAMINE TO COUNTERACT SLEEP DEPRIVATION IN AVIATORS

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The purpose of this investigation was to establish the efficacy of dexedrine for sustaining aviator performance despite 64 hours of extended wakefulness. Although earlier flight studies yielded favorable results with no significant side effects, they were restricted to sleep deprivation periods of only 40 hours. Due to requirements for longer periods of sustained wakefulness, it was necessary to study the efficacy of dexedrine in maintaining aviator performance during 3 days and 2 nights without sleep. To accomplish this, computerized evaluations of aviator flight skills were conducted at regular intervals as subjects completed standardized flights in a UH-60 helicopter simulator, under both dexedrine and placebo. Laboratory-based assessments of cognitive, psychological, and central nervous system status were completed as well. Dexedrine (10 mg) was given prophylactically (prior to signs of fatigue) at midnight, 0400, and 0800 on both deprivation days in one cycle, and placebo was given on both days in the other.

Results indicated that simulator flight performance was maintained by dexedrine for up to 58 hours, while performance under placebo deteriorated significantly. The drug was most beneficial at 0500 and 0900 on the first deprivation day, but it continued to attenuate impairments throughout 1700 on the second deprivation day (after 58 hours awake). Dexedrine likewise lessened the slowing of response times, the impairments in problem identification, and the reductions in performance capabilities that were evident in the cognitive data under placebo. The positive effects of dexedrine were noticeable after only 22 hours of sustained wakefulness but were most evident between 0500 and 1200 on both deprivation days (the times at which performance under placebo suffered the most). These were the same times at which the differences between dexedrine and placebo were most apparent in the flight data. Dexedrine suppressed the increases in slow-wave electroencephalogram (EEG) activity (associated with impaired alertness), which began to occur under the placebo condition after 23 hours of continuous wakefulness. The medication then attenuated a further increase in slow EEG activity that was present throughout 55 hours (and sometimes 59 hours) of deprivation. At the same time, dexedrine (compared to placebo) clearly sustained self-perceptions of vigor, alertness, energy, and talkativeness, while reducing problems with fatigue, confusion, and sleepiness. Mood declines were observed after 20 hours without sleep under the placebo condition,

and these were followed by further decrements that were most noticeable after 48 hours of continuous wakefulness. Ratings actually improved under dexedrine at several times. Recovery sleep was slightly less restful under dexedrine even though the last dose was 15 hours before bedtime (dexedrine has an average half-life of 10.25 hours). Thus, at least two nights of recovery sleep should be required after dexedrine is used to delay sleep for 64 hours.

There were no clinically significant side effects that led to the discontinuation of any participant; however, one subject experienced an increase in diastolic blood pressure that would have been cause for concern had it not decreased when the subject was retested in a prone position. Some aviators complained of palpitations and "jitteriness" under dexedrine, but this did not detract from their performance. One of the subjects became very excitable and talkative under the influence of dexedrine, but he did not become reckless or dangerous.

In summary, prophylactic dexedrine administration substantially reduced the impact of sleep loss in the early morning hours and, for the most part, preserved performance for the remainder of the day in a 64-hour bout of continuous wakefulness. The beneficial effects of dexedrine are most apparent during the circadian trough where performance and alertness under placebo are the worst. Thus, when proper restorative sleep is not available due to operational constraints, dexedrine should be considered an effective countermeasure; however, it should not be used as a substitute for sleep. Proper crew rest management must remain a top priority to preserve our tactical advantage on the battlefield.

EFFECT OF NAPS AND CAFFEINE ON ALERTNESS DURING SLEEP LOSS AND NOCTURNAL WORK PERIODS

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This work was performed at the Long Beach Veterans Administration Medical Center and the San Diego Naval Health Research Center and supported by a Merit Review Grant from the Department of Veterans Affairs, the Sleep–Wake Disorders Research Institute, and the Naval Medical Research and Development Command, Department of the Navy, Bethesda, Maryland, under Research Work Unit 61153N MR. 04101-03-6003. The views presented in this paper are those of the authors. No endorsement by the Department of the Navy has been given or should be inferred.

Methods

Three studies involving 176 male college students or naval recruits have examined alertness and performance over extended periods of sleep loss. Subjects

were chosen to be in good health, to have normal sleep habits, and to be moderate daily caffeine users (250 mg or less). In the first study, groups either (1) went for 64 hours with no sleep or caffeine, (2) had prophylactic naps of 2, 4, or 8 hours prior to sleep loss, or (3) received caffeine at 150, 300, or 400 mg during sleep loss. In the second study, subjects had a 4-hour prophylactic nap prior to sleep loss and then additionally received caffeine at 200 mg (eleveine) during the night. In the third study, subjects either had a 4-hour prophylactic nap prior to sleep loss and received 200 mg of caffeine (eleveine) during the night or had four 1-hour naps during the night.

Results

The results of the first study showed a dose–response effect for length of prophylactic nap and caffeine. Alertness and performance during sleep loss were significantly improved compared to the placebo no-nap group. Alertness was increased most by 8 hours of sleep. The improvement after caffeine use was more similar to that seen after 2–4 hours of additional sleep, except that the effects of caffeine were limited by its metabolic half-life. None of the interventions were able to overcome the profound loss of alertness on the second night of sleep deprivation. The results of the second study indicated that the beneficial effects of caffeine and prophylactic naps were additive (i.e., a prophylactic nap followed by nocturnal use of caffeine left nocturnal alertness and performance at daytime baseline levels). The third study showed that a prophylactic nap followed by nocturnal use of caffeine was superior in maintaining nocturnal performance compared to a series of nocturnal naps, perhaps because the nocturnal naps resulted in sleep inertia.

B

Previous Recommendations on Caffeine from the Committee on Military Nutrition Research

The following is an excerpt from: Institute of Medicine. 1994. *Food Components to Enhance Performance.* Washington, DC: National Academy Press. Pp. 32–34, 56–57.

CAFFEINE

The literature on the effects of caffeine on behavior, performance, and health is extensive and somewhat contradictory (for reviews see, for example, Bergman and Dews, 1987; Graham 1987; Hughes et al., 1988; Jarvis, 1993; Smith et al., 1994a,b). David Penetar and colleagues (chapter 20) present a new study on the effects of caffeine on cognitive performance, mood, and alertness in human subjects who had been sleep-deprived, and summarize current knowledge about the use of such supplements. Caffeine is known to exert its central nervous system-mediated effects by blockade of adenosine receptors. Its stimulant effects when compared with those of other drugs such as amphetamines are weak, but most studies to date suggest that it tends to delay sleep, reduce the deterioration of performance associated with fatigue and boredom, and decrease steadiness of the hands, particularly when performance is already partially degraded by repetitive, nonintellectual tasks.

Less well understood are the effects of caffeine in reversing changes caused by sleep deprivation. To clarify these issues, three doses of caffeine (150, 300, and 600 mg/70 kg of body weight) were assessed among normal healthy males after 2 days of sleep deprivation. Cognitive performance, mood, alertness, vital signs, serum caffeine concentrations, and plasma catecholamine levels were also assessed.

Cognitive performance was measured using a computerized assessment battery. Choice reaction time (for 8 hours) significantly improved after caffeine administration, although tests of code substitution and immediate and delayed recall were unaffected.

Mood was assessed by ratings on a profile of mood states questionnaire. Significant increases in vigor were reported for 2 hours after taking the dose, with decreases in fatigue and confusion. Also, significant improvements in mood for 2 hours postdose were reported on visual analog scales for increased alertness, confidence, energy level, and talkativeness and decreased sleepiness. However, anxiety and jitteryness/nervousness also increased. At 12 hours postadministration, ratings for increased energy levels, decreased sleepiness, and jitteryness/nervousness remained elevated.

Alertness, assessed by the modified multiple sleep latency test, also improved for 4.5 hours after caffeine administration, returning to 50 percent of rested levels when the highest doses were used. Oral body temperature remained elevated for 12 hours and blood pressure (diastolic) for one hour, but neither rate nor systolic blood pressure were elevated.

It was concluded that large doses of caffeine reversed sleep deprivation-induced degradation in cognitive performance, mood, and alertness without serious side effects. These data were consistent with those represented in most other studies reviewed. Therefore, Penetar et al. (Chapter 20) recommended that caffeine be included in rations at 250 mg per tablet and that it be made available to soldiers for maintaining performance during specific military operations. The authors did not study individuals with habitually high levels of caffeine ingestion; it would be useful to determine whether the effects of the doses of 300–600 mg noted in this study were as pronounced in individuals with markedly higher levels of typical intakes.

Sustaining optimal soldier performance is recognized to depend on other measures as well. The first is training, so that tasks can be performed with a minimal level of cognitive effort, cross-training so that individuals can substitute for each other, developing and adhering to appropriate work and rest cycles, exercising wise leadership so that unnecessary demands are not placed on subordinates, and modification of systems to minimize errors. Second is enforcing sleep discipline so that the sleep-deprived individual sleeps as much as he or she can and in as hygienic a manner as possible.

The relationship between caffeine intake and health outcomes, particularly cancer incidence, cardiovascular disease (CVD), and effects on fertility, and pregnancy and child outcome, has been the focus of many studies. While data from individual studies have been contradictory, reviews tend to conclude that there is no significant association or negligible/transient effects relating moderate caffeine consumption and cancer, CVD, fertility, and osteoporosis (see, for example, AMAC, 1984; Cooper et al., 1992; Gordis, 1990; Joesoef et al., 1990; Johansson et al., 1992; Lubin and Ron, 1990; Olsen, 1991; Rosenberg, 1990; Schairer et al., 1986; Wilson et al., 1989). However, reports continue to demonstrate that caffeine intake causes an elevation in blood pressure (Smith et al., 1994a,b). Although the blood pressure elevation produced by caffeine has been interpreted as transient and within the range produced by typical activities

(HHS, 1988; Myers, 1988), blood pressure bears monitoring in any future studies of performance enhancement with caffeine supplementation. Recent reports that assess the safety of caffeine consumption during pregnancy have continued to produce conflicting information (Eskenazi, 1993; Infante-Rivard et al., 1993; Mills et al., 1993). These data indicate that high levels of caffeine intake (> 300 mg/d) potentially increase the risk of spontaneous abortion and intrauterine growth retardation during pregnancy (Mills et al;, 1993). The risk to pregnant women of low levels of caffeine intake is uncertain. Further, women often do not realize they are pregnant and/or do not receive prenatal care until after the time period when most spontaneous abortions occur. Should the Army pursue further research in performance enhancement using caffeine products, these health issues must be carefully considered.

In summary, continued research on the mechanisms for the evident effects of caffeine on cognitive performance, mood, and alertness and how these may be enhanced in combination with other dietary measures is warranted. Of particular interest is how to maximize positive effects when performance is already degraded. Individual differences, expectancy, and placebo effects need further elucidation. In the meantime, practical applications of demonstrated effects in ration planning may be in order.

COMMITTEE RECOMMENDATIONS REGARDING FOOD COMPONENTS PROPOSED BY THE ARMY

1. The following components have clearly demonstrated their ability to enhance performance under appropriate simulated conditions and should be evaluated in appropriate delivery systems.

Caffeine. Caffeine functions as a weak stimulant that, in low doses, tends to delay sleep and reduce the deterioration of performance associated with fatigue and boredom. At higher doses caffeine reverses the sleep deprivation-induced degradation in cognitive performance, mood, and alertness. The long experience with the use of coffee suggests that caffeine is safe at levels required to achieve the desired effects, and its effects are reversible over time. **The primary issues that need to be answered in providing caffeine are the appropriate carrier that should be used to provide the supplement and the amount required to achieve the desired benefit in those both habituated and nonhabituated to it.** Since it would not be desirable to inhibit sleep when operations permit, the timing of ingestion and availability of the caffeine-containing food component should be evaluated.

C

Biographical Sketches

COMMITTEE

JOHN E. VANDERVEEN (*Chair*) is the former director of the Food and Drug Administration's (FDA) Office of Plant and Dairy Foods and Beverages in Washington, D.C. His previous position at the FDA was director of the Division of Nutrition at the Center for Food Safety and Applied Nutrition. He also served in various capacities at the U.S. Air Force School of Aerospace Medicine at Brooks Air Force Base, Texas. He has received accolades for service from the FDA and the Air Force. Dr. Vanderveen is a member of the American Society for Clinical Nutrition, American Institute of Nutrition, Aerospace Medical Association, American Dairy Science Association, and the American Chemical Society; a fellow of the Institute of Food Technologists; and an honorary member of the American Dietetic Association. He has served as the treasurer of the American Society of Clinical Nutrition and as a member of the Institute of Food Technology's National Academy of Sciences Advisory Committee. Dr. Vanderveen holds a B.S. in agriculture from Rutgers University in New Jersey and a Ph.D. in chemistry from the University of New Hampshire.

LAWRENCE E. ARMSTRONG is an associate professor of exercise science at the University of Connecticut. He has joint appointments in the Department of Physiology and Neurobiology and the Department of Nutritional Sciences. Dr. Armstrong received his Ph.D. in human bioenergetics–exercise physiology from Ball State University. His research interests include thermoregulation, fluid–electrolyte balance, energy metabolism, exercise physiology, and the human heat illnesses. He previously served as a research physiologist at the U.S. Army Research Institute of Environmental Medicine. He is a fellow of the American

search Institute of Environmental Medicine. He is a fellow of the American College of Sports Medicine and a member of the Federation of American Societies for Experimental Biology and the Aerospace Medical Association.

GAIL E. BUTTERFIELD was director of nutrition research for Palo Alto Veterans Affairs Health Care System in California; a lecturer in the Department of Medicine, Stanford University Medical School; visiting assistant professor in the Program of Human Biology, Stanford University; and director of nutrition in the Program in Sports Medicine, Stanford University Medical School. Her previous academic appointments were at the University of California, Berkeley. Dr. Butterfield belonged to the American Institute of Nutrition, American Society for Clinical Nutrition, American Dietetic Association, and American Physiological Society. She was a fellow of the American College of Sports Medicine (ACSM), served as chair of the Pronouncements Committee, and was on the ACSM Board of Trustees; she also was president and executive director of the southwest chapter of that organization. She was a member of the Respiratory and Applied Physiology Study Section of the National Institutes of Health and had served on the editorial boards of the following journals: *Medicine and Science in Sports and Exercise*, *American Journal of Clinical Nutrition*, *Health and Fitness Journal of ACSM*, *Canadian Journal of Clinical Sports Medicine*, and *International Journal of Sports Nutrition*. Dr. Butterfield received her A.B. in biological sciences, M.A. in anatomy, and M.S. and Ph.D. in nutrition from the University of California, Berkeley. Her research interests included nutrition in exercise, effect of growth factors on protein metabolism in the elderly, and metabolic fuel use in women exposed to high altitude. She died suddenly on December 27, 1999.

WANDA L. CHENOWETH is a professor in the Department of Food Science and Human Nutrition at Michigan State University. Previously, she held positions as teaching associate at the University of Iowa and University of California, Berkeley. Other work experience includes positions as research dietitian and head clinical dietitian at University of Iowa Hospitals and as research dietitian at the Mayo Clinic. She is a member of the American Society for Nutritional Sciences, American Dietetic Association, and Institute of Food Technology. She serves as a reviewer for several journals, including the *Journal of the American Dietetic Association*, *American Journal of Clinical Nutrition*, and *Journal of Nutrition*, and is a member of the Associate Editorial Board of *Plant Foods for Human Nutrition*. She has served on a technical review committee for the Diet, Nutrition, and Cancer Program of the National Cancer Institute and as a site evaluator for the Commission on Evaluation of Dietetic Education of the American Dietetic Association. Her research interests are in the areas of mineral bioavailability and clinical nutrition. Dr. Chenoweth completed a B.S. in dietetics from the University of Iowa, dietetic internship and M.S. in nutrition at the Uni-

versity of Iowa, and a Ph.D. in nutrition at the University of California, Berkeley.

JOHANNA T. DWYER is the director of the Frances Stern Nutrition Center at New England Medical Center, professor of medicine and community health at the Tufts University School of Medicine, and professor of nutrition at Tufts University School of Nutrition in Boston. She is also senior scientist at the Jean Mayer U.S. Department of Agriculture (USDA) Human Nutrition Research Center on Aging at Tufts. Dr. Dwyer is the author or coauthor of more than 100 research articles and 185 review articles published in scientific journals. Her work centers on life-cycle-related concerns such as the prevention of diet-related disease in children and adolescents and maximization of quality of life and health in the elderly. She also has a long-standing interest in vegetarian and other alternative life-styles. Dr. Dwyer is a past president of the American Institute of Nutrition, past secretary of the American Society for Clinical Nutrition, and past president and current fellow of the Society for Nutrition Education. She served on the Program Development Board of the American Public Health Association from 1989 to 1992 and is a former member of the Food and Nutrition Board of the Institute of Medicine, and a member of the Technical Advisory Committee of the Nutrition Screening Initiative, and the Board of Directors of the American Institute of Wine and Food. As a Robert Wood Johnson Health Policy Fellow (1980–1981), she served on the personal staffs of Senator Richard Lugar (R-Indiana) and Senator Barbara Mikulski (D-Maryland). Dr. Dwyer has received numerous honors and awards for her work in the field of nutrition, including the 1996 W.O. Atwater Award of the USDA and the J. Harvey Wiley Award from the Society for Nutrition Education. She gave the Lenna Frances Cooper Lecture at the annual meeting of the American Dietetic Association in 1990. Dr. Dwyer is currently on the editorial boards of *Family Economics* and *Nutrition Review* and the advisory board of *Clinics in Applied Nutrition*; she is a contributing editor to *Nutrition Reviews*, as well as a reviewer for the *Journal of the American Dietetic Association*, *American Journal of Clinical Nutrition*, and *American Journal of Public Health*. She received her D.Sc. and M.Sc. from the Harvard School of Public Health, an M.S. from the University of Wisconsin, and her undergraduate degree with distinction from Cornell University.

JOHN D. FERNSTROM is professor of psychiatry, pharmacology, and behavioral neuroscience at the University of Pittsburgh School of Medicine, and director of the Basic Neuroendocrinology Program at the Western Psychiatric Institute and Clinic. He received his B.S. in biology and his Ph.D. in nutritional biochemistry from the Massachusetts Institute of Technology (MIT). He was a postdoctoral fellow in neuroendocrinology at the Roche Institute for Molecular Biology in Nutley, New Jersey. Before coming to the University of Pittsburgh,

Dr. Fernstrom was an assistant and then associate professor in the Department of Nutrition and Food Science at MIT. He has served on numerous governmental advisory committees. He presently is a member of the National Advisory Council of the Monell Chemical Senses Center, chairman of the Neurosciences Section of the American Society for Nutritional Sciences (ASNS), and a member of the ASNS Council. He is a member of numerous professional societies, including the American Institute of Nutrition, the American Society for Clinical Nutrition, the American Physiological Society, the American Society for Pharmacology and Experimental Therapeutics, the American Society for Neurochemistry, the Society for Neuroscience, and the Endocrine Society. Among other awards, Dr. Fernstrom received the Mead-Johnson Award of the American Institute of Nutrition, a Research Scientist Award from the National Institute of Mental Health, a Wellcome Visiting Professorship in the Basic Medical Sciences, and an Alfred P. Sloan Fellowship in Neurochemistry. His current major research interest concerns the influence of the diet and drugs on the synthesis of neurotransmitters in the central and peripheral nervous systems.

ROBIN B. KANAREK is professor of psychology and professor of nutrition at Tufts University in Medford, Massachusetts, where she also is the chair of the Department of Psychology. Her prior experience includes research fellow, Division of Endocrinology, University of California, Los Angeles School of Medicine, and research fellow in nutrition at Harvard University. In addition to reviewing for several journals, including *Science*, *Brain Research Bulletin*, *Journal of Nutrition*, *American Journal of Clinical Nutrition*, and *Annals of Internal Medicine*, she is a member of the editorial boards of *Physiology and Behavior* and the *Tufts Diet and Nutrition Newsletter* and past editor-in-chief of *Nutrition and Behavior*. Dr. Kanarek has served on ad hoc review committees for the National Science Foundation, National Institutes of Health, and U.S. Department of Agriculture nutrition research, as well as the Member Program Committee of the Eastern Psychological Association. She is a fellow of the American College of Nutrition, and her other professional memberships include the American Institute of Nutrition, New York Academy of Sciences, Society for the Study of Ingestive Behavior, and Society for Neurosciences. Dr. Kanarek received a B.A. in biology from Antioch College in Yellow Springs, Ohio, and an M.S. and Ph.D. in psychology from Rutgers University in New Brunswick, New Jersey.

ORVILLE A. LEVANDER is a research chemist for the U.S. Department of Agriculture (USDA) Nutrient Requirements and Functions Laboratory in the Human Nutrition Research Center in Beltsville, Maryland. He was resident fellow in biochemistry at Colombia University's College of Physicians and Surgeons, and research associate at Harvard University's School of Public Health. Dr. Levander served on the Food and Nutrition Board's Committee on

Dietary Allowances. He also served on panels of the National Research Council's Committees on Animal Nutrition and on the Biological Effects of Environmental Pollutants. He was a member of the U.S. National Committee for the International Union of Nutrition Scientists and temporary adviser to the World Health Organization's Environmental Health Criteria Document on Selenium. Dr. Levander was awarded the Osborne and Mendel Award from the American Institute of Nutrition. His society memberships include the American Institute of Nutrition, American Chemical Society, and American Society for Clinical Nutrition. Dr. Levander received his B.A. from Cornell University and his M.S. and Ph.D. in biochemistry from the University of Wisconsin, Madison.

ESTHER M. STERNBERG is chief of the Section on Neuroendocrine Immunology and Behavior and director of the Integrated Neural Immune Program of the National Institute of Mental Health Intramural Research Program at the National Institutes of Health (NIH). Dr. Sternberg received her M.D. degree and trained in rheumatology at McGill University, Montreal, Canada. She did postdoctoral training at Washington University, Barnes Hospital, St. Louis, Missouri, in the Division of Allergy and Immunology. She was subsequently a Howard Hughes Associate and instructor in the Department of Medicine at Washington University and Barnes Hospital before joining NIH. Dr. Sternberg is internationally recognized for her ground-breaking discoveries in the area of central nervous system–immune system interactions. She has received the Arthritis Foundation William R. Felts Award for Excellence in Rheumatology Research Publications, has been awarded the Public Health Service Superior Service Award, and has been elected to the American Society for Clinical Investigation in recognition of this work. Dr. Sternberg is also internationally recognized as a foremost authority on the L-tryptophan eosinophilia–myalgia syndrome (L-TRP-EMS). She was the first to describe this syndrome in relation to a similar drug L-5-hydroxytryptophan and published this landmark article in the *New England Journal of Medicine* in 1980. She received the Food and Drug Commissioner's citation for her work elucidating the pathogenesis of this syndrome.

MARY I. POOS (*Food and Nutrition Board [FNB] Staff, Study Director*) is project director for the Committee on Military Nutrition Research. She joined the FNB of the Institute of Medicine (IOM) in November 1997. She has been a project director for the National Academy of Sciences since 1990. Prior to officially joining the FNB staff, she served as a project director for the National Research Council's Board on Agriculture for more than seven years, two of which were spent on loan to the FNB. Her work with the FNB includes senior staff officer for the IOM report, *The Program of Research for Military Nursing* and study director for the reports, *A Review of the Department of Defense's Program for Breast Cancer Research* and *Vitamin C Fortification of Food Aid Commodities*. Currently, she also serves as study director to the Subcommittee

on Interpretation and Uses of Dietary Reference Intakes. While working with the Board on Agriculture, Dr. Poos was responsible for the Committee on Animal Nutrition and directed the production of seven reports in the *Nutrient Requirements of Domestic Animals* series, including a letter report to the commissioner of the Food and Drug Administration concerning the importance of selenium in animal nutrition. Prior to joining the National Academies she was consultant–owner of Nutrition Consulting Services of Greenfield, Massachusetts; assistant professor in the Department of Veterinary and Animal Sciences at the University of Massachusetts, Amherst; and adjunct assistant professor in the Department of Animal Sciences, University of Vermont. She received her B.S. in biology from Virginia Polytechnic Institute and State University and a Ph.D. in animal sciences (nutrition–biochemistry) from the University of Kentucky; she completed a postdoctoral fellowship in the Department of Animal Sciences Area of Excellence Program at the University of Nebraska. Dr. Poos's areas of research interest include protein and nitrogen metabolism and nutrition–reproduction interactions.

SPEAKERS

MICHAEL H. BONNET is professor of neurology at Wright State University of Medicine in Dayton, Ohio. At the Sleep Laboratory in the Department of Neurology at the Department of Veterans' Affairs Hospital in Dayton, Dr. Bonnet conducts research in the areas of sleep deprivation, sleep fragmentation, and insomnia.

JACK L. BRIGGS is the senior food technologist for the Department of Defense Combat Feeding Program, U.S. Army Soldier and Biological Chemical Command, at the Natick Soldier Center. Previously he held positions as a food scientist for Carnation Co., senior scientist at Lipton, and director of research and development at Brilliant Seafood. He received his master's degree in biochemistry from Colorado State University. In his present position as senior food technologist, he is responsible for planning and conducting applications engineering and development activities for ration components. In addition, he coordinates and advises on special technical problems related to the Department of Defense procurement of operational ration components. Currently, Mr. Briggs is working on the formulation and fabrication of novel foods with performance enhancement potential.

JOHN A. CALDWELL is an experimental psychologist and the director of sustained operations research at the U.S. Army Aeromedical Research Laboratory, where he conducts a variety of research on the performance of helicopter pilots. His studies are aimed at fully understanding the effects of sleep deprivation and aviator fatigue and developing countermeasures for use in the operational aviator environment. He conducts both simulator and in-flight pilot per-

formance studies to enhance the efficiency, safety, and well-being of aviators in sustained operations. His efforts have been published in more than 80 separate articles in peer-reviewed scientific journals and laboratory technical reports. Dr. Caldwell is an adjunct faculty member at the School of Aerospace Medicine and the Aviation Pre Command Course at Fort Rucker, and he frequently lectures at safety briefings and scientific symposia. He is a member of the National Sleep Foundation's Speakers Bureau on operator fatigue and frequently consults with various organizations on the effects of fatigue on pilots and methods for overcoming the adverse impact of fatigue in the aviation environment.

ROLAND R. GRIFFITHS is a professor of behavioral biology and professor of neuroscience at the Johns Hopkins University School of Medicine in Baltimore, Maryland. He received his Ph.D. in psychopharmacology from the University of Minnesota in 1972. Excluding abstracts and short reports, the total number of Dr. Griffiths' publications exceeds 200, and he has published more than 25 articles directly related to caffeine use in humans and caffeine dependence.

STEPHEN G. HOLTZMAN is a professor of pharmacology at Emory University School of Medicine in Atlanta, Georgia. In addition, he holds an appointment as a collaborative scientist in the Division of Neuroscience, Yerkes Regional Primate Research Center at Emory. He received his Ph.D. in pharmacology at the University of Michigan in 1969. Dr. Holtzman has won numerous honors and served on various committees related to drug abuse and dependence.

JOHN L. IVY is professor and coordinator of the Exercise Science Program in the College of Education, Department of Kinesiology and Health, and the College of Pharmacy, Division of Pharmacology, at the University of Texas in Austin. In 1998 he was awarded the Margie Gurley Seay Centennial Professorship. Other honors include a fellowship in the American Academy of Kinesiology and Physical Education, Dean's Fellowship for Excellence in Research, the Judy Spence Frank Endowed Fellowship for Excellence, and membership in Sigma Xi, the Scientific Research Society. Dr. Ivy was associate editor of *Research Quarterly for Exercise and Sports,* and currently serves on the editorial boards of *Medicine and Science in Sports and Exercise*, *American Journal of Physiology, Endocrinology and Metabolism*, *Journal of Optimal Nutrition*, *International Journal of Sports Nutrition*, and *Diabetes Forecast.*

RICHARD F. JOHNSON is a research psychologist in the Military Performance Division at the U.S. Army Research Institute of Environmental Medicine (USARIEM), Natick, Massachusetts. He received his Ph.D. in psychology (1970) from Brandeis University, where he was both a National Aeronautics and Space Administration trainee and a Woodrow Wilson dissertation fellow. Prior to joining USARIEM in 1984, he served as a captain in the U.S. Army Medical

Service Corps (1970–1972), was a National Institute of Mental Health grantee (1972–1976), and was a research psychologist with the U.S. Army Natick Research and Development Laboratories (1976–1983). He is a senior lecturer in psychology at Northeastern University and has published in the areas of psychophysiology, experimental research methodology, and stress. He is a fellow of both the American Psychological Association and the American Psychological Society, and is a past president of the Natick Chapter of Sigma Xi, the Scientific Research Society. His current research interests include the effects of environmental extremes and military operational demands on vigilance, psychomotor behavior, and subjective response.

GARY H. KAMIMORI is a research physiologist in the Department of Neurobiology and Behavior, Division of Neuropsychiatry, at Walter Reed Army Institute of Research.

MARY A. KAUTZ has been a research psychologist in the Department of Neurobiology and Behavior, Division of Neuropsychiatry, at the Walter Reed Army Institute of Research (WRAIR) since January 1998. She came on active duty as a direct commissioned officer in October 1997. She holds a Ph.D. in experimental psychology from the American University in Washington, D.C., and has completed two postdoctoral fellowships—one at Johns Hopkins University School of Medicine and a second at Bowman Gray School of Medicine of Wake Forest University. Her interests prior to coming on active duty included behavioral psychopharmacology research with benzodiazapines and alcohol in nonhuman primates. While at WRAIR, her research focus has been on determining militarily relevant relationships between variables, including physiological measures of brain activity, sleep, arousal, cognitive performance, and drugs (particularly stimulants as they are used to enhance cognitive performance following extended periods of sleep deprivation).

HARRIS R. LIEBERMAN is deputy chief of the Military Nutrition and Biochemistry Division of the U.S. Army Research Institute of Environmental Medicine (USARIEM) in Natick, Massachusetts. Dr. Lieberman is an internationally recognized expert in the area of nutrition and behavior and has published more than 90 original, full-length papers in scientific journals and edited books. He has been an invited lecturer at numerous national and international conferences, government research laboratories, and universities. Dr. Lieberman received his Ph.D. in physiological psychology in 1977 from the University of Florida. Upon completing his graduate training he was awarded a National Institutes of Health fellowship to conduct postdoctoral research at the Department of Psychology and Brain Science at the Massachusetts Institute of Technology (MIT). In 1980 he was appointed to the research staff at MIT and established an interdisciplinary research program in the Department of Brain and Cognitive Sciences to

examine the effects of various food constituents and drugs on human behavior and brain function. Key accomplishments of the laboratory included the development of appropriate methods for assessing the effects of food constituents and other subtle environmental factors on human brain function and the determination that specific foods and hormones reliably alter human performance. In 1990 Dr. Lieberman joined the civilian research staff of USARIEM where he has continued his work in nutrition and behavior. He has addressed the effects of various nutritional factors, diets, and environmental stress on animal and human performance, brain function, and behavior. His research program has focused on developing and applying a variety of emerging technologies to sustaining and enhancing human performance.

DAVID M. PENETAR currently is the commander of the U.S. Army Research Institute of Environmental Medicine, Natick, Massachusetts. He earned his Ph.D. in psychopharmacology from the University of Minnesota in 1977. His research experience includes the assessment of sleep deprivation and caffeine effects on cognitive performance conducted while assigned to the Walter Reed Army Institute of Research.

W.K. PRUSACZYK received his B.A. and M.S. degrees in psychology and his Ph.D. in exercise physiology from the University of Georgia. He then went on active duty in the U.S. Army and was stationed at the U.S. Army Research Institute of Environmental Medicine in Natick, Massachusetts. After serving three years in the Military Ergonomics Division, Dr. Prusaczyk left active duty and began work at the Naval Health Research Center (NHRC), San Diego, California. While at NHRC his early work focused on thermoregulation and thermal protection systems for Naval Sea–Air–Land (SEAL) personnel. After five years of work in the field with SEALs, Dr. Prusaczyk assumed the position of head, Applied Physiology Division, in the Human Performance Department at NHRC, managing broad research projects in thermal physiology, occupational physiology, and body composition. In 1997 Dr. Prusaczyk was promoted to head, Human Performance Department. His current research interests are in thermal physiology and protective systems, occupational physiology, and performance enhancement methodologies.

CHRISTINE SCHLICTING is from the Naval Submarine Medical Research Laboratory at the Naval Submarine Base, New London, Connecticut.

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HIGH-ENERGY, NUTRIENT-DENSE EMERGENCY RELIEF FOOD PRODUCT



INSTITUTE OF MEDICINE

High-Energy, Nutrient-Dense Emergency Relief Food Product

Subcommittee on Technical Specifications for a
High-Energy Emergency Relief Ration

Committee on Military Nutrition Research

Food and Nutrition Board

INSTITUTE OF MEDICINE

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The serpent has been a symbol of long life, healing, and knowledge among almost all cultures and religions since the beginning of recorded history. The serpent adopted as a logotype by the Institute of Medicine is a relief carving from ancient Greece, now held by the Staatliche Museen in Berlin.

Cover photo courtesy of Marjatta Tolvanen.

"Knowing is not enough; we must apply.
Willing is not enough; we must do."
—Goethe



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This report has been reviewed in draft form by individuals chosen for their diverse perspectives and technical expertise, in accordance with procedures approved by the NRC's Report Review Committee. The purpose of this independent review is to provide candid and critical comments that will assist the institution in making its published report as sound as possible and to ensure that the report meets institutional standards for objectivity, evidence, and responsiveness to the study charge. The review comments and draft manuscript remain

confidential to protect the integrity of the deliberative process. We wish to thank the following individuals for their review of this report:

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The subcommittee presents its special appreciation to the Institute of Medicine staff who facilitated the work and the preparation of this report: Ricardo Molins, study director; Mary Poos, study director of the Committee on Military Nutrition Research; Gail Spears, who edited the report; Leslie Vogelsang, research assistant; Tazima Davis, senior project assistant; and Allison Yates, director of the Food and Nutrition Board.

Barry Zoumas, Chair
Subcommittee on Technical
Specifications for a High-Energy
Emergency Relief Ration

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Executive Summary

As the world enters a new millennium, natural disasters and international or internal conflicts continue to displace hundreds of thousands of people. The human and physical needs of disaster and other emergency victims—deprived of or uprooted from their homes, on the road or in crowded camps and exposed to harsh elements—are paramount in efforts to provide a significant measure of relief. In response to the plight of refugees, the United States has provided assistance in many ways to victims of such emergencies the world over, most notably via food programs.

To a large extent, the emergency food relief assistance provided by the U.S. government has been channeled through the U.S. Agency for International Development (USAID) and the Department of Defense (DOD). The bulk of medium- and long-term food relief contributed by USAID has traditionally been in the form of commodity foods. However, USAID and DOD also participate in rapid, short-term food relief operations that require special high-energy, self-contained food products not currently manufactured in the United States. Such products constitute the vanguard of food relief and are designed for use over the normally short period of time needed to establish a more permanent, stable, food-based relief pipeline. Because of legislative restrictions on the use of some federal appropriations, only limited purchases of such products can be made by USAID from food manufacturers outside the United States. The availability of science-based technical specifications for use in calls for bids from U.S. food manufacturers, therefore, is of the essence not only to allow procurement of the most appropriate product, but also to do so in the United States.

BACKGROUND AND CHARGE TO THE COMMITTEE

The present study was conducted by an ad hoc subcommittee of the Committee on Military Nutrition Research. The Subcommittee on Technical Specifications for a High-Energy Emergency Relief Ration was established by the Food and Nutrition Board of the Institute of Medicine in response to a request from USAID and DOD to develop technical specifications for a product for use in food relief after natural disasters or other emergency situations around the world. The specifications are to be used by both agencies in their calls for bids from U.S. food manufacturers to supply such a product.

The charge to the subcommittee was as follows: Based on information on the nutritional requirements of target populations, food/nutrition specifications, product descriptions of similar rations already in use (e.g., emergency biscuits), and recommendations by refugee nutrition experts, what are the committee's recommendations to these questions:

1. What are the specifications for a cost-effective emergency ration bar for uprooted people in emergency situations that meets all of the following criteria:
   a. satisfies all nutrient requirements for a population of all ages over 6 months
   b. appropriate for use as the sole source of subsistence for up to 15 days
   c. acceptable to people of any ethnic and religious background
   d. can be eaten on the move without preparation steps
   e. can, without significant cost increase, be prepositioned in harsh environments for at least 3 years
   f. can, without significant cost increase, withstand an airdrop without endangering persons on the ground

2. Specifications should include consideration of each of these categories:
   a. *nutritional composition*, including macro- and micronutrient content and water content
   b. *food properties*, including nutrient stability, food consistency, palatability, and organoleptics
   c. *universal acceptance*, especially cultural acceptability to refugees and displaced persons
   d. *configuration*, size, color, and shape
   e. *packaging* for shipping, long-term stability (3 years), stability for airdrop, and ease of use
   f. *feasibility of manufacture*
   g. *commodity cost within limitations of average relief operations*

3. Make recommendations on special circumstances when this ration should and should not be used (and any provisions for simple alternatives or supplements), including:
   a. severely malnourished groups of refugees or liberated prisoners of war who may have specific micronutrient deficiencies or protein deficiency
   b. populations with a high prevalence of disease, such as acquired immune deficiency syndrome or diarrheal diseases
   c. individuals in harsh environmental conditions.

METHODS

The subcommittee met twice during the study. The first meeting was held at the U.S. Army Soldier Systems Center, Natick, Massachusetts, to allow the subcommittee to benefit from the experience of the DOD Combat Feeding Program, Performance Enhancement and Food Safety Team, and thus gather information on processes, ingredients, and packaging systems used by the U.S. Army in developing products similar to the desired emergency food product (EFP). Also at this meeting, USAID representatives described the agency's worldwide emergency food relief programs, and three USAID consultants discussed two background papers commissioned for this purpose by the agency. Moreover, the consultants contributed invaluable information on field conditions during emergency food relief operations that were taken into consideration by the subcommittee in its deliberations. The second and final subcommittee meeting was held in Washington, D.C.

REPORT ORGANIZATION

The report contains four chapters. Chapter 1 summarizes the project scope, its rationale, and the background for the need and uses of an EFP. It describes the types of emergencies and populations expected to benefit from such a product, and the special circumstances inherent to food relief operations after natural or man-made disasters, famines, massive displacement of people, and other emergencies that must be considered in defining the nutritional, chemical, and physical characteristics the EFP should have.

Chapter 2 describes the basic assumptions underlying the energy level chosen for the EFP, the calculations of macro- and micronutrient levels, and, in some instances, the recommended origin or modality of the nutrients to be used. The daily energy requirement recommended for planning emergency aid rations by a 1995 IOM report (2,100 kcal/day) was adopted by the subcommittee. Fundamental assumptions made by the subcommittee in determining the nutritional content of the EFP are given in Box ES-1.

Each macro- and micronutrient specification is discussed individually in Chapter 2. However, the subcommittee's recommendation for each is presented

BOX ES-1 Assumptions Used in Developing the Nutrition Composition of the EFP

- Potable water is provided as a top priority and is available with the EFP.
- Individuals will eat to meet their energy requirements.
- The product is to be consumed by all age groups, except infants less than 6 months of age; thus the product is not to be used in lieu of breast feeding, which is encouraged to at least 1 year of age with complementary use of the EFP after 6 months of age.
- It is not to be used as a therapeutic product and is not appropriate for severely malnourished individuals.
- It may constitute the sole source of food for target recipients for up to 15 days.
- Recipients are likely to be at least mildly malnourished and/or suffer from mild to moderate diarrhea and other debilitating diseases brought about by unsanitary conditions and exacerbated by stress.
- The recipient population may have nutrient needs comparable to well-nourished individuals in spite of smaller body weights due to maintaining muscle and visceral mass at the expense of body fat.
- The product should provide a nutrient density that will meet or exceed the nutrient recommendations as specified by the recommended intakes (IOM, 1997, 1998, 2000, 2001; NRC, 1989) which are designed to meet the needs of almost all individuals in each life stage and gender group (with the exception of infants) without exceeding Tolerable Upper Intake Levels (IOM, 1997, 1998, 2000, 2001).
- Nutrient needs of pregnant and lactating women are not included in the calculations, but it is assumed they will consume more than the daily ration based on individual needs for additional energy beyond the average of 2,100 kcal/day.

in tabular form in Chapter 4 (as Table 4-1) for ease of use by the agencies and potential manufacturers.

Chapter 3 discusses the preservation, processing, and packaging techniques that manufacturers should use in preparing the EFP so that it will attain the required stability under the expected conditions of delivery and use. These conditions might include extreme temperatures, rough handling, improvised storage, and the possible need to airdrop the product from low altitude. The recommendations from Chapter 3 are presented in the form of a performance specification for use by the agencies in preparing a call for bids in Chapter 4. The subcommittee views the technical specifications recommended in this report as optimal, but recognizes that the sponsoring agencies may be forced to consider developing EFPs prepared and packaged in less desirable ways if cost becomes the primary consideration.

RECOMMENDATIONS

There are five characteristics critical to development of a successful EFP. These are listed in order of importance. The EFP must be:

1. Safe
2. Palatable
3. Easy to deliver
4. Easy to use
5. Nutritionally complete.

In terms of decisions in the development of a prototype, this order of importance of the EFP should guide decisions about trade-offs between competing characteristics. In addition, it is recognized that the EFP must meet economical considerations; although considered in developing the specifications, it was beyond the scope of this report to weigh technical and nutritional advantages versus cost in a cost–benefit analysis. In addition to the recommended levels of each macro- and micronutrient presented in Table ES-1, the following recommendations are made:

- **Microbiological stability.** Preservation techniques that include combinations of low water activity values and some preservative(s) are the best approach to achieve microbiological stability of the EFP.
- **Chemical stability and nutrient retention.** A water activity level lower than 0.4 in the EFP is necessary to ensure protection against nutrient degradation. Microencapsulation of selected components and nutrients, particularly vitamin E together with highly unsaturated lipids, ascorbic acid, and iron (as FeNa EDTA), and other minerals is essential to minimize adverse lipid oxidation and nutrient losses. Antioxidants could be used in combination with microencapsulation depending on the ingredients used to prepare the EFP.
- **Flavor and color.** Based on anecdotal information, is suggested that only a sweet flavor and natural colors be used. The product, if dispersed in water, must not resemble milk. However, potential manufacturers should be encouraged to propose other flavors for the EFP, but these flavors should be tested for acceptability as described below.
- **Ingredients.** The ingredients used to prepare the EFP must provide the nutritional profile and other characteristics defined in the specifications. However, because the product will be distributed among multiple ethnic and cultural groups, alcohol or animal products other than milk may not be used. Use of milk solids must be limited so that lactose levels are not in excess of amounts known to be tolerated by individuals who are lactose maldigestors. Foods containing known allergens, such as peanuts, should be avoided. Some

TABLE ES-1 Nutritional Content of the Emergency Relief Food Product (EFP)[a]

Nutrient	Limiting Group	Minimum Required Nutrient Density per 1,000 kcal[a]	Amount per Single (233 kcal; 50g) EFP Bar
Fat	N/A		9–12 g
Protein[b]	51+ yr, men		7.9 g
Carbohydrate	N/A		23–35 g
Sodium[c]	2–5 yr, children	1.3 g	300 mg
Potassium[c]	2–5 yr, children	1.7 g	396 mg
Chloride[c]	2–5 yr, children	2.0 g	466 mg
Calcium	9–13 yr, children	768 mg	180 mg
Phosphorus	9–13 yr, children	740 mg	172 mg
Magnesium	14–18 yr, boys	190 mg	45 mg
Chromium	—	13 µg[d]	3 µg
Copper	51+ yr, women	560 µg[d]	131 µg
Iodine	1–3 yr, children	105 µg	25 µg
Iron[e]	19–50 yr, women	16 mg[d]	3.8 mg
Manganese	1–3 yr, children	1.4 mg	0.33 mg
Selenium	14–18 yr, girls	28 µg	6.5 µg
Zinc	14–18 yr, boys	10.5 mg[d]	2.4 mg
Vitamin A	14–18 yr, boys	500 µg[d]	117 µg
Vitamin D	51–70 yr, women	5.2 µg[d]	1.2 µg
Vitamin E	14–18 yr, girls	16 mg[d]	2.2 mg
Vitamin K	19–50 yr, men	60 µg	14 µg
Vitamin C	51+ yr, men	100 mg[d]	11.1 mg
Thiamin	1–3 yr, children	1.2 mg[d]	0.28 mg
Riboflavin	14–18 yr, boys	1.2 mg[d]	0.28 mg
Niacin	14–18 yr, boys	11.2 mg NE[d]	2.6 mg NE
Vitamin B_6	51+ yr, women	1.2 mg[d]	0.28 mg[e]
Folate[f]	14–18 yr, girls	310 µg DFE[d]	72 µg DFE
Vitamin B_{12}	14–18 yr, girls	12 µg[d]	2.8 µg
Pantothenic acid	14–18 yr, girls	3.9 mg[d]	0.9 mg
Biotin	51+, women	24 µg[d]	5.6 µg
Choline	51+, men	366 mg[d]	85 mg

[a] Ration set at 2,100 kcal/d (IOM, 1995).
[b] From NRC (1989); based on reference weights from IOM (1997) and estimated energy expenditure from Table 2-3.
[c] Values based on estimated requirements or desirable intakes (NRC, 1989).
[d] Adjusted from baseline nutrient density value; see text for explanation.
[e] Based on 10% iron bioavailability.
[f] If folate is provided as synthetic folate, which is more readily absorbed, these numbers should be divided by 1.6.
SOURCE: IOM (1997, 1998, 2000, 2001).

recommended ingredients are the following in order to provide nutrients as specified (see Table ES-1):

- cereal base: wheat flour, corn, oat flakes or flour, rice flour
- protein: soy products, such as concentrates or isolates; milk solids, casein, or derivatives; mixture of cereal base and protein must have a Protein Digestibility-Corrected Amino Acid Score ≥ 1.0
- lipid sources: partially hydrogenated soybean or cottonseed oil, flaxseed oil (source of omega-3 fatty acids), canola oil, sunflower oil
- sugars: sucrose, glucose, high-fructose corn syrup, maltodextrins
- baking and leavening agents, if needed
- vitamin and mineral premix as specified in the nutrient profile.

• **Testing prototypes for acceptability.** All EFP prototypes should be tested for acceptability under conditions similar to those used by the U.S. Army for General Purpose Survival Packets and Meal Ready-to-Eat, Individual. The suggestion made by the U.S. Army to use its facilities at various overseas locations for testing the EFP among local populations, so that its acceptability by populations having diverse ethnic and cultural backgrounds can be established, is heartily endorsed.

• **Packaging.** All packaging components used in the EFP must be capable of withstanding a wide range of temperatures and other physical abuse. Separate or additional packaging may be necessary for EFP airdrop operations. Pulp-based material with a moisture barrier coating should be used for the EFP primary packaging. A pouch constructed of a trilaminate consisting inside out of polyolefin, aluminum foil, and polyester or nylon should be the secondary packaging to keep oxygen at less than 2 percent throughout the 3-year shelf life of the product. Optional presentations of the EFP other than that for airdrop configuration could include a reusable, semi-rigid polyolefin, multi-ration container appropriately designed to allow secondary uses such as storage or water transport. Alternatively, a metal outer package, such as a tinplate box with an easy-to-remove cover, would be of great value to recipients during emergencies.

• **Product configuration.** The recommended 2,100-kcal/day energy level should be provided by an EFP weighing approximately 450 g. This ration should be configured as nine equal portions having the shape of bars, each scored across the width of the bar to provide two 116-kcal portions upon breaking it. A daily supply of nine bars should be packaged under a nitrogen flush or vacuum into one trilaminate pouch to provide the barrier against oxygen and moisture needed for extended shelf life. Five daily rations should be packaged into a single bundle so that 5-days worth of food for a single individual or a 1-day feeding of a five-member family may be distributed as a unit. Eight bundles of five EFPs each should be placed into a shipping container of corrugated

construction or constructed of metal, so that they can be recycled for use as storage or water containers. Shippers would be assembled onto a pallet for transport.

- **Production methods.** The EFP must be prepared using Good Manufacturing Practices and all sanitary regulations and practices applicable to ready-to-eat food products.

- **Testing for quality assurance and control.** Testing of the EFP must be conducted throughout the expected shelf life of the product and under conditions of delivery and storage simulating actual use, to ascertain the initial content and stability of nutrients throughout the expected 3-year shelf life. Standard methodologies for determining vitamin and mineral content of the EFP should be used, and appropriate procedures, such as those used for nutritional labeling, must be applied.

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1
Introduction

PROJECT DESCRIPTION AND SCOPE

The Subcommittee on Technical Specifications for a High-Energy Emergency Relief Ration was established by the Food and Nutrition Board, Institute of Medicine (IOM) of The National Academies under the oversight of the standing Committee on Military Nutrition Research. The subcommittee was formed to address a request from the U.S. Agency for International Development (USAID) of the Department of State and the Department of Defense (DOD). These agencies asked IOM to develop technical specifications to be used in their solicitation of bids from the U.S. food industry for the production of a high-energy, nutrient-dense emergency relief food product (EFP). The scope of the subcommittee's task centered on defining the specifications of an EFP that would satisfy the nutritional requirements of populations of all ages above 6 months, be appropriate for use as the sole source of subsistence for up to 15 days, be acceptable to a wide spectrum of cultures and ethnic and religious backgrounds, be eaten on the move without need for preparation, be stable for at least 3 years, and be amenable to land or air delivery.

The specifications requested for an EFP encompassed the following: nutritional composition, including water and macro- and micronutrient content; food properties related to stability, consistency, and sensory properties and acceptability; product configuration, size, color, shape, and primary and secondary packaging for long-term prepositioning under severe environmental conditions and for land or air delivery; feasibility of manufacture; quality assurance and control parameters; and estimated cost.

In addition, the subcommittee was asked to make recommendations on when the EFP should and should not be used. Finally, it was requested that the report present all relevant product characteristics and other processing and quality control parameters in performance specification format.

ORIGIN OF THE STUDY

Feeding refugees or disaster victims depends on being able to deliver a nutritionally appropriate diet quickly and at low cost. USAID, through its recently created Democracy, Conflict and Humanitarian Assistance Bureau (which includes the former Bureau for Humanitarian Response), assists foreign countries in famine and disaster relief by providing food rations for distribution. Similar humanitarian aid is provided by DOD in emergency situations. This food relief is often the only source of food available to affected individuals during the initial period after such natural disasters as hurricanes or earthquakes, or during emergencies such as evacuations or fleeing from combat zones.

The energy value, nutritional composition, and sensory appeal of emergency food rations are of utmost importance in meeting the nutritional needs of recipients. In general, emergency food relief has traditionally relied on distribution of bulk food such as grains or corn- or wheat-based mixes that require preparation prior to consumption. The aim of the present study, in contrast, is to provide specifications for a stand-alone product that can be delivered and used as a sole source of food while a more permanent, stable food relief system is established. It is possible that the EFP could be used later in circumstances other than emergencies as a supplemental source of nutrients to more traditional diets.

In addition to the overriding importance of having a food ration that provides the required energy level, protein, vitamins, minerals, and other essential nutrients, sensory appeal is an important factor to be considered when developing formulations that may be acceptable to a wide spectrum of cultures. Maintaining quality and appropriate package design, in turn, are critical to meeting the food relief objective because the rations must be able to endure very harsh conditions during handling and storage with minimal nutrient losses. The size and type of packaging has also proven to be important to avoid diversion of the EFPs to military use during emergencies involving civilian populations and combatants. Because of logistic problems during many emergencies, it is also essential that packaging of the EFPs be specially designed to withstand an airdrop without being destroyed or harming recipients on the ground.

Although the United States is a major contributor to global food relief, U.S. companies do not currently manufacture the type of food products necessary for the initial stages of emergency food relief. As a result, and because USAID is required to purchase only U.S. products with Public Law (P.L.) 480 funds, purchases of such products from European manufacturers (see Chapter 3) can only be made by USAID using other limited funds. The availability of specifications

for use in solicitations of bids from the U.S. food industry, therefore, will allow not only procurement of the most appropriate product, but also one that is manufactured within the United States.

THE NEED FOR AND USES OF A HIGH-ENERGY, NUTRIENT-DENSE EMERGENCY RELIEF FOOD PRODUCT

Emergencies Requiring Relief Operations

Disasters requiring food relief operations include natural disasters, man-made disasters, and complex humanitarian emergencies. Natural disasters are those caused by fire, flood, drought, earthquake, and disease outbreak, whereas man-made disasters are caused by human error, as in industrial accidents. Complex humanitarian emergencies are usually the result of or complicated by armed conflict, genocide, or rural famines, and tend to cause massive population displacements aggravated by the lack or collapse of basic services (Keely et al., 2001). The extent and level of complexity of these emergencies may be further compounded by natural weather phenomena such as droughts or by unique circumstances such as the presence of large populations of prisoners of war. Some 30 complex humanitarian emergencies existed worldwide at the end of 1999 (Keely et al., 2001).

Natural disasters and their impact on people are on the increase, according to U.S. relief organizations. Causes identified by USAID (2001a) include the continuous degradation of natural environments that magnify the impact of natural events, and population increases in coastal areas and other regions exposed to floods, eruptions, landslides, and other geological or meteorological threats (USAID, 2001b). Examples of environmental degradation contributing to natural disasters are destruction of forests, desertification, and overall climate change. The number of natural disasters in the 1990s—designated by the United Nations General Assembly in its resolution 44/236 as the International Decade for Natural Disaster Reduction—tripled that seen in the 1960s. The Office of the U.N. Emergency Relief Coordinator estimated that from 1970 to 1990 some 800 million people were affected by natural disasters, including more than 3 million deaths, with cumulative economic losses in the order of $30 to $50 billion per year (UNEP, 1992).

According to USAID, almost 2 billion people were affected by natural disasters globally during the 1990s (USAID, 2001b). In 1999 alone, 212 million people were affected by hurricanes, typhoons, earthquakes, and floods that required immediate response from national and international relief organizations. This number did not include the hundreds of millions of people affected by droughts and their sequel of famines, many of whom abandoned their homes, villages, and regions in search of food for survival, nor the some 35 million

people uprooted by 25 armed conflicts in 27 countries that year. Among the latter, 21 million were classified as internally displaced persons (IDPs), while the other 14 million, having crossed international boundaries, were classified as refugees (Crisp, 2000; USCR, 2000). Statistics from the office of the United Nations High Commissioner for Refugees (UNCHR) for 2000 list 21 million people as "refugees and others of concern"; 12 million of these were refugees (UNHCR, 2001).

Emergency relief is provided by the United States and other donors in regions of the world where natural disasters occur and the affected country does not have the capacity to cope with destruction of the public service infrastructure. It is also provided when complex humanitarian emergencies induce massive population displacements through or into areas where public services are nonexistent or insufficient (Keely et al., 2001). Many situations requiring emergency relief arise in the least developed, poorest areas of the world, where human populations are frequently afflicted with chronic malnutrition and various debilitating diseases such as dysentery and malaria (de Onis, 2000; Snow et al., 1999). The threat to life as well as the psychological distress associated with the loss of homes and livelihoods, and sometimes the horrors of combat situations, are other important contributing factors to the overall weakness and poor physical state of populations in need of emergency relief (Burkholder et al., 2001). These often result in high mortality rates, particularly among children in developing countries and the elderly in more developed areas (Keely et al., 2001).

Although it has been said that the only common denominator in emergencies requiring relief is that they are all unique and different, the need for foods with acceptable quality attributes and in quantities appropriate to sustain those affected is also common to all, as is the need to deliver such food promptly and at low cost. It is generally agreed among relief organizations that the quality of food relief provided to affected individuals during the initial stages of an emergency is a determinant in minimizing mortality rates. It is during flight and the time immediately after arrival in camps or other relief stations that the highest mortality takes place (Sphere Project, 2000). It is also during the first stage of emergencies when people who are on the move or under the trauma of arrival in camps do not have appropriate food preparation utensils and facilities, and hence must rely on ready-to-eat EFPs. This has been the rationale behind the development of various compact EFPs currently produced in other countries (Grobler-Tanner, 2001; Young et al., 1988).

Target Populations for an Emergency Relief Food Product

Although food relief situations involve people of all ages, there are differences in the composition of populations affected by various types of emergencies. Natural and man-made disasters, on one hand, affect the entire population in the disaster area. Complex emergencies, on the other hand, may affect groups

within a population in ways that differ depending on gender, age, or ethnic group. Situations that involve combat, for example, may result in displacement of women and children while older boys and adult men stay behind. UNHCR provisional statistics indicate that there were slightly more women than men among refugee and IDP populations in 2000 (52 vs. 48 percent, respectively). Infants aged 0 to 4 and youngsters aged 5 to 17 years constituted more than 14 and 31 percent, respectively, of the total refugee population, whereas the proportion corresponding to the elderly (aged 60 and above) constituted 8 percent. The largest group was, by far, adults aged 18 to 59, who comprised 46 percent of refugees (UNHCR, 2001).

Population composition in terms of age, sex, health, nutritional status, activity level, and climate are important considerations to ensure that food relief properly addresses the nutritional needs of intended recipients. However, clear international guidelines currently available for estimating food rations for refugees refer mostly to foods provided through the stable supply pipeline that relief agencies establish after the initial stages of an emergency. Little has been published on the appropriate composition of a high-energy, nutrient-dense EFP for use at the onset of emergencies before the food supply system has been established.

The energy level of emergency food rations, in contrast, has been defined. A 1995 report by IOM, also sponsored by USAID, estimated the mean per capita energy requirements (EMPCER) for planning emergency food aid rations at 2,100 kcal (IOM, 1995). This level refers to the average daily energy requirement of individuals in a "typical" population in developing areas of the world, engaged in a light level of physical activity. The estimated EMPCER in the report was based on the following assumptions:

> (1) the population is distributed as indicated in the World Population Profile 1994 report for developing countries; (2) the average height of adult males of 170 cm and of adult females of 155 cm, which are the approximate heights of average males and females in sub-Saharan Africa and slightly greater that those of adults in South and Southeast Asia; (3) the weights of these adults are at the median for U.S. adults of the stated heights; and (4) the total energy expenditure of the adults is 1.55 and 1.56 times the BMR [basal metabolic rate] for males and females, respectively, which is consistent with a light level of activity. (p. 24)

The report did not elaborate on potential food sources to provide that level of energy or on formulations for such rations.

Given that the EFP under study is for use during the initial stages of all types of emergencies when there would be few or no other sources of food or when the prevailing conditions would not be amenable to preparation of other foods, the EFP composition must satisfy the nutritional needs of the subgroup in the population with the greatest needs. In so doing, it can then be assumed that

the needs of other population groups—with the notable exception of nursing infants up to 6 month of age, for whom human milk is best—would also be covered by the EFP. USAID requested that the EFP specified by the subcommittee not be a therapeutic food product, although, as mentioned earlier, its eventual use as a supplemental source of nutrients in other feeding programs is possible.

U.S. Food Relief Programs and Emergency Relief Operations

The United States is the largest donor of humanitarian assistance. The U.S. government contributes to emergency relief and humanitarian assistance in response to natural disasters, man-made disasters, and complex humanitarian emergencies mainly through USAID. This agency's 2000 Performance Report (USAID, 2000a) lists promoting humanitarian assistance as its goal number 6 and states that in 1999, "... the Bureau for Humanitarian Response's Office of U.S. Foreign Disaster Assistance (OFDA) responded to 65 declared disasters in more than 63 countries. These included 17 complex humanitarian emergencies, 41 natural disasters, and 7 man-made disasters." As a result, $294 million was allocated in 1999 to these efforts compared to $186 million in 1998. In addition, the Office of Food for Peace provided $513 million in food assistance for these declared disasters (USAID, 2000b)

Other U.S. agencies coordinate disaster response with USAID, including the Centers for Disease Control and Prevention of the U.S. Department of Health and Human Services, the U.S. Geological Survey of the U.S. Department of the Interior, the National Oceanic and Atmospheric Administration of the U.S. Department of Commerce, and DOD. DOD often provides logistic and personnel support to U.S. relief operations, particularly when rapid airlift of emergency food and medical supplies is necessary.

USAID's Office of Food for Peace takes a leading role in defining the modality and extent of the U.S. food aid response. Food aid is procured from U.S. suppliers and shipped from the United States to the emergency sites. If there is an ongoing food relief program sponsored by USAID in a country neighboring the emergency, available food may be rapidly transferred to the emergency area.

From the operational standpoint, USAID normally provides emergency food assistance directly through U.S. private voluntary organizations (PVOs) and local nongovernmental organizations (NGOs), or indirectly through the World Food Program (WFP). USAID or WFP handles the logistics and the PVOs or NGOs identify the recipients and their needs. However, rapid response in the aftermath of catastrophic events is often undertaken with the cooperation of DOD.

The United States provides emergency food assistance through two programs, Title II of the Food for Peace Program under P.L. 480, which is administered by USAID, and under a surplus disposal program administered by the U.S. Department of Agriculture (USDA), section 416(b) of the Agricultural

Development Act of 1949. All food used in the food assistance programs, including 36 commodities (USAID, 2001b) as well as all other food products—including such foods as the EFP under study—are procured solely by USDA's Commodity Credit Corporation through public solicitation of bids from food producers, distributors, or manufacturers.

The importance of having specifications for a high-energy, nutrient-dense EFP for manufacture in the United States lies in the severe regulatory restrictions that USAID faces for purchasing non-U.S. products. Because there are no American suppliers of EFPs for the agency to purchase under P.L. 480, such products must be purchased abroad using very limited funds available to the agency for non-U.S. purchases. Purchases are made as the need arises, thus incurring long delays in the delivery of desperately needed food relief during emergencies. These factors not only severely limit the amounts that can be purchased, but in the past have forced USAID to forego stockpiling and prepositioning of EFPs at strategic locations around the world for rapid delivery. In contrast, funds from Title II could be used to purchase up to several hundred metric tons of EFPs needed per year if EFPs from U.S. manufacturers were available, and they could be prepositioned to optimize emergency response. Thus, the availability of a U.S.-manufactured EFP that is easily delivered and consumed without further preparation, and appropriately formulated to fulfill the nutritional requirements of individuals undergoing severe physical and mental stress, would undoubtedly facilitate a wider and more rapid response to emergencies by U.S. relief agencies. It could also mean the difference between life and death to thousands of individuals. Developing specifications for such a product is consistent with the passionate appeal for appropriate food for refugees made by Mason and colleagues (1992) and it is also, by any humanitarian measure, a worthy endeavor.

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2
Nutrient Content and Special Considerations

This chapter presents the rationale for the levels of individual nutrients recommended for the emergency food product (EFP) described in this report, and discusses additional issues to be considered.

> The goal of an EFP is to reduce morbidity and mortality among displaced persons by providing a nutritionally complete food that will be adequate as a sole source of nutrients for as long as 15 days from the recognized time of displacement. It should provide nutrition for the period between initial displacement and establishment of a more stable food supply line.

The EFP should be consumed with an ample quantity of water to ensure that the osmotic load provided by the EFP is diluted. This report assumes that emergency relief agencies will provide potable water supplies as a top priority. This assumption is based on assurances provided by the United States Agency for International Development.

There are five characteristics critical to the development of a successful EFP, listed in order of priority: (1) safe, (2) palatable, (3) easy to deliver, (4) easy to use, and (5) nutritionally complete. This order of priority should guide decisions about competing characteristics in developing a prototype EFP.

TABLE 2-1 The Population Distribution from Two Reports Providing Demographic Information used to Determine Nutritional Needs for Disaster Responses

Sub-Saharan Africa[a]		The Sphere Project[b]	
Age Group (yr)	% of Population	Age Group (yr)	% of Population
0–3	10	0–4	12
4–6	7	5–9	11
7–9	7	10–14	11
10–17	17	15–19	9
18–60	48	20–59	49
> 60	7	60+	7
		Pregnant	2
		Lactating	3
		Male/female	51/49

[a] Jamison and Hobbs (1994).
[b] Sphere Project (2001).

INTRODUCTION

The nutritional advantages of a single EFP as opposed to two or more products are evident. Providing a limited selection of commodity-type foods may increase the risk of malnutrition because nutritional components that are found in only one of the foods (e.g., ascorbic acid) may be absent from the diet if that food is not selected. Under emergency conditions, diets are invariably highly monotonous, and often relief foods quickly become a medium of exchange and are commonly sold or traded for other foods, water, firewood, alcohol, and a variety of other goods and services. If a nutritionally complete food ration is divided among two or more different foods, or if foods are targeted to specific individuals such as children or pregnant women, then certain foods are more likely to be exchanged. This type of exchange can deprive the population of a portion of the profile of nutrients provided by the emergency food ration and increase the risk of malnutrition. Providing a single ration product would reduce this risk.

CHARACTERISTICS OF TARGET POPULATIONS

Characteristics of potential target populations were considered in determining the nutrient composition of the EFP. As shown in Table 2-1, some target populations may have as much as 23 percent of the population below 10 years of age and 12 to 17 percent below 5 years of age (Jamison and Hobbs, 1994; Sphere Project, 2001). Refugee groups fleeing from military conflicts may have

TABLE 2-2 Estimated Mean Per Capita Energy Requirement (EMPCER) by Body Size of Adults

	Sub-Saharan Africa	South and Southeast Asia	United States
Male height, weight	170 cm, 63.5 kg	165 cm, 60.1 kg	180.4 cm, 78.1 kg
Female height, weight	155 cm, 50.0 kg	153 cm, 49.0 kg	163.7 cm, 55.3 kg
EMPCER	2,076	2,045	2,194

SOURCE: Institute of Medicine (IOM, 1995b).

women and children as a large proportion of the population, with only a small proportion of women pregnant or lactating.

Data from the Nutrition Collaborative Research Support Program (CRSP) in Kenya (Calloway et al., 1992; Neumann and Harrison, 1994; Neumann et al., 1991), as well as data from sub-Saharan Africa (Sphere Project, 2001) and South and Southeast Asia (James and Schofield, 1990), indicate that people from these areas have smaller body sizes than those in Western populations (Table 2-2).

While the EFP might have nonemergency uses (e.g., as a complementary food for breast-fed children 7 to 12 months of age), it has been designed as a sole food source for periods of 2 to 15 days. It is likely that the recipient population will be in poor nutritional status and may have some wasting, appetite depression, and malabsorption. The goal of this report is to provide recommendations for a product that would meet the needs of diverse populations.

General Assumptions

Given the goal outlined above, the following assumptions are made regarding the recipient population:

- The relief food product is the only food consumed.
- Individuals eat to meet their energy requirement.
- Individuals in the target population are of smaller stature and body mass than similarly aged groups in the North American population (this is the same premise used in an earlier report from the Food and Nutrition Board, *Estimated Mean per Capita Energy Requirements for Planning Emergency Food Aid Rations* [IOM, 1995b]).
- All individuals over the age of 6 months will consume the product.

Estimating Energy Requirements

The energy content of the EFP should be determined by the energy needs of the recipient population. However, because the EFP must be manufactured prior to knowing where it will be needed, the population's energy needs will not be known. Recommended intakes for nutrients from recent reports in the United States and Canada are typically used as the standard for nutrient requirements and thus nutrient content (IOM, 1997a, 1998, 2000, 2001), but, as discussed earlier, energy consumption per individual may be less in the EFP target population than in the United States or Canada due to lower body weights for similar subgroups. Furthermore, because the EFP is a single food meant to support a heterogeneous population, nutrient content must be determined on an energy density basis.

Estimating Energy Requirements of the Population

A potential basis for calculating the energy requirements for a refugee population is provided in the Institute of Medicine report, *Estimated Mean per Capita Energy Requirements for Planning Emergency Food Aid Rations* (IOM, 1995b). The goal of this report was to establish an estimated mean per capita energy requirement (EMPCER) when little was known about the characteristics of the population. Energy requirements for 14 age and gender groups, plus pregnant and lactating women, were estimated based on body mass and assumptions about energy needs in pregnancy and lactation obtained in two refugee populations. The estimated energy requirements for adults were calculated based on an estimate of basal metabolic rate (BMR) and a physical activity level (PAL). To estimate BMR, the report used equations developed by the Food and Agriculture Organization/World Health Organization/United Nations University (FAO/WHO/UNU, 1985). An average height of 170 cm for adult men and 155 cm for adult women was assumed (the average of adult men and women in sub-Saharan Africa; see Table 2-2). These average heights are slightly greater than those of adults in South and Southeast Asia (Table 2-2) and less than those of the U.S. population. The weights used for the estimates of BMR were the median weight for U.S. adult males of 170 cm (63.5 kg) and females of 155 cm (50 kg). The U.S. median weights (NRC, 1989) were used to provide a conservative estimate of the EMPCER for populations in most developing countries (IOM, 1995b).

For individuals under 18 years of age, values were based on data from affluent populations. Although the individuals from whom these data were derived were larger (and therefore assumed to have a greater BMR) than many children and adolescents from refugee populations, this "extra" allotment for children in developing countries was deemed appropriate on the basis that the additional food would allow some compensatory growth (IOM, 1995b). Both the adult and child values were recognized as overestimates of energy requirements, but were justified in order to establish a conservative EMPCER.

The resulting EMPCER in the report was 2,100 kcal/day (after rounding). This number is used below as the basis for the total energy content of the EFP.

Estimating Energy Requirements for Specific Life Stage and Gender Groups

The IOM (1995b) report estimated energy requirements for specific life stage and gender groups, as described above. However, it was determined that using that approach was inappropriate for determining the content of the EFP for three reasons. First, the approach could lead to underestimates of nutrient density needed because the nutrient density is based on an assumed energy intake. If energy intake is less than expected, the nutrient density will be too low to meet the micronutrient requirements. Second, the life stage and gender groups do not correspond to the current groups used in the Dietary Reference Intake (DRI) reports (IOM, 1997a, 1998, 2000, 2001). Third, the FAO/WHO/UNU (1985) equations used for infants and children under age 5 are now recognized as flawed (Butte, 1996; Torun et al., 1996).

For the above reasons, estimates of energy requirements for each life stage category were recalculated and are shown in Table 2-3. For individuals 4 years of age and older, estimated energy requirements were obtained by first calculating individual BMRs based on the age, sex, weight, and physiological status of each individual (FAO/WHO/UNU, 1985). Individual energy requirements were then calculated using the same PAL values (women: 1.56, men: 1.55) that were used by IOM (1995b).

With the exception of infants aged 7 through 12 months, the BMR and energy requirements were derived using anthropometric data from individuals in the Kenya Nutrition CRSP (Calloway et al., 1992; Neumann and Harrison, 1994; Neumann et al., 1991). Because the Kenya Nutrition CRSP did not collect anthropometry on children aged 6 through 12 months, the value for this age group was the mean weight of rural infants aged 9 months from the Mexico Nutrition CRSP (Allen et al., 1992).

The Kenya data set contains anthropometry on 1,717 individuals aged 0 to 65 years. As is common in much of the developing world, most adults and children in this population were smaller than U.S. individuals, the result of early growth stunting (Martorell and Habicht, 1986) (Figure 2-1). Additionally, the rural Kenyan population was subject to periodic food shortages and were relatively thin (Neumann and Harrison, 1994).

Estimating Energy Requirements for Infants and Children. Recent research using doubly labeled water to measure energy expenditure suggests that values derived from the FAO/WHO/UNU 1985 equations are inflated for infants and young children (Butte, 1996; Butte et al., 2000; de Bruin et al., 1998; Prentice et al., 1988). Therefore, the energy requirements for infants 9 months of age (representing the 7- through 12-month-old group) and children 2 years of age

TABLE 2-3 Median Weights, Estimated Basal Metabolic Rate (BMR), and Energy Requirements of a Rural Kenyan Population[a]

Age	Gender	Weight (kg)	BMR (kcal/d)	Energy (kcal/d)	Estimated Number of Emergency Food Product (EFP) Bars[b] per day
7–12 mo[c,d]	Both	7.0	371	578	1–2[e]
1–3 yr[d]	Both	10.2	571	855	3–4
4–8 yr	Both	19.4	936	1,456	6–7
9–13 yr	Both	26.5	1,086	1,693	7–8
14–18 yr	Boys	42.0	1,378	2,136	9
	Girls	40.9	1,238	1,931	8–9
19–50 yr	Men	54.3	1,509	2,339	9–10
	Women	51.0	1,264	1,972	8–9
51+ yr	Men	56.1	1,451	2,249	9–10
	Women	47.0	1,237	1,929	8–9

[a] Weights from Kenya Nutrition CRSP (Calloway et al., 1992).

[b] Each EFP bar has approximately 233 kcal; 9 bars = 2,100 kcal = one average ration per day. Each can be broken in half to yield 116 kcal. This allows distribution to young children.

[c] Weights from Mexico Nutrition CRSP (Allen et al., 1992).

[d] BMR estimate based on equations of Butte and coworkers (2000).

[e] It is assumed that the EFP would be used as a complementary energy source to human milk and therefore would provide 50 percent of the estimated energy need.

(representing the 1- through 3-year-old group) were calculated according to the formula of Butte and coworkers (2000):

Energy requirements (MJ/d) = 0.321 + 0.013 × age (mo) - 0.047 × sex + 0.139 × feeding group + 0.277 × weight,

where sex is coded as 1 for boys, and 2 for girls, and feeding group is coded as 1 for breast-feeding (nearly all children in the Kenyan and Mexican populations). Values for boys and girls were later averaged.

The Butte equations were based on breast-fed children in the United States and yielded values of similar magnitude to those derived for Mexican infants and young children: 638 kcal/day for 0- through 9-month-old infants and 843 kcal/day for 1- through 2-year-old toddlers (Butte et al., 2000). The resulting energy estimates are lower than those used in the IOM (1995b) report (800 and 1,350 kcal/day, respectively), because the IOM values are based on the energy requirements of children derived from the FAO/WHO/UNU (1985) equations and U.S. body weights.

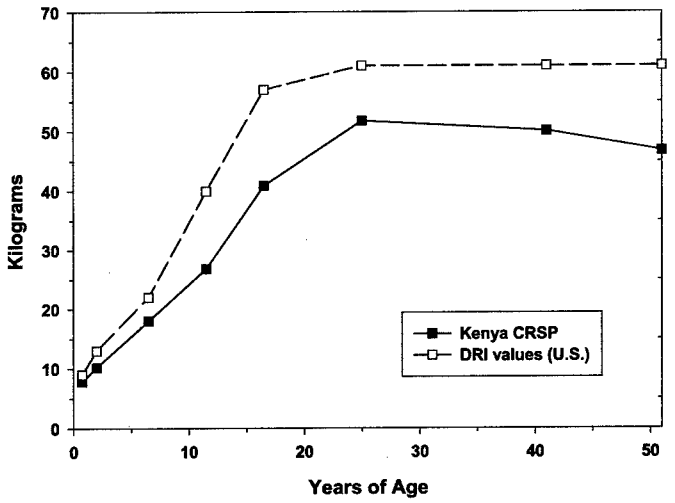


FIGURE 2-1 Reference weights of DRI life stage groups (U.S. population), and weights of rural Kenyans.

Estimating Energy Requirements for Pregnancy and Lactation. Although adequate nutrition during pregnancy and lactation are of concern in refugee populations, the EFP is designed to meet energy requirements based on the assumption that pregnant or lactating women as well as others with higher energy needs (i.e., due to physical activity or rapid growth) will consume additional food bars to meet these needs.

In 1985, FAO/WHO/UNU recommended an increased energy intake of 285 kcal/day during pregnancy. However, the actual increased energy needs during pregnancy vary widely by trimester (Prentice et al., 1996) and by population (Prentice and Goldberg, 2000). For example, the total additional energy needed during pregnancy in The Gambia has been estimated at about 7,000 kcal, or about 25 kcal/day (Prentice and Goldberg, 2000). Moreover, Prentice and colleagues (1996) have proposed that maternal energy metabolism during pregnancy may be lower as measured by change in BMR in women in developing countries versus those in affluent populations. This is believed to be due to their smaller body size. If true, then pregnant women in some emergency feeding situations may not need to consume 285 kcal beyond their nonpregnant, nonlactating energy requirement (or 1 to 2 additional food bars over the 9-bar ration). This number is near to the estimated daily increment of 229 kcal/day during the

second trimester, when pregnancy energy requirements appear to be intermediate (Prentice et al., 1996).

FAO/WHO/UNU (1985) also recommended an additional 500 kcal/day during lactation, which assumed an additional 200 kcal/day obtained from maternal fat stores. Prentice and colleagues (1996), based on an extensive review of the literature, recommended an increment of 480 kcal/day for mothers of infants 1 through 6 months of age with previous weight loss.

CHARACTERISTICS OF THE EMERGENCY RELIEF FOOD PRODUCT

Given the estimated energy requirements (Table 2-3), the proposed energy density for the EFP is 4 to 5 kcal (17 to 21 kJ)/g. To obtain this energy density, an EFP low in water (see Chapter 3) with 35 to 45 percent fat along with 10 to 15 percent protein is required (see sections below). Palatability of the EFP is a primary concern, and should dictate the final choice of ingredients (see Chapter 3). It is assumed that pregnant and lactating women will consume more than the average requirement of 2,100 kcal as needed to support pregnancy and lactation.

Nutrient Content

The methodology for determining the appropriate amount of each nutrient to be included in the EFP is summarized in Box 2-1, followed by a more detailed explanation and rationale for the approach adopted for each nutrient.

A starting premise for determining the appropriate nutrient content of the EFP is that the upper limit of an individual's food intake is somewhat constrained by his or her total energy requirement, while the lower limit is set by many factors, including appetite, access to food, trading of food, and an individual's ability to make his or her own food decisions. When food intake is lower than energy requirements, the nutrient density may need to be adjusted, thus highlighting a need for testing prototype EFPs developed from the specifications presented in this report.

The recommended intakes (either recommended dietary allowance [RDA] or adequate intake [AI]) as specified in the recent reports on DRIs (IOM, 1997a, 1998, 2000, 2001) were used. These reports provide recommended intakes for vitamins and minerals for 16 life stage and gender groups, plus pregnancy and lactation. It should be noted that these DRIs were established based on a selected criterion or criteria of adequacy consistent with good health, as opposed to the mere prevention of overt deficiencies. Thus the values obtained may be higher than those previously recommended by WHO. RDAs were calculated from the estimates of average requirements (EAR) using an estimate of the variability among individuals in the requirement. In most cases, the coefficient of variation of nutrient requirements was assumed to be 10 percent. The RDA is set at two

BOX 2-1 Summary of Methodology to Determine the Nutrient Content of the EFP

1. Use the Adequate Intakes (AI), Recommended Dietary Allowances (RDA), and Tolerable Upper Intake Levels (UL) as developed by IOM (1997a, 1998, 2000, 2001) or, for protein, FAO/WHO (2000).
2. Use 2,100 kcal/person as the target for the population, but evaluate the amounts needed based on estimated energy expenditure for different subgroups of the population.
3. Select the life stage and gender group that has the highest nutrient needs relative to estimated energy needs for each nutrient. This group is designated the limiting subgroup.
4. Determine the nutrient density for the limiting subgroup utilizing the AI or RDA by dividing the recommended intake of the nutrient by the energy requirement determined for that subgroup (see Table 2-3).
5. Adjust the nutrient density value of the limiting subgroup based on probable malabsorption, bioavailability assumptions, potential nutrient interactions, and properties related to the plant sources of ingredients utilized in the EFP.
6. Determine for each nutrient if the requirement for the limiting subgroup exceeds the UL for any other age group.
7. Adjust the proposed nutrient level, if necessary, to ensure that the UL is not reached for other age groups. If the rationale used allows some subgroups to exceed the UL, so state. Provide a maximum level for the EFP based on the UL.
8. Recommend food ingredients that would prevent interactions with other nutrients and avoid reaching the maximum value.
9. Describe the assumptions and the scientific rationale underlying the recommended level for each nutrient.

standard deviations above the EAR, and should meet the requirements of almost all of the U.S. and Canadian populations for which it is recommended.

For some required nutrients, it was not possible to establish an intake at which half of a life stage and gender group would be adequately nourished, while the other half would demonstrate signs of inadequacy. Thus an EAR could not be set. However, data were available that could be used to establish a level of intake that appeared adequate for most, if not all, people consuming that amount. This is called the *adequate intake* (AI). AIs are available for a number of the nutrients included in the EFP. Given that the data upon which an AI is

based are less certain, there is more judgment in its derivation. In some cases, the EFP may not provide the AI level due to constraints related to palatability or cost. In this case, the probability that the target population has underlying nutrient deficiencies is assumed, and what is feasible for a product to be used for 10 to 15 days as the sole source of nutrition is determined.

There are also nutrients that are deemed essential for inclusion in an EFP, but for which DRIs have not yet been determined. In this case (i.e., macronutrients and electrolytes), other recommendations for these nutrients (FAO/WHO, 2000; NRC, 1989) were considered in determining the amounts appropriate for the EFP.

Ideally, the formulation of an EFP requires information on variability in actual consumption of the relief food. Since such information is not available at this developmental stage of the product, a few cautionary flags must be raised in the use of the proposed EFP:

- The EFP is not designed to meet all the nutrient needs for pregnancy and lactation; however, due to the energy requirements being conservatively estimated based on energy needs for smaller individuals, it should meet the requirements for most nutrients for almost all women.
- The EFP is not appropriate for severely malnourished individuals who require medical attention. Severe malnutrition is defined in the WHO Sick Child Initiative as quoted by IOM (1995a) as the presence of any one of the following symptoms: visible severe wasting, severe pallor, clouding of the cornea, or edema of both feet.
- The EFP is not a therapeutic nutritional supplement. (A ration distributed to the general population cannot be formulated as a therapeutic diet, as it would present too many risks of excess intake for individuals who were not severely malnourished. Severely malnourished individuals need special help, including fluid and electrolyte replacement therapy, blood transfusions for severe anemia, and medical supervision. This food product is *not* meant to be a substitute for this therapy, but a sustaining ration for people who have been uprooted due to war or natural disaster.)
- The EFP is not a substitute for human milk for infants agers 0 to 6 months.
- The EFP is not designed to meet the needs of young infants; however, it may be combined with water to produce a gruel suitable as a complementary food for older infants (7 to 12 months of age).

Determination of a Minimal Nutrient Density

At the population level, there are a number of individual minimal nutrient densities for each nutrient. If a single food must meet the nutrient requirement of most individuals in the population, this food should have a nutrient density that

meets or exceeds the minimal nutrient densities of most individuals in the population. Since food intake is limited by energy requirements, a high nutrient density is necessary to meet the nutrient requirements of an individual with low energy needs. The approach described here to establish the nutrient content for the EFP provides a complete food for individuals consuming on average as little as 855 kcal/day (1- to 3-year-old age group) to those who may require in excess of the average ration of 2,100 kcal/day (adult men); thus the EFP can be used by a diverse population.

The approach used to determine nutrient density for the EFP is as follows: for each nutrient, a minimal density value was estimated for the life stage and gender group in the population with the highest nutrient requirement relative to their energy requirement using the data on recommended nutrient intakes (Table 2-4) (IOM, 1997a, 1998, 2000, 2001; NRC, 1989; WHO, 2000), divided by the estimated average energy requirement for that life stage and gender group based on data from Kenyan refugee populations (Table 2-3). Neither pregnant nor lactating women were considered as a limiting group because for some nutrients (e.g., iodine, vitamin A) the minimal nutrient density would provide intakes that would exceed the UL (IOM, 1997a) for other groups in the population. Additional assumptions used in setting the minimal nutrient density include:

- The relief food is the only food consumed.
- Individual energy consumption equals energy requirement.
- The food product should provide a nutrient density that will meet the nutrient requirements of almost all members of each life stage and gender group without exceeding the UL for any group.

These assumptions err in the direction of providing more of a nutrient than may be necessary unless energy consumption does not meet energy requirements. In most cases, the RDA values used were calculated from EARs which were originally estimated from only a few individuals with assumed variations in requirements, and then extrapolated to other age and gender groups using conservative approaches. Most of the estimates of AIs were based on mean intakes for healthy population groups that did not demonstrate any indicators of inadequacy of the nutrient, and thus could easily be overestimates of actual requirements for subgroups.

Finally, in the case of many nutrients, the minimal nutrient density was subsequently modified upward in order to ensure that possible interactions with other nutrients or storage conditions, poorer bioavailability, or assumed presence of diarrhea or disease in the recipient population were taken into account. Since increased amounts of nutrients will increase the cost and potentially may affect palatability and shelf life of the EFP, and palatability is the major factor that ensures adequate energy consumption, slight reductions in these recommended amounts may be necessary.

TABLE 2-4 Unadjusted Baseline Minimal Nutrient Density Values Using Recommended Intakes

Nutrient	Limiting Group	Baseline per 1,000 kcal[a]	Basis for Recommended Intake
Fat	N/A	39–50 g	Providing an energy density of 4–5 kcal/g
Protein[b]	51+ yr, men	34 g	Balance studies
Carbohydrate	N/A	100–125 g	Seven to 12 of the 23–35 g of total carbohydrate should be from sugars for adequate palatability
Sodium[c]	2–5 yr, children	1.3 g	Maximum level of intake
Potassium[c]	2–5 yr, children	1.7 g	Level estimated to meet minimum requirements
Chloride[c]	2–5 yr, children	2.0 g	Level estimated to be equimolar to sodium
Calcium	9–13 yr, children	768 mg	Based on maximal calcium retention
Phosphorus	9–13 yr, children	740 mg	Based on factorial approach
Magnesium	14–18 yr, boys	190 mg	Amount needed to maintain magnesium balance
Chromium	—	13.5 μg	Based on amounts in well-balanced diets/1,000 kcal
Copper	51+ yr, women	470 μg	Biochemical indicators of copper status
Iodine	1–3 yr, children	105 μg	Balance studies
Iron[d]	19–50 yr, women	9 mg	Based on iron requirement (estimated basal losses, increase in hemoglobin mass, increase in nonstorage iron, increase in storage iron) plus assumed iron absorption
Manganese	1–3 yr, children	1.4 mg	Average intake in healthy population
Selenium	14–18 yr, girls	28 μg	Maximizing plasma glutathione peroxidase activity
Zinc	14–18 yr, boys	5.2 mg	Level needed to match exogenous losses
Vitamin A	14–18 yr, boys	420 μg RAE	Level needed to maintain adequate stores
Vitamin D	51–70 yr, women	5.2 μg	Maintain serum 25(OH)vitamin D levels
Vitamin E	14–18 yr, girls	7.8 mg	Level needed to prevent hydrogen peroxide-induced hemolysis

continued

TABLE 2-4 Continued

Nutrient	Limiting Group	Baseline per 1,000 kcal[a]	Basis for Recommended Intake
Vitamin K	19–50 yr, men	~60 µg	Average intakes in adequately nourished population groups
Vitamin C	51+ yr, men	40 mg	Level needed to maintain near-maximal neutrophil concentration with minimal urinary loss
Thiamin	1–3 yr, children	0.6 mg	Level needed for normal erythrocyte transketolase activity
Riboflavin	14–18 yr, boys	0.6 mg	Level needed to maintain normal erythrocyte glutathione reductase activity and urinary riboflavin excretion
Niacin	14–18 yr, boys	7.5 mg NE	Level needed to maintain adequate niacin metabolism as measured by excretion of metabolites
Vitamin B_6	51+ yr, women	0.8 mg	Level needed to replete depleted stores
Folate[e]	14–18 yr, girls	207 µg	Level needed to maintain normal homocysteine, red cell folate concentrations
Vitamin B_{12}	14–18 yr, girls	1.2 µg	Level needed to maintain normal B_{12} levels and hematological status in adults
Pantothenic acid	14–18 yr, girls	2.6 mg	Average intake in healthy population
Biotin	51+, women	16 µg	Average intake in healthy population
Choline	51+, men	244 mg	Level needed to maintain normal liver enzyme levels in young adults

[a] Estimated energy requirements for each limiting group taken from Table 2-3.
[b] From NRC (1989); based on reference weights from IOM (1997a) and estimated energy expenditure from Table 2-3.
[c] Values based on estimated requirements, desirable intakes, or maximal intakes (NRC,1989).
[d] Based on 10% iron bioavailability.
[e] If folate is provided as synthetic folate, which is more readily absorbed, these numbers should be divided by 1.6.
SOURCE: IOM (1997a, 1998, 2000, 2001).

In order to individualize and facilitate the use of the EFP to the extent possible, it is designed to be consumed in multiple subunits so that it is possible to consume from 117 kcal (one-half of a scored 233-kcal EFP bar) to 2,100 kcal (9 EFP bars, which are 1 day's ration) or more (e.g., pregnant or lactating women or individuals with high energy expenditure) over the entire day, yet still contain adequate nutrient levels to meet the needs of smaller individuals with lower energy intakes.

Although there are conflicting data on whether individuals will consume enough of a single, biscuit-type food product to meet their energy requirements (Brown et al., 1995; Sanchez-Griñan et al., 1992), it is assumed for the purpose of this report that individuals, at least for a short period of time, will consume enough EFPs to meet their energy requirements. The nutrient content of the EFP is based on this assumption.

NUTRIENTS INCLUDED IN THE EMERGENCY RELIEF FOOD PRODUCT SPECIFICATIONS

For each nutrient or nutrient group that follows, the assumptions, including the minimal nutrient density, the limiting groups, and how the RDA, AI, or other values were utilized are discussed. Since the EFP will be used for a wide range of age groups, in those cases where maximum values were set, they were developed from the UL values included in the DRI reports (IOM 1997a, 1998, 2000, 2001).

Energy-Yielding Nutrients

Fat, protein, and carbohydrates comprise the energy nutrients. The rationale for the fat, protein, and carbohydrate levels in the EFP are discussed below.

Dietary Fat

The recommended fat content of the EFP is 35 to 45 percent of calories and takes into consideration the following:

- the quantity of fat needed to provide a food of sufficient energy density to meet energy requirements, to be lightweight, and to be palatable;
- the quantity of fat needed to ensure adequate absorption of fat-soluble vitamins;
- the quality of fat needed to provide an adequate supply of essential fatty acids; and
- the ability to protect fat from oxidation and degradation under severe storage and transport conditions.

The maximum fat content of the EFP is limited by the minimal requirements for other macronutrients, vitamins, and minerals (Jéquier, 1999; Koletzko, 1999). The principal mechanisms for increasing the energy density of a food are to either reduce water content or to increase fat content. Because fat on a weight basis is 2.25 times as energy dense as either carbohydrate or protein, a high-fat product will weigh less than lower-fat products of similar water and energy content. The reduced weight of an energy-dense food also has advantages with respect to storage and transport. Furthermore, infants and young children have comparatively high energy requirements per kilogram of body weight (Koletzko, 1999) and have limited capacities to consume food. Therefore, very-low-fat diets increase the risk of inadequate energy intakes that would result in inadequate intakes of some micronutrients in young children. FAO/WHO (1994) suggested diets of children under 2 years of age should contain 30 to 40 percent of energy from fat.

Satiation. High-fat foods are readily over-consumed, and experimental studies suggest little effect of fat per se on satiation (feeling of fullness) when energy density of the meal is held constant (Rolls, 2000; Rolls and Bell, 1999; Saltzman et al., 1997; Stubbs et al., 1996; van Stratum et al., 1978). These results suggest that an energy-dense food, regardless of fat content, is less likely to induce satiation, and therefore is likely to promote consumption of greater amounts of energy. In the case of a refugee population, in which anorexia may be common, the provision of a higher-fat, nutrient-dense food may be an important means of ensuring adequate energy intake.

Palatability. The fat content of a food can have a significant influence on its sensory properties and the quantity of the food that is consumed (Drewnowski, 1997). Fat contributes to flavor, mouth feel, moistness, and other textural properties, depending on the food and the type of fat. Relatively little research has been published concerning the influence of fat content on the palatability of products similar to the proposed EFP. Recently, Abdallah and coworkers (1998) asked 102 men to rate the pleasantness of 39 commercially available cookies and cakes. Sugar content was the best predictor of pleasantness. However, the highest ratings of pleasantness occurred with foods that were high in both sugar and fat. Moisture content bore little relationship to pleasantness after statistically controlling for the fat and sugar content of the products. Others have investigated the sensory effects of reducing the fat content of five cookies. Only a reduction of fat by 50 percent of its original recipe was associated with declines in sensory ratings (Drewnowski et al., 1998). In both studies, subjects were much more sensitive to variability in sugar content than in fat content.

Fat Intake and Absorption of Fat-Soluble Vitamins. The absorption of fat-soluble vitamins and provitamins is dependent on fat in the diet. However, the precise quantity of dietary fat needed for efficient absorption of fat-soluble

vitamins is poorly understood. A common rule of thumb is that fat energy should not fall below 10 percent of total energy (Jéquier, 1999). Thus, the fat content of the EFP is more than adequate to promote absorption of fat-soluble vitamins.

Type of Fat. As the nutritional quality of diets in developing countries improves, the availability and the percentage of energy in the diet contributed by fat increases (Tagle, 1988). The greatest concern in developing the EFP regarding type of fat is to include fats/oils that will provide the greatest stability in terms of storage of the finished product, without the inclusion of fat of animal origin. For long-term health, other aspects of dietary fat, such as the proportion of essential fatty acids or the inclusion of long chain polyunsaturated fatty acids (LC-PUFAs) is of interest as well. However, with the limited time that the EFPs will be used (15 days or less), cost and storage requirements of the finished product limit the advisability of including some of these specific fatty acids.

Polyunsaturated Fatty Acids. Polyunsaturated fatty acids are necessary for normal health in adults and normal development in the fetus and infant (Uauy et al., 1999). The essential fatty acids, α-linolenic acid (LNA, *n*-3) and linoleic acid (LA, *n*-6), present in various vegetable oils, are precursors for the other *n*-3 and *n*-6 LC-PUFAs. In animal models, synthesis of docosahexaenoic (DHA) and arachidonic acid (AA) from their essential fatty acid precursors are decreased by experimental protein and energy malnutrition (Lopez-Pedrosa et al., 1998; Marin et al., 1995) and observational studies in infants have documented associations between protein–energy malnutrition (PEM) and signs of *n*-6 fatty acid deficiency (Decsi et al., 1998; Holman et al., 1981; Koletzko et al., 1986; Leichsenring et al., 1995; Marin et al., 1991; Smit et al., 1997).

Studies indicate that children with sickle cell anemia and with zinc and copper deficiencies appear to have impaired ability to utilize LA and LNA (Cunnane, 1981; Enomoto et al., 1998). Research has shown considerable regional variability in the LC-PUFA content of human milk of women in developing countries, presumably due to variability in diets (Chulei et al., 1995; Koletzko et al., 1992; Laryea et al., 1995; Okolo et al., 2000; Rocquelin et al., 1998; Schmeits et al., 1999; VanderJagt et al., 2000; Xiang et al., 1999). Of relevance to some developing country populations is the fact that high LA intakes from specific vegetable oils (e.g., corn oil) may decrease the synthesis of DHA from LNA. The recommendation that a ratio of LA to LNA between 5:1 and 10:1 has been made (FAO/WHO, 1994), and seems reasonable and fairly easy to obtain from vegetable oil sources.

Although an EFP having at least 35 percent of calories from vegetable oil sources will probably not be totally devoid of such fatty acids, the constraints of manufacturing, required storage life, and the impact of oxidized unsaturated fat on flavor dictate against addition of these fatty acids.

Vitamin E, PUFA, and Oxidation. Because of their susceptibility to oxidation, very high intakes of PUFA, without a correspondingly high intake of antioxidants, can lead to vitamin E deficiency (Valk and Hornstra, 2000). Fortunately, most commonly consumed vegetable oils are good sources of vitamin E (IOM, 2000) and have relatively high vitamin E:PUFA ratios (Dupont et al., 1990). Recommendations to provide adequate vitamin E intakes in high PUFA diets have been made, and vary from 0.4 (NRC, 1989) to 0.6 mg (FAO/WHO, 1994) of α-tocopherol per gram of PUFA.

Maximum Fat Content of the EFP. The upper limit of fat for the EFP is recommended to be 45 percent of energy in order to produce a stable product that would not be unduly affected by oxidation.

Fat intakes in developing countries are often quite low and come from a small number of principal dietary sources. Average fat intakes of school-aged children ranged from 10 percent of energy (in rural Kenya where animal products are consumed in relatively small amounts) to 25 percent of energy in peri-urban Egypt (Beaton, 1995). In Kenya, 40 percent of the fat in the diet was polyunsaturated, much of it from corn oil (Calloway et al., 1992). In The Gambia, children's intake of fat as a percent of energy declined from birth and stabilized at 24 months of age, when the average intake of energy from fat was 15 percent (Prentice and Paul, 2000), with most of the fat coming from groundnuts and cereals. This maximum level of fat exceeds the fat content of diets normally consumed in many developing countries, but should enhance palatability of the EFP.

In summary, recommendations regarding the fat content of the EFP are as follows:

- Total fat should comprise 35 to 45 percent of energy.
- Saturated fat should comprise at least 10 percent of energy.
- Total PUFA should be 7 to 10 percent of energy.
- The ratio of linoleic acid to α-linolenic acid should fall between 5:1 and 10:1 derived from a mixture of vegetable oils.

Protein and Amino Acid Requirements

Protein is essential for all physiological functions. Although two structural proteins, collagen and elastin, comprise about half of the proteins in the adult body, the protein associated with muscle, visceral organs, and blood is the most dynamic and most affected by poor nutritional status (Crim and Munro, 1994). Adults with good nutritional status and in protein balance turn over about 300 g of protein/day (Stein, 1995); growth during childhood and pregnancy increases this turnover. The body has no readily identifiable reserves of amino acids essential for protein synthesis. Loss of 30 to 40 percent of total body protein invariably results in death from starvation (Cahill, 1970). Rapid losses due to lack

TABLE 2-5 Recommended Amino Acid Pattern of an Emergency Relief Food Product (EFP)

Nutrient	Amount[a] (mg/kg body weight [BW])	Amino Acid (mg/g Protein)[c]
Protein[b] (g/kg BW)	1.0	—
Isoleucine	31	28
Leucine	73	66
Lysine	64	58
Methionine + cysteine	27	25
Phenylalanine + tyrosine	69	63
Threonine	37	34
Tryptophan	12.5	11
Valine	38	35
Histidine[c]	8	19

[a] The amino acid requirement for children 2 years of age was used (NRC, 1989).
[b] Total protein based on 1 g/kg body weight, using reference body weights from the Dietary Reference Intake reports (IOM, 1997a).

of food in emergency situations can thus result in serious health consequences over relatively short periods of time.

PEM may be present in populations that are likely to be recipients of the EFP (Young and Jaspars, 1995). For instance, an August 1989 survey of the Hartisheik A camp in Ethiopia indicated that 15.5 percent of reported cases of death in children less than 5 years of age were due to PEM and general malnutrition (CDC, 1990). The EFP target populations may have reduced energy intakes and low protein intakes, resulting in negative energy and nitrogen balances (Fjeld et al., 1989), reduced growth and/or lactation volume, and loss of body weight and muscle mass (Golden, 1994; Golden et al., 1977; Rice et al., 2000; Young and Jaspars, 1995). Limited muscle mass has been documented by lower body weights and mid-arm circumferences (Collins, 2000; De Onis et al., 2000; Young and Jaspars, 1995). Decreased skeletal muscle mass decreases functional capabilities (Dudley et al., 1989) and may impact the ability to perform normal life functions, as documented with PEM (Day and DeHeer, 2001; Kalra et al., 2001). Thus the EFP must provide adequate protein of appropriate quality.

Protein requirements include two components: the need for amino acids and for total protein (NRC, 1989). The EFP should meet both of these needs. The essential amino acid requirements for 2-year-old children identified by WHO (FAO/WHO/UNU, 1985), and subsequently adopted by the National Research Council (NRC, 1989), serve as the minimum amino acid pattern to use for the

Amount/233 kcal Food Bar (g)	Amount/1,000 kcal of EFP (g)	Amount/2,100 kcal Ration (g)
8	34	71
0.22	0.95	1.99
0.52	2.23	4.69
0.46	1.96	4.12
0.20	0.84	1.78
0.50	2.13	4.47
0.27	1.15	2.41
0.09	0.37	0.78
0.28	1.18	2.48
0.15	0.64	1.35

[c] Amino acid patterns for children 2 to 5 years of age from FAO/WHO/UNU (1985).

EFP (34 g/1,000 kcal, or 8 g/EFP bar) along with the generally recommended amount of total protein of 1 g/kg body weight (see Table 2-5). Although the protein content may be slightly low for young children (their RDA is 1.2 g/kg body weight [NRC, 1989]), the recommendation must take into consideration that a higher protein level per kilocalorie may be too high for adults and may not be as palatable (Young et al., 1985). ***A maximum of 15 percent of total calories as protein is recommended to prevent renal load problems and thirst promotion*** (Briend and Golden, 1993). Thus, the amount of protein recommended for the EFP is a compromise. Although the pattern of amino acids will meet the essential amino acid needs of the young child, the total protein may be limiting.

Because the EFP may be the sole food source for as long as 15 days, ***the protein should have a protein digestibility-corrected amino acid score (PDCAAS) of 1.0 or better*** (FAO/WHO, 1989). The protein and amino acids could be provided by a combination of soybean protein isolates or concentrates and grains such as wheat, and complemented with milk solids (NRC, 1989). If milk solids are used, some amount of lactose would be included, but the level should be kept below 17 g/1,000 kcal (see "Lactose," below).

There is abundant research demonstrating the effectiveness of combinations of plant proteins such as those from soybeans and wheat flour in meeting essential amino acid needs along with total protein (Brown et al., 1982; Clegg, 1960; Dahlin and Lorenz, 1993; Friedman and Brandon, 2001; Grange et al., 1994). Wheat flour has good digestibility and provides the physico-chemical properties

for a palatable food product but is limiting in lysine content. Soy protein has lysine and is a high-quality protein, but may be limiting in methionine or sulfur amino acids for children (Friedman and Brandon, 2001). Other legume protein sources may not be sufficient. For example, the combination of wheat flour, chickpeas, and milk powder has a PDCAAS of 0.73 (FAO/WHO, 1989), which is low in lysine. Amino acids should be provided in the EFP only as intact protein and not as free amino acids. ***Supplementing with amino acids is not recommended as it will affect taste and increase cost, and can lead to problems of imbalance without adequate premixing.***

Subsequent food processing should not affect protein quality. For instance, heat used in extrusion could reduce the lysine availability of the product (Clegg, 1960; Dahlin and Lorenz, 1993). ***Protein content in the final EFP should be within 10 percent of specifications.***

Carbohydrates

Carbohydrates include monosaccharides (glucose, fructose, and galactose); disaccharides (maltose, sucrose, and lactose); oligosaccharides (maltodextrins); and polysaccharides—starch (amylose and amylopectin)—and nonstarch (cellulose, xantham, pectins, and carrageenans) (Bemiller and Whistler, 1996). Carbohydrates serve several functions as components of the EFP. They provide energy, sweetness, and desirable physical properties of the product, and are necessary for sodium absorption to maintain electrolyte status. There are also maximum levels beyond which undigested and unabsorbed carbohydrates result in gastrointestinal problems due to gas production by intestinal bacteria. Carbohydrates and fat are the two major energy sources provided by the EFP; ***carbohydrate should be provided primarily as starch associated with the grains and/or legumes used as protein sources and to meet specific requirements for taste, palatability, stability, and metabolic function*** (FAO/WHO, 1998).

Sweetness and Physical Properties. Cookie-like products (e.g., slightly sweet biscuits) have proven to be most acceptable for a wide spectrum of cultures during various emergencies where relief food products have been used, although compressed food bars such as the Norwegian BP-5 were also acceptable (Grobler-Tanner, 2001). The only flavor found to be acceptable to widely diverse populations was sweetness (Drewnowski, 1997; Young et al., 1985). Therefore, nutrient composition recommendations for the EFP include sugars such as sucrose or corn syrup to provide sweetness and to improve the texture of the EFP. The specifications for the EFP limit total sugar levels, however, as described in the following subsections. Most of the carbohydrate in the EFP will be in the form of starch.

Glucose. A high incidence of diarrhea and malabsorption, commonly due to poor sanitation, is associated with uprooted populations (UN Subcommittee on Nutrition, 2001). Provision of potable water is the highest priority in emergency relief efforts (UNHCR, 2000), with the EFP as the primary source of electrolytes. Therefore, the emergency food product should provide glucose and sodium in quantities that will optimize intestinal absorption when consumed with ample water, yet not be so high as to be malabsorbed (Santosham et al., 1987).

Ability to absorb glucose in the small intestine and transport it with sodium remains intact during acute diarrhea (Hirschhorn, 1980). The EFP should provide 6 g of glucose for each 1 g of sodium to promote gastrointestinal uptake of sodium (Santosham et al., 1987). The sodium recommendation is 1.4 g/1,000 kcal, thus resulting in a requirement for 8.6 g of free glucose/1,000 kcal. However, the total monosaccharide level must be less than 25 percent of carbohydrates, by weight, to prevent osmotic diarrhea and elevation of the osmotic load. Use of maltodextrins to provide 8.6 g of free glucose is recommended due to the cost of free glucose compared to maltodextrins.

Lactose. Milk solids may be used in the EFP, but the level of milk sugar—lactose—needs to be considered. Because there may be a high incidence of adult lactase deficiency in the populations receiving the EFP, consumption of excessive lactose might be a concern if it led to abdominal discomfort, flatulence, abdominal bloating, and diarrhea (Scrimshaw and Murray, 1988). Secondary lactase deficiency also has been shown to be associated with acute gastroenteritis, malnutrition, acquired immune deficiency syndrome enteropathy, and diarrhea of infectious origin in both adults and children (Riley and Marsh, 1998; Scrimshaw and Murray, 1988). Such lactase deficiency may be transient or chronic in nature. For these reasons, ***use of lactose as a carbohydrate source is not recommended.*** Because milk solids provide high-quality protein and often are readily available for emergency feeding programs, their use as a protein source in the EFP may be desirable.

Controlled studies have shown that the majority of individuals demonstrated to be lactose maldigestors do not experience symptoms with 1 cup of milk or the equivalent amount of lactose (12 g) or more consumed at one time (Scrimshaw and Murray, 1988; Suarez et al., 1995). Many of these studies are based on results following ingestion of single test meals providing varying amounts of lactose, and tolerance to repeated intake of this amount of lactose on the same day and over a extended period of time is less clear. However, the reported milk consumption of individuals shown to be lactose maldigestors often exceeds 1 cup/day (Scrimshaw and Murray, 1988). In a controlled study by Calloway and Chenoweth (1973), four subjects shown to be lactose maldigestors were fed a diet that included 1,000 g of homogenized low-fat milk providing approximately 50 g of lactose in four divided doses for a period of 12 days. Breath hydrogen concentrations were slightly or moderately elevated in two of the subjects at this level of intake but there were few subjective complaints of discomfort due to the diet.

Although the EFP is not intended to be used in treatment of individuals with severe diarrhea or malnutrition, the use of products containing milk in feeding adults and children with these conditions demonstrates the acceptability of including milk in emergency rations. Collins and colleagues (1998) recently reported successful use of a product containing dried skim milk, vegetable oil, vitamins, and minerals as part of the diet given to adult patients with severe malnutrition in Baidoa, Somalia. Although the milk product was diluted in the first few days of treatment, the amount was gradually increased and provided 137 or 95 g of lactose/day. The latter diet was reported as being better tolerated but the investigators attributed this response to the lower protein content of the diet rather than the reduced amount of lactose.

The use of diets containing milk in treating young children with diarrhea has been studied extensively (Brown, 1991; Brown et al., 1991; Penny and Brown, 1992). A meta-analysis of clinical trials that compared the outcomes of young children treated with either lactose-containing or lactose-free diets (Brown et al., 1994) showed an overall treatment failure rate of approximately 22 percent among children treated with lactose-containing diets compared with a treatment failure rate of 12 percent among those who received lactose-free diets. On the basis of these meta-analyses the author concluded that the majority of children with acute diarrhea can safely receive undiluted, lactose-containing milks, which would contain about 12 g/240 ml, distributed over multiple feeding episodes. However, children with severe diarrhea and dehydration may have increased treatment failure rates if they receive undiluted lactose-containing milk and should be managed under close supervision. This concern, however, is not applicable to use of the EFP since it is not intended as a therapeutic treatment for individuals with severe diarrhea or malnutrition.

Based on evidence suggesting that consumption of 12 g of lactose contained in 1 cup of milk would be tolerated by populations with a high prevalence of lactose maldigestion when consumed as part of a meal, if approximately one-third of the daily ration of EFPs (and thus one-third of the lactose) is consumed during each eating episode, the maximum lactose content should be 17 g/1,000 kcal (4 g/EFP bar). Thus, children ages 1 to 3 years consuming 855 kcal/day (Table 2-3) would receive approximately 14.5 g/day or ~5 g/meal episode. This amount of lactose would allow milk solids to provide about one-third of the specified content of protein (34 g/1,000 kcal) and one-half of the calcium (768 mg/1,000 kcal) for the EFP. ***Lactose should only be present in the EFP due to its presence in milk solids—it should not be added.***

Fiber. Generally, fiber is considered essential for human health, and the targeted population should consume fiber-containing foods if possible (NRC, 1989). However, other requirements of the EFP limit the advisability of its providing fiber. First, it is well recognized that individuals living in sub-Saharan Africa and Asia usually consume about 30 g/day of nonstarch polysaccharides, an indication of adequate fiber intake (FAO/WHO, 1998). The EFP will be used for less than 15 days and hence a lack of fiber would not result in a chronic

problem or exacerbate a condition. Furthermore, the energy density of the product needs to be high (e.g., 4.2 kcal/g is the energy density of the BP-5 [Young et al., 1988]) to meet the needs of all age groups in the population, and to facilitate ease of transport and distribution. Consequently, although the EFP will contain some fiber because of its grain and legume constituents, the level of fiber should be limited to provide maximal energy density.

Importance of Carbohydrates for Physical Activity. Individuals in need of the EFP may often be walking long distances on foot, or may be expending a large amount of energy erecting shelters, finding water, finding fuel, or meeting hygiene needs. These factors emphasize the importance of carbohydrate in the EFP in a number of ways. First, during moderate-intensity labor (e.g., less strenuous than a brisk walk, under 5.6 km/h, or at less than 40 to 50 percent VO_{2max}), the primary metabolic fuel is fat with carbohydrate contributing about 25 percent toward total caloric expenditure (Brooks and Trimmer, 1996). However, during the course of several hours of work, muscle and liver glycogen stores can become depleted and the ability to walk or perform physical tasks declines. Adequate dietary carbohydrate intake is necessary to sustain prolonged exercise of more than 1 hour (Ivy et al., 1979) and to allay fatigue.

Second, if an insufficient amount of carbohydrate is consumed on consecutive days by individuals who exercise for prolonged periods, they likely will experience irritability, dizziness, and/or nausea in addition to fatigue (Sherman, 1983). Moreover, carbohydrate stored in muscle and liver tissue as glycogen involves water storage (i.e., 3 g of water/g of carbohydrate). This water is released when glycogen is metabolized and provides a minor, but useful, contribution to meeting fluid needs. Finally, compared to no feeding, carbohydrate intake during exercise increases endurance (Brooks et al., 2000). The EFP is convenient to eat during periods of physical activity, requires no preparation, and does not significantly divert the consumer from essential daily tasks. Individuals can thus benefit from consuming the EFP before and during periods of prolonged activity because it includes 40 to 50 percent of its calories as carbohydrate. This level of carbohydrate allows for an energy-dense ration (35 to 45 percent from fat) and for adequate protein (10 to 15 percent of energy coming from protein).

To summarize carbohydrate requirements for the EFP per 1,000 kcal/day:

- 40 to 50 percent of energy as carbohydrate, at least 50 percent of which is from starch;
- no more than 25 percent of carbohydrates as monosaccharides;
- at least 8.6 g of glucose from maltodextrins to allow for sodium transport;
- no more than 17 g of lactose from milk solids (no free lactose added) per 1,000 kcal;
- primary role for sucrose or corn syrup is to provide palatability and texture; and
- no added fiber in order to provide an energy-dense product.

Water

In situations that require the distribution of emergency rations to distressed populations, water supplies often will be insufficient or contaminated. Since humans can live only few days without water (Brown, 1947a), ***this report assumes that provision of adequate potable water is the first priority of any emergency operation***. Efforts should also be made to educate indigenous group leaders regarding location of water supplies and water purification (e.g., boiling, iodination). Because of concerns over possible water shortages, the EFP is designed to contribute minimally to osmotic load, while providing essential nutrients and energy to meet the needs of most individuals in emergency situations for a short period of time.

The minimal water requirement for a fasting 70-kg adult, resting in a mild environment, is about 800 ml/day (Gamble, 1947). This is by no means consistent with good health. In the United States, for example, the average adult experiences a water turnover (all sources) of approximately 2,500 ml/day. The lowest volume of fluid required to prevent deterioration provides about 300 ml of urinary output per day. Under low-stress conditions this is equivalent to an intake of about 1,000 ml (Johnson, 1964). According to Gamble (1947) and Marriott (1950), when all water intake ceases, the minimum unavoidable water loss approximates 1,500 ml/day (or about 2 percent of body weight). In a tropical or desert climate, fluid losses may range from 300 ml/h (at rest in shade, 35° C) to 900 ml/h (walking in direct sunlight, 40° C) (Adolph, 1947); this results in total water losses of approximately 3 to 10 percent/8 h exposure for a 70-kg adult. Continuous labor in a desert environment can increase the daily water requirement to 11 L/day, primarily due to sweat losses (Brown, 1947b).

Sustained mental and physical performance are incompatible with the loss of more than 7 to 8 percent of body weight as water (Calloway, 1960). When water losses reach 15 to 25 percent of body weight, it is likely that coma, circulatory failure, and death will occur (Adolph, 1947; Leithead and Lind, 1964). The clinical conditions of heat exhaustion, heat cramps, heat syncope, and heatstroke also are influenced or caused by perturbations of fluid–electrolyte balance (Hubbard et al., 1986).

The state of starvation involves considerable dehydration, regardless of environmental stressors. The actual body water deficit depends on the duration of starvation, water availability, body size, energy intake, dietary composition, work output, and environmental conditions. Infections (e.g., bacterial dysentery) are also common in undernourished individuals, and gastrointestinal illness, with vomiting and diarrhea, obviously increases water and electrolyte losses.

Carbohydrate Effect on Water Requirement

When water supplies are insufficient, provision of a minimum of 100 g of carbohydrate in a survival ration is needed (Johnson, 1986). Extensive studies on the composition of survival rations (Calloway, 1960; Gamble, 1947; Grande et al., 1958) have demonstrated that 100 g of carbohydrate constitutes the minimal essential ration amount. This amount of carbohydrate reduced the deficit of body water by lowering the amount of body solutes requiring excretion and by preventing ketosis, thus permitting a reduction in urine volume. The carbohydrate also was essential in maintaining the ability to perform various physical activities by preventing total depletion of glycogen stores, and provides some feeling of satiety.

Protein Effects on Water Requirement

Although muscle wasting is common in starvation, inclusion of a large amount of protein in the EFP is contraindicated because it negatively affects water balance. Assuming maximal renal concentration, the excretion of 1 g of urea nitrogen requires 40 to 60 ml of water. This means that the inclusion of 10 g of dietary nitrogen (equivalent to about 63 g of dietary protein) in a 2,100 kcal diet increases the volume of required water by 400 to 600 ml/day. Further, renal concentrating ability is severely compromised in moderate malnutrition (Golden, 2001).

Figure 2-2 depicts the effects of protein and energy content on obligatory urine volume in a multi-level study. The emergency rations tested contained four energy levels (500, 1,000, 1,500, and 2,000 kcal) and four protein levels (0, 7.5, 15, and 30 percent of total calories). In rations that contained 0 and 7.5 percent protein, increasing the caloric content of the ration from 500 to 2,000 kcal did not increase the obligatory urine volume. However, a ration that contained 30 percent protein approximately doubled the obligatory urine volume when the caloric content increased from 500 to 2,000 kcal (Calloway and Spector, 1954).

Based on these calculations and considering the renal dynamics discussed in the previous paragraph, it appears that ***the 2,000 kcal diet was optimal in terms of osmotic load when it contained 7.5 percent protein*** (approximately 40 g of protein). ***The osmotic load created by 15 percent protein appears to be tolerable and this thus becomes the maximum allowable amount.*** Where water availability is of real concern, lower levels of protein should be considered maximal in developing the EFP.

Salt and Total Dissolved Solids Effects on Water Requirement

Sodium chloride (NaCl) in emergency rations requires consumption of sufficient water to dilute the added osmotic content to the level found in plasma.

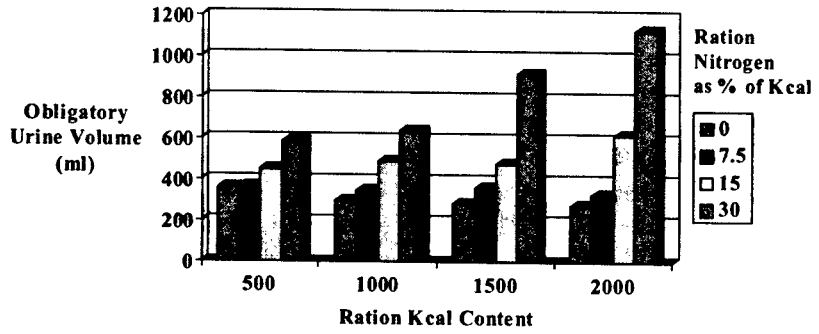


FIGURE 2-2 Influence of caloric and protein content of emergency rations on urine volume. Excretion of 1 g of urea nitrogen requires 40 to 60 ml of water.

Baker and colleagues (1963) examined the minimal water intake that is needed to dilute various amounts of dietary salt. They overloaded study participants with 11.8, 15.8, and 23.8 g of NaCl for 4 days and 32.8 g of NaCl for 10 days in a 23° C environment. Water was plentiful and was consumed ad libitum. Urinary and fecal excreta eliminated 47 percent of the total water intake and 92 percent of the salt intake. Plasma sodium levels remained constant during the course of metabolic tests, exemplifying the efficacy of renal electrolyte control. Evaluation of water balance indicated that 127 ml of water was required to dilute each gram of NaCl in a 70-kg adult leading a sedentary existence in a mild environment. Thus, the 3 g of Na supplied per 2,100 kcal from the EFP would be equivalent to 7.6 g of NaCl and require 965 ml of water.

Similarly, the human water requirement increases as the total number of osmotically active particles increases in the diet. Because underground wells, reservoirs, and streams contain dozens of minerals, the total dissolved solids (TDS) in water must be considered. Daniels and Layton (1983) have considered the concentrations of TDS in natural water sources. Although they recommend a TDS of 1,000 mg/L for field water supplies, many public drinking water sources in the United States have TDS concentrations exceeding 2,000 mg/L. This indicates that natural field water can have a significant impact on the TDS consumed each day. This should be considered when plans are formulated for the provision of water with emergency rations.

Summary of Osmotic Load and Water Requirement

There are a variety of nutrients that can increase osmotic load. Some are intracellular osmotic solutes, such as potassium, magnesium, organic phosphates, and protein; some are extracellular osmotic solutes such as sodium and its anions, chloride, and bicarbonate.

Healthy individuals have good renal control of fluid and electrolytes and maintain body equilibrium within a wide range of fluid, sodium, and potassium intakes. However, given the probable circumstances of a population in need of an EFP, body sodium, potassium, and chloride are important components that need to be monitored when a single food source is used to provide all nutrients. Given that various water sources with high levels of solids may significantly increase the osmotic load (Daniels and Layton, 1983), it is important to minimize to the extent possible that contributed by the EFP.

Electrolytes

Sodium

Sodium is essential for human health for acid–base balance, body water balance, and nerve function, and it contributes to the palatability of foods. The recommendation for sodium is based on general consumption patterns and available recommendations for maximum intakes (NRC, 1989). Although less than 1 g/day is essential for life, the chronic diarrhea that may be expected in populations requiring the EFP, along with perspiration losses due to elevated ambient temperatures and hard work, increase sodium requirements. Furthermore, additional dietary sodium enhances water retention (Shirreffs et al., 1996) and replaces sodium losses due to diarrhea. Individuals working outdoors with elevated ambient temperatures lose between 2.3 to 3.4 g Na/L of sweat (Costill et al., 1976; Dill et al., 1976). Western dietary guidelines suggest sodium intakes of no more than 2.4 g/day (NRC, 1989). Given that the EFP may be used during periods of sustained physical activity or in high ambient temperatures, the EFP should contain a minimum of 1.3 g/1,000 kcal, which is equivalent to 300 mg/EFP bar. This would provide 1 g of sodium for the 1- to 3-year age group with an average weight of 10 kg and consuming 855 kcal/day (see Tables 2-4 and 2-6). The maximum amount is 1.4 g/1,000 kcal.

Potassium

Potassium is essential for fluid balance, nerve transmission, and acid–base balance. Similar to sodium, potassium is lost in sweat, feces (diarrhea), and urine, although sweat losses are considered negligible (NRC, 1989). Golden (2001) suggests that a growing child without pre-existing deficiency may need about 1.3 g of potassium/1,000 kcal (2.7 g/2,100 kcal). The recommendation for

TABLE 2-6 Electrolyte Intake Based on Energy Needs of an Emergency Relief Food Product (EFP)[a]

Age	Gender	Energy Requirement (kcal/day)[b]	Sodium[c] (g/day)	Potassium[c] (g/day)	Chloride[c] (g/day)
7–12 mo[c]	Both	578	0.83	0.96	1.3
1–3 yr	Both	855	1.0	1.42	1.9
4–8 yr	Both	1,456	2.1	2.43	3.2
9–13 yr	Both	1,693	2.4	2.82	3.7
14–18 yr	Boys	2,136	3.1	3.56	4.7
	Girls	1,931	2.8	3.22	4.2
19–50 yr	Men	2,339	3.4	3.90	5.1
	Women	1,972	2.8	3.29	4.3
51+ yr	Men	2,249	3.2	3.75	4.9
	Women	1,929	2.8	3.21	4.2

[a] The EFP contains 1.3 g of sodium, 1.7 g of potassium, and 2.0 g of chloride per 1,000 kcal.
[b] Estimated energy values from Table 2-3.
[c] Based on daily recommendations of no more than 3.0 g of sodium, desirable intake of 3.5 g of potassium, and chloride on an equimolar basis with sodium (NRC, 1989).

the EFP is 1.7 g/1,000 kcal (396 mg/EFP bar), which is given as a desirable intake for adults (NRC, 1989) but much above the minimum requirements, and thus should provide enough to compensate for possible losses due to sweat and mild to moderate diarrhea. The maximum amount is 2.0 g/1,000 kcal (specified content + 20 percent).

Chloride

Chloride is lost in diarrhea, as well as with vomiting due to its high concentration in gastric juice. Chloride is the principal inorganic anion in extracellular fluid, and is essential for maintaining fluid and electrolyte balance (NRC, 1989). Although chloride deficiency is rarely observed, its loss mirrors sodium loss with the exception of that due to vomiting, so it is also important to ensure adequate intakes of chloride for refugee populations, particularly when consuming a single-source food product. The minimum amount contained in the EFP should be 2.0 g/1,000 kcal to match the sodium content on an equimolar basis. This provides 466 mg of chloride/EFP bar. The maximum amount is 2.2 g/1,000 kcal (specified content + 10 percent). This amount is also equimolar to the sodium level, as recommended by NRC (1989) (see Tables 2-4 and 2-6).

Summary of Electrolyte Content

Electrolyte content can influence palatability: added sodium in high amounts results in a very salty-tasting product whereas added potassium in high amounts results in a bitter-tasting product. If electrolyte losses are extensive due to chronic severe diarrhea, then therapeutic electrolyte/fluid supplements should be provided (which is beyond the scope of this report).

The nutrient density recommendations for sodium, potassium, and chloride provide additional amounts beyond the recommended intakes for healthy people (NRC, 1989). Because of its bitter taste, food sources should provide the bulk of the potassium.

Calcium, Phosphorus, and Magnesium

The nutrient content specifications for calcium, phosphorus, and magnesium were derived from the recent evaluation of requirements for these nutrients as part of the DRI process (IOM, 1997). It is assumed that growth stunting is present in the targeted populations (Neumann and Harrison, 1994). There are limited data suggesting that rapid improvement of nutritional status may improve growth, although early stunting is never fully compensated; providing bone-related nutrients early in relief efforts is potentially of benefit. The EFP specifications reflect requirements for children (IOM, 1997a).

Data presented in the DRI report (IOM, 1997a) justify the adequacy of the AI and RDA for calcium and phosphorus, respectively, during adolescence and adulthood as meeting dietary needs during pregnancy and lactation as well. Additional needs are identified during pregnancy for magnesium (IOM, 1997a); however, the individual minimal nutrient density for magnesium (Table 2-4), based on adolescent boys, is actually slightly greater than that derived for pregnancy assuming an additional 200-kcal intake. Thus, additional needs for pregnancy would be met based on the assumption that additional energy (e.g., more EFP bars) would be consumed.

Foods such as soybeans and grains should provide the primary source of these nutrients. However, poor digestibility from plant sources may require some or all of these nutrients to be added to the EFP as direct ingredients in order to provide the specified levels.

Calcium

Dietary calcium is essential for bone, neuromuscular, and cardiovascular health, as well as for many biochemical functions (IOM, 1997a). During calcium deficiency, the key calcium roles in regulatory proteins are protected at the expense of bone calcium. There is a tight regulation of serum calcium levels through exchange from and to the bone, resorption by the kidney, and absorption

from the gastrointestinal tract. Thus, the clinical sign of low calcium status is poor skeletal development, which affects growth, fracture rates, and subsequent rates of osteoporosis.

Bone growth and prevention of osteoporosis are related to chronic intakes of calcium, and there are no data suggesting that suboptimal intakes during a short emergency situation of less than 15 days would have any effect or that supraoptimal intakes during the same short time period would significantly improve bone status. Decreased bone turnover occurs in malnutrition (Branca et al., 1992), but some catch-up (or compensatory) growth is documented with children when adequate overall good nutritional status is restored (Fjeld et al., 1989; Golden, 1994). Although the EFP may provide the only source of nutrients for a very short period of time, the addition of calcium is essential to provide as nutritionally complete a diet as possible.

The minimal nutrient density for calcium is 768 mg/1,000 kcal, which is derived from the AI for children ages 9 through 13 years of 1,300 mg/day (IOM, 1997a). This assumes that these children will consume about 1,700 kcal/day, or 7 to 8 EFP bars (Table 2-3). One bar will contain about 180 mg of calcium.

The source of supplemental calcium used in the EFP should be readily absorbed (certain food sources may decrease the availability of calcium). The role of phytate, oxalic acid, and wheat bran in calcium absorption has been studied (Heaney et al., 1988, 1991; Weaver et al., 1996). Although these compounds decrease calcium absorption, overall there was no significant physiological effect on absorption when provided in a mixed diet (Heaney and Weaver, 1989; Heaney et al., 1990). It is anticipated that cereal grains and legumes will comprise the bulk of the EFP, and thus some sources of phytate will be present. Thus, an increase of 15 percent over the required amount of calcium, 180 mg/EFP bar, is suggested to compensate. The proposed level for calcium may come from supplementation of the food sources to no more than 207 mg/EFP bar (specified content + 15 percent).

The UL for calcium should be considered since the recipient population may have low urinary volumes due to dehydration related to diarrhea and inadequate fluid intakes (Golden, 2001). Urinary loads of calcium must be considered due to calcium interactions with other nutrients that may be deficient in target populations such as iron, zinc, and possibly phosphorus (Golden, 2001). The UL for calcium for adults is 2,500 mg/day based on the adverse effect of milk alkali syndrome seen at higher intake levels (IOM, 1997a). Given the concerns related to renal solute loads discussed earlier, the maximum calcium level should not exceed 885 mg/1,000 kcal.

Phosphorous

The recommendation for phosphorus is based on its function in growth of soft and bone tissues and replacing phosphorus losses, but not on prevention of a

specific sign or symptom of a nutritional deficiency (IOM, 1997a). The phosphorus content of the EFP is set based on the minimal nutrient density of 740 mg/1,000 kcal, which is derived from the RDA of 1,250 mg for boys and girls 9 to 13 years of age based on their estimated energy needs (Table 2-3). One EFP bar of 233 kcal should contain at least 172 mg of phosphorus in an available form. The UL for phosphorus for adults is 4,000 mg based on elevated serum inorganic phosphate levels seen with very high intakes (IOM, 1997a). This level corresponds to a maximum of 1,900 mg of phosphorus/1,000 kcal.

Although potential energy and protein ingredients supply phosphorus for the EFP, the majority of phosphorus from plant foods is in the form of phytic acid, which is less bioavailable (Wyss et al., 1999; Zhou and Erdman, 1995). Furthermore, elevated levels of phytate may impair bioavailability of important trace elements such as zinc. Thus there is a concern about high levels of phytate phosphorus. Method of food processing may also affect mineral availability. Kivisto and colleagues (1986) reported that apparent absorption of magnesium and phosphorus was decreased in an extruded cereal product.

Additional phosphorus to meet the level recommended may be provided by hydrolyzed phytic acid or soluble forms of phosphorus salts such as sodium hypophosphate. The specified range for phosphorus is 740 to 880 mg/1,000 kcal, or 172 to 206 mg/EFP bar (specified content + 20 percent). It is assumed that soybean- and grain-derived ingredients will contribute most of the phosphorus.

Magnesium

Magnesium is found both in bone (about 50 percent), soft tissue, and extracellular fluid. It is a required cofactor for over 300 enzymes, many of which are involved with energy metabolism and cellular replication. Absorption of magnesium from a typical diet is approximately 50 percent, with fiber decreasing absorption (Kelsay et al., 1979), ostensibly due to its phytate content. The RDA for adults is based on balance studies; the minimal nutrient density for magnesium is based on the requirements of 14- to 18-year-old boys. The recommended amount of magnesium for this subgroup is 190 mg/1,000 kcal based on the energy requirement for this group (Table 2-3) and the RDA (410 mg/d) for magnesium (IOM, 1997a). This amount provides 45 mg of magnesium/EFP bar. A higher level in the EFP is allowed if the source is from food ingredients. The maximum content is 230 mg/1,000 kcal (specified content + 20 percent) in order to ensure that total intake of added magnesium salts is below the adult UL of 350 mg/day. The UL applies only to magnesium salts added to foods, and is a level designed to prevent diarrhea associated with magnesium supplementation. Therefore the maximum amount of added magnesium consumed per day should be below this level, with the magnesium content coming primarily from the

TABLE 2-7 Recommended Macromineral Content of an Emergency Relief Food Product (EFP)

Nutrient	RDA or AI[a] for Nutrient Density (mg/d)	Amount/233 kcal Food Bar (mg)	Amount/1,000 kcal of EFP (mg)	Amount/2,100 kcal Ration (mg)
Calcium[b]	1,300	180	768	1,620–1,865
Phosphorus	1,250	172	740	1,555–1,865
Magnesium	410	45	190	400[c]

[a] RDA = recommended dietary allowance, AI = adequate intake.

[b] Calcium recommended intake is an AI rather than an RDA.

[c] The tolerable upper intake level (UL) for magnesium of 350 mg/d applies only to supplemental magnesium, not to magnesium naturally found in foods.

soybean- and grain-derived ingredients. Table 2-7 summarizes the recommended content for calcium, phosphorus, and magnesium in the EFP.

Trace Elements

Chromium

Although chromium has been shown to potentiate the action of insulin in vivo and in vitro (IOM, 2001), specific evidence of deficiency in humans has been reported in only a few isolated cases of patients receiving total parenteral nutrition and in malnourished infants who responded to oral doses of chromium chloride (Hopkins and Majaj, 1967). Because of insufficient evidence to set an EAR for chromium, AIs of 35 μg/day and 25 μg/day for men and women 19 through 50 years of age, respectively, were established based on estimated mean energy intakes. Adverse effects have not been demonstrated with excess intakes of chromium per se from food or supplements; consequently, a UL has not been established (IOM, 2001).

Early interest in chromium supplementation to improve growth and glucose utilization in malnutrition has not been applied in current practice (Carter et al., 1968; Gürson and Saner, 1973). The AI values for chromium were based on estimating average amounts of chromium in well-balanced Western diets (which were found to contain on average 13.4 μg/1,000 kcal) (IOM, 2001). Thus a ration containing a minimum of 13.4 μg/1,000 kcal could be expected to meet or exceed the chromium requirement for all healthy persons in a similar population.

This value is higher than the 1.04 μg/1,000 kcal recommended by the Sphere Project as the desirable nutrient density for refugee diets (Sphere Project, 2001). While there is a lack of evidence of deficiency or toxicity and difficulties in analyzing chromium levels in foods, it is important that a single-source food

product have some chromium present. It is suggested that the minimum chromium content of the EFP be 13 μg/1,000 kcal (3 μg/EFP bar). The maximum content is not specified in the event that higher amounts are naturally present in the EFP ingredients.

Copper

Copper deficiency is frequently observed in malnourished populations, particularly in children with protein–energy malnutrition (Ashour et al., 1999; Donma et al., 1990; Squali Houssaïni et al., 1997). Chronic and protracted diarrhea leading to copper depletion has been recognized as a particular concern in infants (Beshgetoor and Hambidge, 1998) and is also a likely risk factor for marginal copper status in adults. The high prevalence of malnutrition and diarrhea characteristic of many groups that will receive the EFP supports the need for adequate copper intake.

Factorial analysis as well as indicators such as plasma copper concentrations, serum ceruloplasmin concentration, erythrocyte superoxide dismutase activity, and platelet copper concentration, are the basis for determining recommended intakes for copper (IOM, 2001).

The minimal nutrient density value for copper was calculated (see Table 2-4) for the limiting subgroup of women 51 years of age and older. Based on the RDA of 900 μg (IOM, 2001), the value required to prevent inadequate intake in almost all individuals in this group would be 470 μg of copper/1,000 kcal. Recognizing the prevalence of malnutrition and diarrhea that often afflicts populations in need of an EFP, the EFP should contain 20 percent above this amount, or 560 μg of copper/1,000 kcal (131 μg/ EFP bar).

Acute liver failure has been demonstrated in individuals consuming large amounts of copper. The UL is 1,000 μg of copper/day for children ages 1 through 3 years (IOM, 2001), more than double the proposed levels for this age group for the EFP (480 μg/855 kcal). The maximum content is 670 μg/1,000 kcal (specified content + 20 percent).

Iodine

Iodine, a component of thyroxin, is essential for thyroid function and mental development (IOM, 2001). Iodine uptake into the thyroid gland is regulated from the pituitary by thyroid stimulating hormone (TSH). Thus, iodine is part of the regulation of thyroxin production. In iodine deficiency, TSH secretion increases and this may eventually lead to goiter as well as impaired production of thyroid hormones T_3 and T_4, essential factors for energy regulation and postnatal brain development (Hollowell et al., 1998).

In iodine deficiency, including mild deficiency, dietary iodine supplementation has an immediate impact on thyroid function (Moulopoulos et al., 1988).

Iodine deficiency during pregnancy increases risk of poor fetal mental and physical development, including cretinism. Iodine deficiency disorder (IDD) is considered the most common cause of preventable mental retardation. Iodine deficiency is well established as a nutritional problem worldwide, regardless of refugee status (UNICEF, 2000). In 1999, WHO estimated that 740 million people per year in 130 countries were at risk of IDD, including 50 million who have some degree of IDD-related brain damage. Africa, Southeast Asia, and Asia had the highest concentration of individuals at risk (WHO, 1999b). The use of iodized salt, as well as utilization of iodine for water treatment, is common worldwide. From 1990 to 1998, two-thirds of the households living in IDD-affected countries had access to iodized salt; 20 countries had 90 percent of their households with access to iodized salt.

Iodine is stored in the thyroid gland and deficiency does not occur until that store has been depleted (Clugston and Hetzel, 1994). Aside from inadequate iodine intake, protein–calorie malnutrition also may decrease thyroid iodine levels (Ingenbleek and Malvaux, 1974). Iodine turnover is slow in individuals with adequate iodine status (Fisher and Oddie, 1969).

Iodine is rapidly absorbed in the gastrointestinal tract, and excessive iodine is excreted in the urine. One study suggests that during acute diarrhea associated with protein–calorie malnutrition, iodine may be poorly absorbed (Ingenbleek and Malvaux, 1974). Bioavailability of iodine is generally high, although there are data suggesting inhibition of iodine absorption with soy flour (Shepard et al., 1960). In some populations, linamarin found in cassava may block thyroid uptake of iodine, and there are some data indicating that other water-containing humic substances may block thyroidal iodination.

The most successful method to prevent IDD is iodination of table salt. This is the recommendation of WHO, whose major emphasis is on total prevention of IDD through this practice (WHO, 1999b). WHO recommends that in order to provide 150 µg/day of iodine via iodized salt, iodine concentration in salt at the point of production should be within the range of 20 to 40 mg of iodine (or 34 to 66 mg of potassium iodate) per kg of salt (WHO, 1996a). The EFP should contain iodine as iodized salt although the iodine could also be added as calcium iodide, potassium iodide, or potassium iodate. Dietary studies such as that by Melse-Boonstra and coworkers (2000) indicate that most individuals, regardless of economic, rural, or urban status, purchase salt for cooking.

The minimal nutrient density value for iodine is based on the subgroup of children 1 to 3 years of age and is 105 µg/1,000 kcal. Assuming that iodized table salt will be used in the EFP and provide at least 50 percent of the specified sodium content, the EFP will provide more than adequate levels of iodine to prevent IDD. In the United States, iodized salt contains 194 µg of iodine per g of sodium (Venkatesh Mannar and Dunn, 1995). Thus, if the source of all the sodium in the EFP is from iodized salt, the iodine content of the ration per 1,000 kcal would be approximately 250 µg of iodine. If one assumes that half of the

sodium in the EFP may come from nonsalt sources, then the total iodine intake would be half of this, or about 125 µg of iodine/1,000 kcal, which is above the minimum nutrient density needed.

The UL for iodine is based on observations of hypothyroidism, thyroiditis, goiter, and sensitivity reactions (Pennington, 1990). Although little research has been done on refugee populations, iodination of salt in the United States increased the incidence of excessive iodine intake (Hollowell et al., 1998; Pennington, 1990). The UL for children 1 to 3 years of age is 200 µg/day (IOM, 2001), which results in a maximum content of 230 µg/1,000 kcal (specified content + 115 percent). If most of the sodium in the EFP comes from added salt, then it is possible that a mixture of iodized and noniodized salt may be needed to keep the total iodine content below this level. However, if most of the required sodium in the EFP comes from other sources, iodine can be provided in other forms as mentioned previously.

Iron

Anemia due to iron deficiency represents a major public health problem worldwide. It has been estimated that more than 2 billion people (over 33 percent of the world's population) are iron deficient (INACG, 1999). Young children and women of reproductive age are at greatest risk. Programs to control iron deficiency have been implemented in almost all countries; nevertheless, both anemia and iron deficiency remain endemic among many populations (de Benoist, 2001).

Subclinical and clinical consequences of iron deficiency include impaired physical work performance, developmental delays in infants, cognitive impairment, and adverse pregnancy outcomes (IOM, 2001). Although numerous confounding factors make it difficult to establish a clear relationship, iron deficiency has been reported to be associated with reversible abnormalities of immune function and increased risk of infections (Oppenheimer, 2001; Scrimshaw and SanGiovanni, 1997). A recent conference, organized to evaluate the strength of evidence that iron deficiency causes specific functional outcomes, concluded that there is a significant body of evidence to support a causal relationship among iron deficiency, deficits in work productivity, and child development; and among severe anemia, malnutrition, and increased child mortality. However, causal evidence is lacking or contradictory in support of a relationship between iron deficiency and low birth weight and infectious disease (Stoltzfus, 2001).

The potential adverse effects of excess iron intake also are recognized. High-dose iron supplements have been shown to reduce zinc absorption if both are taken without food; however, this inhibitory effect does not occur if they are consumed with food (IOM, 2001). Similarly, high-dose iron supplements often

lead to constipation and other gastrointestinal symptoms when taken without food but usually are not a problem when taken with food (IOM, 2001).

The possibility that high-dose iron supplementation may have adverse effects in individuals with severe malnutrition or infectious diseases also has received attention (Tomkins, 2000). Smith and coworkers (1989) reported an increase in mortality in children with protein–energy malnutrition who received supplements of iron and recommended that iron therapy should not be instituted during the first week of treatment. However, the increase in mortality was not statistically significant and the dosage of iron supplementation was not reported. Based on a comprehensive review of published studies on the relationship between iron and infectious diseases, Oppenheimer (2001) had the following observations of use to health planners: (1) oral iron supplementation has not been shown to cause an increased risk of infection in any age group in nonmalarious countries, (2) oral iron supplementation in malarious regions may carry up to a 50 percent increased risk of clinical malaria if given in therapeutic doses at times of malaria transmission, and (3) oral iron supplementation in therapeutic doses to older immunized children and adults in malarious regions may also carry up to a 50 percent increased risk of other infectious disease. In the studies in malarious regions showing a significant iron-associated increase in risk of nonmalarial infectious morbidity, the dosage of oral iron was 3 mg/kg/day for children 6 months to 6 years of age and 60 mg/day for anemic women.

Review of nine published and four unpublished placebo-controlled, randomized trials of iron supplementation in malarious areas by an expert panel convened by the International Nutritional Anemia Consultative Group led to a consensus statement (INACG, 1999) recommending that oral iron supplementation should continue to be recommended in malarious areas where iron-deficiency anemia is prevalent. It was recognized, however, that present evidence is insufficient to rule out the possibility of an increased risk of malarial illness in some iron-supplemented individuals.

Results of other studies, however, have shown no differences in incidence or severity of conditions such as diarrhea or respiratory infections associated with iron supplementation (Berger et al., 2000; Calder and Jackson, 2000; Oppenheimer, 2001).

It is assumed that as refugees many recipients of the EFP will be iron deficient. Although it is recognized that the deficiency cannot be reversed in 15 days, it is essential that sufficient iron is provided not only to meet basic requirements but also to support the initiation of repletion. The EFP, however, should not contain excess iron, particularly in soluble forms, because excess iron promotes oxidative changes leading to destruction of nutrients such as vitamin C, as well as to the development of rancidity.

Consideration must also be given to the form of iron used in the EFP. Use of iron-EDTA appears to have potential as a fortificant, particularly in diets of low bioavailability. It is less affected by inhibitors of iron absorption and is less

likely to cause organoleptic problems, and its efficacy has been demonstrated in several intervention studies (Bothwell, 1999). The possibility of using microencapsulated iron should also be considered (Jackson and Lee, 1991) to minimize problems such as rancidity and inhibit interaction with other nutrients.

Phytic acid, a known inhibitor of iron absorption, will influence the bioavailability of iron from food products (Reddy et al., 2000). Although various approaches are available to reduce the phytate content of the EFP, the most practical approach appears to be fortification of the product at a level of iron that would ensure a sufficient quantity of absorbable iron. Vitamin C has been shown to offset the inhibitory effect of phytate on iron absorption (Hallberg et al., 1989; Siegenberg et al., 1991), thus providing justification for a liberal content of this vitamin in the product (see later section, "Vitamin C").

Factorial modeling was used to calculate recommended intake levels for iron for older infants, children, and adults (IOM, 2001). Using the recommended intake for adult women during their reproductive years (18 mg/day), the minimal nutrient density (Table 2-4) is 9 mg of iron/1,000 kcal. Pregnant women would need higher amounts (12.4 mg/1,000 kcal). The IOM values are based on an assumed bioavailability of 18 percent for children 1 year of age and older, pregnant women during the first trimester, and nonpregnant adults. A mixed protein diet that includes some heme iron is assumed. For children under 1 year of age, for whom the diet will contain little meat and primarily cereals and vegetables, the bioavailability is assumed to be 10 percent; for pregnant women, due to the increased rates of absorption seen during the second and third trimester, bioavailability is assumed to be 25 percent (IOM, 2001).

The provisional recommended daily iron intakes set by the Joint FAO/WHO Expert Consultation on Human Vitamin and Mineral Requirements (FAO/WHO, 2000) for diets having 10 percent iron bioavailability are considerably higher: for girls 10 to 14 years of age, 33 mg (19.5 mg/1,000 kcal); for older adolescents, 31 mg (16 mg/1,000 kcal); and for adult women of reproductive age, 29 mg (14.7 mg/1,000 kcal). To achieve the young adolescent nutrient density, the iron content would need to be doubled to take into account the lower bioavailability.

Since a generous amount of ascorbic acid is included in the EFP, and use of an iron source that is well tolerated and more bioavailable is recommended, the EFP should contain 16.3 mg of iron/1,000 kcal (3.8 mg/EFP bar), assuming 10 percent bioavailablity. This provides the same amount of iron as 9.1 mg/1,000 kcal assuming 18 percent bioavailablility. Given the concern about excess dietary iron and possible adverse effects on immune function, as well as possible food interactions and oxidative changes, the maximum iron content of the EFP is 17.6 mg/1,000 kcal (specified content + 10 percent). A lower iron content may be necessary if this range leads to unacceptable oxidative changes in the product; however, use of encapsulated iron, as mentioned earlier, could help prevent this problem.

Manganese

Dietary manganese is essential to the action of metalloenzymes involved in the formation of bone and in the metabolism of amino acids, lipids, and carbohydrates. Manganese deficiency has been observed in animals but has only been produced experimentally in humans, thus little data is available related to deficiency in refugee populations. Early signs of inadequate manganese include hypocholsterolemia and a scaling, blistering rash on the upper torso (Friedman et al., 1987; IOM, 2001).

Recommended intakes of manganese are based on median intakes due to insufficient data to determine specific requirements and thus are AIs (IOM, 2001). The minimal nutrient density value is based on the AI for children ages 1 to 3 years (1.2 mg/day) and is 1.4 mg of manganese/1,000 kcal (see Table 2-4). This would provide 0.33 mg of manganese/EFP bar. The maximum content of the EFP is 1.7 mg/1,000 kcal (specified content + 20 percent). Risk of elevated blood manganese concentrations and neurotoxicity are the basis for UL values which range from 2.33 mg/1,000 kcal for children 1 through 3 years of age to 4.70 mg/1,000 kcal for adult males (IOM, 2001).

The level specified for the EFP is much greater than that recommended by the Sphere Project (2001) for a desirable nutrient density of 0.3 μmol/100 kcal (0.2 mg/1,000 kcal) for refugee diets, but it is within the range found in Western diets.

Selenium

The biological role of selenium is as a component of various selenoproteins. Selenoproteins include five glutathione peroxidases, two deiodinases, several thioredoxin reductases, and selenophosphate synthetase 2 (Behne and Kyriakopoulos, 2001). These proteins are important in supporting immunocompetence and defense against oxidative stress, regulation of thyroid hormone action, and regulation of the redox status of vitamin C and other molecules (IOM, 2000).

Different populations vary greatly in their dietary intake of selenium, largely because the selenium content of plant foods depends on the selenium content of the soil where the food is grown. Meat and fish may be more reliable sources of selenium; however, their content can be influenced by the selenium content in feed sources. Intakes of selenium are particularly low in New Zealand, Finland, and parts of China. Low plasma and hair concentrations of selenium and low plasma glutathione peroxidase activity are common in these countries (Thomson and Robinson, 1996; Varo et al., 1994; Xia et al., 1989). Surveys in other parts of the world suggest that marginal or deficient selenium status may be widespread (Fordyce et al., 2000; Kvicala et al., 1999; Maksimović and Djujić, 1997; Ngo et al., 1997). Low selenium intake leading to severe selenium deficiency is recognized as the major factor contributing to the development of Keshan disease, a cardiomyopathy that occurs primarily in

children living in various parts of China (Ge and Yang, 1993). The role of selenium deficiency in Kashin-Beck disease, a degenerative osteoarticular disorder that is endemic to certain low-selenium areas of Tibet, is less clear (IOM, 2000).

Because of the possibility that the selenium status of the population groups receiving the EFP may be marginal or deficient, it can be argued that the ration should provide a generous intake of selenium. Additional support for increasing selenium levels in the EFP is provided by the hypothesized role of selenium in iodine metabolism, oxidative changes associated with protein–energy malnutrition, and viral infections (Ashour et al., 1999; Beck et al., 2001; Contempre et al., 1992; Fechner et al., 2001; Golden, 1998; Levander and Beck, 1999; Ngo et al., 1997; Sive et al., 1993; Tatli et al., 2000; Vanderpas et al., 1990).

The recommended intakes for selenium are based on the criterion of maximizing plasma glutathione peroxidase activity (IOM, 2000) and on data from two intervention studies, one in China and one in New Zealand. Compared to the RDA values (55 μg/day for girls 14 to 18 years old [IOM, 2000]), intakes recommended in a preliminary FAO/WHO (2000) report are considerably lower. Based on the limiting minimal nutrient density (see Table 2-4) of 28 μg of selenium/1,000 kcal (6.5 μg/EFP bar), the minimum amount of selenium should be at this level for the EFP. The maximum content is 34 μg/1,000 kcal (specified content + 20 percent).

Attempts to identify evidence of selenium toxicity in populations living in seleniferous areas of the world have given conflicting results (Reilly, 1996). Residents of seleniferous ranches in South Dakota or Wyoming with intakes as high as 724 μg/day showed no adverse effects associated with their high intakes (Longnecker et al., 1991). In contrast, endemic selenium toxicity occurring in China led to biochemical abnormalities at selenium intakes over 750 μg/day and changes in nails and hair in susceptible individuals at intakes of at least 910 μg/day (Yang et al., 1989). Other signs of chronic toxicity included lesions of the skin, gastrointestinal tract, and nervous system (IOM, 2000).

Hair and nail brittleness and loss are the endpoints on which the UL for selenium is based (IOM, 2000). The maximum nutrient density is 103 μg/1,000 kcal based on the UL for children 4 to 8 years of age. The specified range is well under this maximum. If it is necessary to add selenium to the ration in addition to that provided by the major ingredients, it is recommended that it be in the form of selenomethionine, due to its greater bioavailability compared to selenate and selenite (IOM, 2000). Selenomethionine is the form that has been used in supplementation trials.

Zinc

Low zinc intakes and marginal or deficient zinc status are found frequently in developing countries, particularly in young children (Zinc Investigators'

Collaborative Group, 2000). However, lack of reliable laboratory biomarkers has made it difficult to accurately estimate the prevalence and severity of zinc deficiency (Hambidge, 2000). Evidence for the existence of inadequate zinc intakes has come largely from zinc supplementation trials (Hotz and Brown, 2001). Consumption of plant-based diets, especially those having a high content of phytic acid, is considered a major factor contributing to zinc deficiency (Gibson et al., 1997; Hambidge et al., 1998).

Zinc intakes recommended by FAO/WHO (2000) vary depending on the estimated bioavailability of dietary zinc. Diets are classified as having high (56 percent), moderate (35 percent), or low (15 percent) zinc bioavailability, based on the dietary content of animal and fish protein, calcium (less or greater than 1 g of calcium/day), and daily molar ratios of phytate to zinc (less than 5, 5 through 15, and greater than 15) (WHO, 1996b).

Zinc bioavailability of the traditional diets consumed by various groups of potential EFP recipients may vary considerably. Using the WHO model, estimates of zinc absorption have ranged from 15 percent for diets in Malawi, Kenya, and Guatemala to 30 percent for diets in Ghana, Guatemala, and Egypt (Gibson and Ferguson, 1998). Thus, if based on dietary zinc content alone, the zinc status of these populations might be expected to differ appreciably; however, other factors such as limited amounts of food and persistent diarrhea may lead to marginal zinc deficiency even in those populations consuming diets low in phytate, a known binder of zinc.

An increasing number of supplementation trials have demonstrated the value of increased zinc intake in promoting linear and ponderal growth in children. For example, a meta-analysis of 25 zinc supplementation trials showed that zinc supplementation had an overall positive effect on change in height (Brown et al., 1998). Subjects in these trials ranged in age from birth to 13 years, with a mean age of 3.6 years. The mean dose of zinc used for supplementation was 14 mg/day (1.5 to 50 mg/day).

A positive effect on growth, however, was not observed in all studies (Friis et al., 1997; Kikafuna et al., 1998). Inconsistent results might be attributed to inclusion of older children whose rate of growth is slower and to the presence of multiple deficiencies that would not be expected to respond to supplementation with a single nutrient (Hotz and Brown, 2001). Some evidence suggests that zinc supplementation may lead to increased activity levels in young children (Sazawal et al., 1996) and improved neuropsychological performance in school-age children (Penland, 2000).

Because zinc deficiency is associated with diarrhea and impaired immune response (IOM, 2001), there is considerable interest in the possible therapeutic or preventive role of zinc in infectious diseases and diarrhea in children in developing countries. A recent study reported a pooled analysis of all available published and unpublished randomized controlled trials of the effect of supplemental zinc in children less than 5 years of age with acute or persistent diarrhea

(Zinc Investigators' Collaborative Group, 2000). In the acute diarrhea trials, zinc-supplemented children had a 15 percent lower probability of continuing diarrhea on a daily basis. In the persistent diarrhea trial analysis, zinc supplementation resulted in a 24 percent lower probability of continuing diarrhea and a 42 percent lower rate of treatment failure or death.

Reports of low dietary intakes of zinc and marginal zinc nutriture in pregnant women are of concern (Fitzgerald et al., 1993; Huddle et al., 1998; Kirksey et al., 1994). Although observational studies have produced strong associations between poor maternal zinc status and various indicators of poor pregnancy outcome, supplementation trials have not produced strong or consistent results in support of dietary zinc supplementation (Caulfield et al., 1998).

The minimal nutrient density value for zinc is 5.2 mg/1,000 kcal, based on boys 14 to 18 years of age (Table 2-4). The RDA for zinc (11 mg/day for adolescent boys) is based on estimates that between 30 and 40 percent of dietary zinc would be absorbed (IOM, 2001). It is doubtful that this level of absorption would occur with the EFP given the plant-based diet with the expected level of phytate. Thus, to cover the potential for reduced absorption due to the type of food ingredients, and due to the presence of gastrointestinal problems in the potential recipient populations, the baseline value is increased by 100 percent, resulting in the specified level of zinc in the EFP of 10.4 mg/1,000 kcal (2.4 mg/EFP bar). This level seems justified on the basis of the demonstrated positive effects of zinc supplementation on growth and in the treatment and prevention of diarrhea in malnourished children

Based on estimates of the zinc content of muscle and changes that occur during malnutrition, Golden (2001) has proposed a level of 13 mg of zinc/1,000 kcal for emergency refugee rations. He concluded that this amount would allow individuals who do not have an initial deficit of zinc to gain at least 5 g/kg of body weight/day even with a diet having low zinc bioavailability. A nutrient density of 13 mg of zinc/1,000 kcal is similar to that in other emergency relief foods such as Corn Soy Blend and Unimix, and lower than that in BP-5 Compact Food or F100 (Golden, 2001). The level chosen for the EFP bar is similar to the desirable nutrient density of 9 mg/1,000 kcal recommended by the Sphere Project (Sphere Project, 2001).

The ULs for zinc, 40 mg/day for adults with lower values for children, are adjusted on a weight basis (IOM, 2001). Other reference values developed as safe upper limits of zinc intake (WHO, 1996b) are higher. The UL for zinc for children ages 1 through 3 years (IOM, 2001) is 7 mg/day, which is less than the proposed level for the EFP for this age group (which totals 8.9 mg/day, based on the estimated energy expenditure for children ages 1 through 3 years).

The UL for zinc is based on the adverse effect of excess zinc on copper status, recognizing that the studies reported were primarily in adults (IOM, 2001). Although this level of zinc in the EFP appears to be of possible concern, the expectation that less will be absorbed also holds true for the UL, and thus a

higher amount will in all likelihood not increase the risk that adverse consequences to copper status will occur, particularly when copper is also added to the EFP and the EFP will be fed over a short period of time. However, given the concern about the UL, it is important that the specifications for the EFP be tightly controlled; the maximum content is 11.4 mg/1,000 kcal (specified content + 10 percent).

Additionally, the molar phytate:zinc ratio of the EFP should be less than 10 because higher ratios are associated with suboptimal zinc status (Bindra et al., 1986; Oberleas and Harland, 1981). Consideration needs to be given to the form of zinc added to the EFP. Zinc carbonate and zinc oxide are insoluble and poorly absorbed, whereas zinc sulfate and zinc acetate appear to be better utilized (Allen, 1998) and would be the preferred ingredient.

Vitamins

Vitamin A

Vitamin A is required for normal vision, gene expression, reproduction, embryonic development, growth, and immune function (IOM, 2001). Problems associated with vitamin A deficiency reflect these functions. For example, vitamin A deficiency causes blindness as a consequence of xerophthalmia. Vitamin A deficiency is also associated with increased risk for infectious diseases (Underwood and Arthur, 1996). Conversely, infection may contribute to development of vitamin A deficiency as a result of decreased food intake as well as decreased absorption, increased utilization, and possibly increased excretion (Nalubola and Nestel, 1999).

Vitamin A deficiency is a significant public health problem in many parts of the world, especially in Africa and Southeast Asia (WHO, 2001). Clinical vitamin A deficiency affects at least 2.8 million preschool children in more than 60 countries, and it is estimated that subclinical deficiency is a problem in at least 250 million people (Stephenson et al., 2000). School-age children and pregnant women also are affected. An estimated 250,000 to 500,000 children become blind each year as a consequence of severe vitamin A deficiency (WHO, 2001). Importantly, improving the vitamin A status of deficient children, ages 6 months to 5 years, increases their chances of survival, as shown by the meta-analysis of eight studies in which the risk of mortality from diseases such as measles and diarrhea decreased by 23 percent (Beaton et al., 1994).

Many, but not all, studies have shown a beneficial effect of vitamin A supplementation in infectious disease. Meta-analyses by Fawzi and coworkers (1993) and Glasziou and Mackerras (1993) showed a significant reduction in mortality from infectious diseases in children given vitamin A. The value of vitamin A supplementation in improving morbidity, however, is less clear, and results of various studies have been equivocal. Supplementation has been shown

to reduce the severity of measles and have a beneficial effect on measles-related pneumonia. However, a beneficial effect on nonmeasles respiratory infections has not been demonstrated (Villamor and Fawzi, 2000). WHO (1999a) has recommended vitamin A supplementation in the management of uncomplicated measles in areas of known deficiency as well as in all cases of complicated measles.

Vitamin A deficiency may be an important factor contributing to poor maternal performance during pregnancy and lactation (Ladipo, 2000; Underwood and Arthur, 1996), as well as growth deficits in children (Hadi et al., 2000; West et al., 1988). Vitamin A is also important in iron metabolism. Impaired mobilization of iron stores was found in the presence of vitamin A deficiency (Lynch, 1997), and a significant increase in mean hemoglobin concentration has been shown in anemic school children given vitamin A supplements (Fishman et al., 2000; Mwanri et al., 2000). Furthermore, the results of a recent study suggest that vitamin A may enhance the absorption of iron from cereal-based meals, possibly by preventing phytic acid from binding iron in the cereals (Layrisse et al., 2000).

The adverse effects of high vitamin A intake are well recognized. Most cases of toxicity have occurred because of high-dose supplements taken over a period of months or years. The possible teratogenicity of high vitamin A levels consumed during the first trimester of pregnancy is of particular concern. However, the threshold at which risk occurs is controversial. Most of the data on birth defects associated with excess vitamin A consumption involve doses equal to or greater than 7,800 μg of preformed vitamin A/day (IOM, 2001), although Rothman and colleagues (1995) showed a significantly increased risk for malformation of cranial structures originating from neural crest cells in the infants of women who consumed more than 4,500 μg/day of preformed vitamin A from food and supplements during the first trimester of pregnancy. The ULs for women 14 through 18 and 19 through 50 years of age are 2,800 and 3,000 μg/day of preformed vitamin A, respectively (IOM, 2001).

The UL for infants is 600 μg/day, based on case reports of infants who developed bulging fontanels as a result of receiving high-dose vitamin A supplements (IOM, 2001). This value is only slightly higher than the AI of 500 μg/day for infants 7 through 12 months of age based on estimated intakes for infants receiving human milk and complementary foods (IOM, 2001). Similarly, the differences between the ULs for children 1 through 8 years of age and their corresponding RDAs are relatively small.

Recommendations for the vitamin A content of diets intended for refugee feeding vary. Beaton (1995) recommended 380 μg of retinol equivalents(RE)/1,000 kcal as a goal for fortification of the total diet for refugee feeding. This amount would be expected to meet the needs of at least 95 percent of

TABLE 2-8 Vitamin A Content Based on Energy Needs of an Emergency Relief Food Product (EFP)

Age	Gender	Energy Requirement (kcal/d)[a]	Recommended Dietary Allowance for Vitamin A (μg RAE/day)[b]
7–12 mo	Both	578	500 (AI)
1–3 yr	Both	855	300
4–8 yr	Both	1,456	400
9–13 yr	Both	1,693	600
14–18 yr	Boys	2,136	900
	Girls	1,931	700
	Girls, pregnant	2,131	750
19–50 yr	Men	2,339	900
	Women	1,972	700
	Women, pregnant	2,172	770
51+ yr	Men	2,249	900
	Women	1,929	700

[a] Estimated energy values (from Table 2-3).
[b] Taken from IOM, 2001.

individuals and in theory would lead to a 3-month liver reserve. The Sphere Project (2001) suggested that 500 μg RE/day can be used for planning purposes in the initial stages of an emergency. In contrast, Golden (2001) recently recommended that 2,000 μg of retinol/1,000 kcal be added to a ration intended for emergency feeding.

The vitamin A content of diets and products used for refugee relief also varies considerably. For example, the approximate vitamin A content of the BP-5 Compressed Compact Food is 1,025 μg of preformed vitamin A/1,000 kcal (Golden, 2001); Corn/Soy Blend (new), 1,850 μg; and Unimix, 1,635 μg (Beaton, 1995).

The minimal nutrient density value calculated for vitamin A and based on the RDA (900 μg RAE/day [IOM, 2001]) is for boys 14 through 18 years of age (Table 2-4). The value is 420 μg RAE/1,000 kcal. Because the vitamin A status of many of the potential recipients of the EFP may be marginal or deficient and because vitamin A is important in situations involving infectious diseases and diarrhea, the baseline value of 420 μg/1,000 kcal is likely to be too low for many

Tolerable Upper Intake Level for Preformed Vitamin A (μg/day)	Intake of Vitamin A from EFP[c] Based on Estimated Energy Requirements (μg/day)
600	289–578
600	427–855
900	728–1,426
1,700	846–1,693
2,800	1,068–2,136
2,800	966–1,931
2,800	1,066–2,131
3,000	1,170–2,339
3,000	1,170–2,339
3,000	1,086–2,172
3,000	1,124–2,249
3,000	964–1,929

[c] The EFP contains between 500 and 1,000 μg of preformed vitamin A/1,000 kcal (Table 2-4 and IOM, 2001).

crises. It is recommended that the EFP contain a minimum of 500 μg of preformed vitamin A/1,000 kcal (117 μg/EFP bar). The maximum content is 1,000 μg of preformed vitamin A/1,000 kcal (specified content + 100 percent). Carotenoids possibly present in food ingredients do not contribute to this total due to concern for variable rates of absorption and bioconversion.

Based on assumed energy intakes, the minimum amount exceeds the U.S. and Canadian recommended intakes for all individuals (see Table 2-8). At the maximum amount, intakes of pregnant women would not exceed the UL of 3,000 μg/day of preformed vitamin A (IOM, 2001), although the intake of children 1 through 8 years of age may be 50 percent above their respective UL for the vitamin. However, the UL is designed to represent chronic intake—it is not meant to apply to malnourished individuals who are recipients of fortification or supplementation programs for the prevention and treatment of vitamin A deficiency (IOM, 2001).

If one of the initial activities in a relief program was to give children a high-dose vitamin A supplement (Golden, 2001), it could be argued that the high content of the EFP would not only be unnecessary but also might increase the risk of adverse effects. In view of the sponsors' proposed use of this product such a

possibility is unlikely. High-dose supplements are in the range of 60,000 μg of preformed vitamin A administered as a single dose every 4 to 6 months (NRC, 1987); thus the extra amount ingested from food would represent a very small percentage of the total amount given, and consumption of the EFP is recommended for no more than 15 days. It is very important, however, that the content of vitamin A in the EFP be monitored carefully to be within the specifications given.

Vitamin D

Vitamin D (cholecalciferol) is required for calcium absorption, normal muscle function, and bone growth (IOM, 1997a). Although human requirements can be met with adequate exposure to sunlight (Holick, 1994), it is difficult to monitor and ensure adequate bioconversion. The need for dietary vitamin D for survivability or bone growth for a 15-day period for which the EFP is intended is not clear. However, based on the expectation that the target population is prone to deficiency and a dietary source will enhance the absorption and utilization of calcium, it is recommended that the EFP contain vitamin D. Although not reliably documented, vitamin D deficiency resulting from long periods of wearing full body clothing and living in environments with significant air pollution—especially from dust—is possible.

Clearly, when refugees live close to the equator, vitamin D synthesis is probably occurring when environmental and cultural conditions allow adequate skin exposure to the sun. Because of the complexity of estimating true vitamin D needs in this population, the recommendation for vitamin D content of the EFP is the AI (IOM, 1997a). Populations in need of the EFP can be expected to have a high percentage of individuals under 50 years of age. While the AI for vitamin D is 5 μg of cholecalciferol/day for the adult population 50 years of age and younger, it is 10 μg/day for those between 51 and 70 years of age, and 15 μg/day for those over 70 years of age (IOM, 1997a). The amount of vitamin D for the EFP is proposed to be 5.2 μg/1,000 kcal (1.2 μg/EFP bar), based on the needs for those over 50 years of age; those over 70 years of age were thought to be too small a group within refugee populations to be used as the basis for the vitamin D content of the EFP.

Little information is found concerning the bioavailability of vitamin D in malnourished individuals, so no adjustments were made in the recommendation. The types of ingredients likely to be used in the EFP strongly support the addition of vitamin D as cholecalciferol as there may well be no other dietary source included unless fortified milk solids are used as a protein source. Although dictated by cost, utilization of this form also reduces the potential for toxicity (as compared to $1,25(OH)_2D_3$, the biologically active form of vitamin D). The EFP is not formulated as a therapeutic ration, thus individuals with hepatic or renal disease would need to be treated separately.

The UL for vitamin D is 50 μg of cholecalciferol/day (~24 μg/1,000 kcal), and is based on hypercalcemia at higher levels of intake on a chronic basis (IOM, 1997a). Since the recipient population is likely to have some sun exposure, this limit should be strictly adhered to, as there is a risk in displaced populations—due to dehydration—of compromised urinary function (Briend and Golden, 1993). The maximum content is 5.8 μg/1,000 kcal (specified content + 10 percent).

Vitamin E

Vitamin E, the primary fat-soluble antioxidant in the body, is essential for proper immune system function and for maintenance of cell membranes. Deficiencies of vitamin E have been reported in malnourished individuals (Golden, 2001). Furthermore, diarrhea and malabsorption are likely to be present in the populations served by the EFP. Absorption of vitamin E is known to be low and varies from 21 to 86 percent depending on the presence of any defects that lead to impaired absorption. Impaired absorption was taken into account in developing the recommended dietary intakes (IOM, 2000). Assuming that a smaller percentage of the dietary vitamin E in the EFP will be absorbed due to possible malabsorption, and recognizing that girls 14 to 18 years of age have the greatest nutrient density need, 20 percent is added to the minimal nutrient density value estimated for this age group (Table 2-4) of 7.8 mg of d-α-tocopherol/1,000 kcal, to provide the amount for the EFP of 9.4 mg/1,000 kcal (2.2 mg/EFP bar). The level of vitamin E should provide adequate antioxidant activity to protect against the oxidation of PUFAs after absorption. Therefore, an additional 6.6 mg of vitamin E is added to protect the maximum amount of PUFA at 10 percent of energy (which is 11 g/1,000 kcal), equivalent to 0.6 mg of vitamin E/g of PUFA.

Since it is recommended that the vitamin E be encapsulated, it is not necessary to provide additional d-α-tocopherol beyond the level specified above to serve as an antioxidant for the PUFA present in the bar. If it is not encapsulated, additional vitamin E or other antioxidants will need to be added to protect against lipid oxidation and subsequent destruction of the vitamin over the shelf life of the EFP.

The required level of d-α-tocopherol (9.4 mg/1,000 kcal) is well below the UL for all groups (1,000 mg total α-tocopherol/day [IOM, 2000]).

Vitamin K

Vitamin K functions as a cofactor for the blood clotting cascade, and a deficiency is marked by reduced levels of blood clotting factors such as prothrombin factors X, IX, VII, and protein C. Vitamin K is a cofactor for carboxylation of glutamyl residues on proteins to form ϒ-carboxyglutamyl proteins (Gla). Osteocalcin, a Gla protein, is essential for bone formation (IOM, 2001; Olsen, 1994).

Deficiencies of vitamin K are rare in most parts of the world, and there are no data validating vitamin K deficiencies in refugee populations. One study from India showed that breast-fed infants with diarrhea had low levels of prothrombin, suggesting that vitamin K deficiency is independent of antibiotic therapy (Kumar et al., 2001). Children with protein–energy malnutrition also have low prothrombin levels (Hassanein and Tankovsky, 1973), but this deficiency is better treated with an increase in dietary protein than with vitamin K. Besides dietary sources of vitamin K, it has been assumed that the microflora of the gastrointestinal tract synthesize menaquinone. With antibiotic therapy—or in the newborn with a sterile gastrointestinal tract—there is a decrease in vitamin K availability (Kumar et al., 2001). However, the contribution of the bacterially produced vitamin K is unknown (IOM, 2001). Thus, dietary sources are recommended, and vitamin K should be added to the EFP.

Blood clotting is important to survival, especially when there are multiple opportunities for injury due to military-type conflicts as well as the continued movement of many refugee populations. Thus, even a 15-day period of vitamin K supplementation may improve survivability by reducing blood loss due to prolonged clotting time following injury. There are data suggesting some benefit for nursing mothers to consume vitamin K to increase levels in their milk. Due to the limited data, however, the AI is the basis for the vitamin K content of the EFP.

Food composition data suggest that soybean oil (193 μg of vitamin K/100 g) could provide the vitamin K required in the EFP, thereby limiting the need for addition of vitamin K (USDA, 1994). Median and mean intakes of vitamin K have been estimated to be 80 to 120 and 60 to 210 μg/day, respectively, for adult men, the most limiting group (IOM, 2001). Levels that result in deficiency are much lower. Given little concern regarding intake above the AI, the minimum content for the EFP is set at 57 μg/1,000 kcal (14 μg/EFP bar). No maximum level is set as little evidence of adverse effects of overconsumption has been identified, except in those taking prescription anticoagulants.

Vitamin C

The function of vitamin C in protecting against oxidative stress, its necessity for wound healing, and its likely role in maintaining normal immune function (IOM, 2000) make the vitamin particularly critical for recipients of emergency rations. Vitamin C also is known to enhance the absorption of nonheme iron, which is especially important in populations where iron deficiency is a major nutritional problem, particularly among women and children (IOM, 2000).

Outbreaks of scurvy have been reported in refugee populations during the past three decades, often in populations entirely dependent on emergency food rations found to provide less than 2 mg/day of vitamin C (IOM, 1997b). It is

difficult to estimate the actual number of scurvy cases that occur, due partly to lack of adequate surveillance systems in refugee camps, but also because of the frequent existence of multiple deficiencies. Populations under siege or on the move are more likely to encounter problems obtaining fresh fruits and vegetables, the major food sources of vitamin C. Populations in some parts of Africa may have marginal intakes of vitamin C for considerable periods of time before an emergency situation occurs.

The RDA for vitamin C for adults (75 mg/day for women, 90 mg/day for men) is based on the amount needed to maintain near maximal neutrophil ascorbate concentrations with minimal urinary excretion of the vitamin (IOM, 2000). Recommended intakes for children and adolescents are derived from adult values based on body weight. The nutrient density needed to meet the needs of the most limiting group (men over age 50 years; see Table 2-4) is approximately 40 mg/1,000 kcal (IOM, 2000). This level would provide the recommended intakes of vitamin C for healthy adults and exceed those for children.

The vitamin C status of EFP recipients is assumed to be marginal given the likelihood that previous diets were low in fruits and vegetables and the occasional observation of scurvy in some refugee populations. It is also possible that storage in higher heat conditions and possible oxidation may destroy some of the vitamin C present in the EFP. Therefore, it is recommended that the vitamin C content of the EFP be 2.5 times the baseline minimal nutrient density, or 100 mg/1,000 kcal (23.3 mg/EFP bar). This level is similar to that in the BP-5 Compact Food (87 mg/1,000 kcal; Golden, 2001), and slightly lower than that in the USAID Corn/Soy Blend (106 mg/1,000 kcal [IOM, 1997b]).

Given that the ULs for children 1 through 3 and 4 through 8 years of age are 400 mg/day and 650 mg/day, respectively, it is unlikely that levels will be above the UL unless premixing problems arise. Thus, for specifications, the maximum vitamin C content of 200 mg/1,000 kcal is suggested (specified content + 100 percent).

Vitamin C is the most labile of the water-soluble vitamins, and is easily oxidized in the presence of moisture, heat, and light. It is anticipated that significant losses of vitamin C may occur during storage, but the use of an ethylcellulose-encapsulated vitamin C should provide for minimum storage losses (IOM, 1997b). With the overage recommended, adequate vitamin C will be present for the recipient.

Thiamin

Thiamin is centrally involved in carbohydrate metabolism, nucleic acid and fatty acid synthesis, and membrane and nerve conduction. Anorexia, tiredness, and weight loss are early symptoms of thiamin deficiency; more severe thiamin deficiency leads to cardiovascular and neurological symptoms, including mental changes (Brown, 1990). In adults, beriberi (severe thiamin deficiency) is

characterized by varying degrees of peripheral neuropathy and cardiovascular involvement while sustained deficiency leads to death. In young infants (2 to 3 months of age), beriberi is characterized by cardiac symptoms, cyanosis, vomiting, and dyspnea; death can occur within hours of the onset of symptoms (Tanphaichitr, 1994). In young, breast-fed infants, beriberi is due to the thiamin deficiency of the mother. Because ethanol is a thiamin antagonist, chronic and heavy alcohol consumption can lead to thiamin deficiency, manifested as Wernicke-Korsakoff syndrome (Zubaran et al., 1997). The biological half-life of thiamin is approximately 9 to 18 days (Ariaey-Nejad et al., 1970). Therefore, consumption of thiamin-poor diets rapidly leads to poor thiamin status. Thiamin deficiency (unspecified) in individuals with poor initial thiamin status has been noted in refugee situations within 2 weeks (Golden, 2001).

Losses of thiamin during cooking may be considerable due to high temperatures and discarding of cooking water (Kimura et al., 1990). Additionally, thiamin is destroyed by sulfite and chlorite, such as sodium hypochlorite, a disinfectant commonly added to water in refugee camps (Dwivedi and Arnold, 1973; Stammati et al., 1992)

Parasitic infections have been shown to be associated with poorer thiamin status in a sample of young Egyptian men (Hussein et al., 1989). Additionally, thiamin deficiency has been reported among children with severe gastroenteritis (Truswell et al., 1972).

Based on the RDA for the limiting group of children ages 1 to 3 years (0.6 mg/day [IOM, 1998]), the minimal nutrient density necessary to meet recommended intakes is 0.6 mg/1,000 kcal (Table 2-4). The thiamin content of the EFP should be the amount that conservatively meets nutritional requirements under adverse conditions. Due to possible gastrointestinal problems in the target population, and potential destruction of the vitamin due to long-term storage and temperature, the recommended thiamin content is doubled to 1.2 mg/1,000 kcal (0.28 mg/EFP bar). No UL has been set for this nutrient as there are no data on adverse effects from food or supplement intake (IOM, 1998). The maximum content is 1.4 mg/1,000 kcal (specified content + 20 percent).

Riboflavin

Riboflavin plays a central role in energy metabolism because of its role as the precursor for the coenzymes flavin mononucleotide (FMN) and flavin–adenine dinucleotide (McCormick, 1990, 1994). Both coenzymes function as catalysts for redox reactions, and are involved in numerous metabolic pathways. Riboflavin coenzymes are necessary for the functioning of the electron transport chain. Symptoms of riboflavin deficiency can include painful lesions of the lips and mouth, peripheral nerve dysfunction, and inflammation of the tongue. In general, deficiencies of riboflavin are associated with deficiencies of other nutrients (IOM, 1998).

Significant rates of riboflavin deficiency have been documented in a wide range of populations, including The Gambia (Reddy et al., 1987), northeast Thailand (Pongpaew et al., 1995), Malaysia (Shahar et al., 1999), Guatemala (King et al., 1997), and Zimbabwe (Wacker et al., 2000). In an emergency situation, prior consumption of animal products and green vegetables (major sources of riboflavin) may be limited or absent; therefore riboflavin deficiency can be assumed to be present, particularly if the crisis has been long.

In general, the bioavailability of riboflavin is quite high, although both riboflavin and FMN can form complexes with a variety of substances, including ascorbic acid, tryptophan, zinc, copper, and iron (Jusko and Levy, 1975). Riboflavin is heat stable, and therefore cooking losses are generally minimal, but the vitamin is susceptible to destruction via oxidation and exposure to light.

Diarrhea and other factors that decrease transit time can cause poor absorption (McCormick, 1994). Enhanced losses of riboflavin can occur with catabolic nitrogen losses, and protein–energy malnutrition can be associated with reduced absorption and utilization of riboflavin (McCormick, 1994). Systemic infection, even without gastrointestinal involvement, can increase the riboflavin requirement (McCormick, 1994).

Since riboflavin plays a central role in energy metabolism, the requirement should theoretically be related to energy intake and expenditure. Belko and coworkers (1983, 1984, 1985) examined the effects of dieting and moderate exercise (2.5 to 5 hr/wk) on the riboflavin status of overweight women and found that both activities increased the riboflavin requirement. In The Gambia, the seasonality of riboflavin intake may be associated with changes in energy intake and balance (Bates et al., 1994). Riboflavin requirements may also be increased by a high carbohydrate:fat ratio (Boisvert et al., 1993). Diets of this type are common in developing countries, and are frequently found in refugee situations.

The baseline minimal nutrient density value calculated for riboflavin and based on the RDA (1.3 mg/day [IOM, 1998]) was for boys 14 through 18 years of age (Table 2-4). The value is 0.6 mg/1,000 kcal. Under emergency conditions, riboflavin status may often be compromised by weight loss, heavy exercise, diarrhea, and multiple nutritional deficiencies. Therefore, the baseline value is likely to be too low for many crises. In support of this hypothesis, Bates and coworkers (1989) reported that riboflavin intakes of 1.8 to 2.5 mg/day were required to return a group of Gambian subjects to an acceptable mean erythrocyte glutathione reductase-activity concentration (EGRAC) of 1.3 to 1.4. Furthermore, Belko and coworkers (1985), studying a group of overweight women on low-calorie diets (1,200 to 1,250 kcal/day), found that riboflavin intakes of 1.0 mg/1,000 kcal were associated with elevated EGRAC levels, while a diet containing 1.2 mg/1,000 kcal provided statistically significant improvements in EGRAC values.

The importance of riboflavin in energy metabolism and its potential destruction by heat and light, and the apparent lack of adverse effects of chronic

consumption at higher than recommended levels (no UL has been established), suggest that the content of the EFP can be safely doubled to 1.2 mg/1,000 kcal (0.28 mg/EFP bar). This level should be enough to cover any additional requirements due to physical activity and/or diarrhea, and is comparable to the riboflavin content of Unimix (1.1 mg/kcal) and Corn-Soy Blend (1.3 mg/1,000 kcal), although lower than F100 (2.0 mg/1,000 kcal) (Golden 2001). The maximum content is 1.4 mg/1,000 kcal (specified content + 20 percent).

Niacin

Niacin, through its coenzymes nicotine adenine dinucleotide and nicotine adenine dinucleotide phosphate, plays a central role in energy metabolism, fatty acid and steroid synthesis, DNA repair, and calcium mobilization (Swendseid and Jacob, 1994). Severe niacin deficiency gives rise to the classic deficiency syndrome, pellagra, which is characterized by dermatitis, diarrhea, dementia, and death. Neurological symptoms include apathy, depression, and memory loss. Changes in the digestive track can lead to vomiting, diarrhea, and constipation. Early signs of mild deficiency can include ill-defined gastrointestinal problems, weakness, and lassitude (IOM, 1998). Although the prevalence of mild and marginal deficiencies has not been well documented, rates are likely to be relatively high in some maize- and sorghum-consuming populations (in which niacin deficiency is typically found due to low niacin content along with low levels of tryptophan), particularly during the "hungry season" (the weeks or months when the produce from the previous harvest is fully consumed and the next harvest is not yet ready). The body can convert the amino acid tryptophan to niacin with about 60 mg of tryptophan being needed to produce 1 mg of niacin, although this may vary by as much as 30 percent (IOM, 1998).

Niacin can be obtained either by consumption of preformed niacin or by conversion of tryptophan to niacin. Niacin bioavailability varies according to the form of niacin and the food matrix. In developing countries, many people obtain most of their niacin from grains, legumes, and green leafy vegetables, and by synthesis of niacin from tryptophan. In mature maize, and to a lesser degree in wheat and other cereals, niacin is bound to complex carbohydrates and small peptides and is biologically unavailable (WHO, 2000). Only about 30 percent of niacin in maize is bioavailable; however, heat treatment of maize under alkaline conditions, as is traditionally done in Mexico, greatly increases niacin bioavailability (Carpenter and Lewin, 1985).

Pellagra has often been observed in refugee populations. In 1989 to 1990, an outbreak of pellagra occurred among Mozambican refugees living in Malawi (Malfait et al., 1993). During an 8-month period, nearly 18,000 of 286,000 refugees (incidence = 6.3 percent) were affected (CDC, 1991). Incidence of pellagra

TABLE 2-9 Recommended Dietary Allowances (RDAs) and Tolerable Upper Intake Levels (ULs) for Niacin

Age (yr)	RDA (mg of NE[a]/d)	UL[b] (mg/d)	Niacin Intake from the EFP Bar (mg NE/d)
1–3	6	10	10
4–8	8	15	16
9–13	12	20	19
14–18, boys	16	30	24
14–18, girls	14	30	22
Adults, men	16	35	26
Adults, women	14	35	26

[a] NE = niacin equivalents.
[b] As nicotinic acid/niacinamide added to foods or in supplements only (IOM, 1998).
[c] Based on a maximum content of 2.9 mg NE/EFP bar (or 12.4 mg/1,000 kcal).
SOURCE: IOM (1998).

was nearly eight times higher for women than men, but children under 5 years of age were relatively unlikely to be affected. This epidemic was precipitated by a disruption in local groundnut supply, a source of niacin for the population. More recently, an outbreak of pellagra in Angola affected both refugee and local populations (Baquet et al., 2000). Most cases occurred in women (83 percent), with relatively few children under 15 years of age afflicted (18 percent of cases). Other pellagra outbreaks were documented during the 1980s and 1990s in Nepal, Zimbabwe, Angola, Malawi, and Mozambique (WHO, 2000).

Because of the central role of niacin in energy metabolism, niacin requirements bear a theoretical relationship to energy. However, no research has examined the influence of energy expenditure or intake on niacin requirements (IOM, 1998). Additionally, inadequate iron, riboflavin, or vitamin B_6 status reduces the efficiency of the conversion of tryptophan to niacin, although the magnitude of these effects has not been established (IOM, 1998).

The limiting subgroup for niacin is boys 14 to 18 years of age (Table 2-4), with a minimal nutrient density needed of 7.5 mg of niacin equivalents (NE)/1,000 kcal. The UL for niacin refers only to nicotinic acid or nicotinamide added to foods or taken as supplements. Thus concern about adverse effects would only arise with the amount of nicotinic acid or nicotinamide added to the EFP.

Because niacin deficiency is likely to be highly prevalent in many refugee populations, the minimal nutrient density is increased by 50 percent to 11.2 mg NE/1,000 kcal (2.6 mg NE/EFP bar). Table 2-9 provides the estimated amount of niacin intakes from the EFP based on the energy intakes estimated in Table 2-3. Given that not all the NE included in the EFP will be as an added ingredient, it is probable that the UL for nicotinic acid/niacinamide will not be exceeded at

this level of total niacin intake. However, it is important that the level contained in the EFP be carefully monitored and not be exceeded by more than 10 percent. The maximum content is 12.4 mg/1,000 kcal (specified content + 10 percent).

Golden (2001) has recommended 18 mg/1,000 kcal of niacin. The niacin content of other emergency products ranges from 10 mg/1,000 kcal (F100) to 27 mg/1,000 kcal (Oxford SK8 biscuit). The recommended level for the EFP is within the range of these other recommendations.

Vitamin B_6

Vitamin B_6, a group of six compounds of which the pyridoxine forms are the most prevalent in plant-based foods, is important in a wide variety of metabolic processes, including normal protein metabolism and glucose production (IOM, 1998). Adequate vitamin B_6 status is also required for optimal conversion of tryptophan to niacin. Hemoglobin synthesis is dependent on adequate vitamin B_6 status, and severe deficiency of vitamin B_6 can lead to hypochromic, microcytic anemia. Poor vitamin B_6 status is associated with compromised cell-mediated immune function. In cases of severe deficiency, infants can suffer convulsions, while symptoms in adults include depression, confusion, irritability, stomatitis, and cheilosis (IOM, 1998).

Little research has been conducted to examine the prevalence of vitamin B_6 deficiency in developing countries. In periurban Egypt, 38 percent of 70 women had low vitamin B_6 levels in breast milk, and low values were associated with poorer mother–infant interaction (McCullough et al., 1990). In Indonesia, approximately 40 percent of rural third-graders had plasma pyridoxal phosphate values (the most widely used vitamin B_6 status index) indicative of deficiency (Setiawan et al., 2000). Vitamin B_6 deficiency was observed in 26 percent of young female Chinese textile workers (Ronnenberg et al., 2000). In Europe, the SENECA study found that more than 50 percent of the elderly in some geographical areas had vitamin B_6 deficiency (Haller et al., 1991). The results of these studies suggests that pre-existing deficiencies of vitamin B_6 can be assumed to be present in refugee populations.

Vitamin B_6 is available from a wide range of plant and animal foods. The forms of vitamin B_6 in eggs, fish, and poultry are highly bioavailable. Plant pyridoxines are less bioavailable and may decrease absorption of the more bioavailable forms of the vitamin (Gregory, 1998). In developing countries, the principal sources of vitamin B_6 are likely to be starchy staples and legumes. Food processing and storage adversely influence the vitamin B_6 content of some foods (Leklem, 1996). Vitamin B_6 in food is unstable under neutral or alkaline conditions.

Since vitamin B_6 is absorbed by a nonsaturable, passive process that occurs primarily in the jejunum, the presence of parasites or diarrhea may have little effect on vitamin B_6 absorption, although it has not been well explored. In order

to support the role of vitamin B_6 in amino acid metabolism, some investigators have proposed an increased requirement for vitamin B_6 coincident with increasing protein intake. Several studies have documented a relationship between increased protein intake and decreased vitamin B_6 status. However, the precise mathematical relationship remains unclear (IOM, 1998). Several studies have examined the influence of physical activity on vitamin B_6 status and metabolism, and have shown little or no relationship (Manore, 2000), although exercise is theoretically linked to increased vitamin B_6 requirements.

Based on the RDA of 1.5 mg/day for the limiting subgroup of women 51 years of age and older (IOM, 1998), a minimal nutrient density value was calculated (see Table 2-4). Under the assumptions of the method, the value required to prevent inadequate intake in almost all individuals in this life stage and gender group would be 0.8 mg of vitamin B_6/1,000 kcal. Given concern about losses in food processing and storage, the EFP should contain 50 percent more, or 1.2 mg/1,000 kcal (0.28 mg/EFP bar).

Large doses of oral vitamin B_6 have been associated with a range of negative outcomes. The UL is 30 g/day as pyridoxine for children ages 1 through 3 years (IOM, 1998), much higher than the proposed level for the EFP. Therefore, adverse effects related to vitamin B_6 should not be a problem. The maximum content is 1.4 mg/1,000 kcal (specified content + 20 percent).

Folate

Folate is a collective term for a family of compounds that are structurally and functionally related to pteroylmonoglutamic acid. Folate is involved physiologically in DNA synthesis, purine synthesis, and amino acid interconversions, including the synthesis of methionine from homocysteine (IOM, 1998). Folate deficiency leads to megaloblastic anemia, elevated homocysteine, increased risk of neural tube defects, and possibly increased risk of other congenital disorders, cancer, and vascular disease (IOM, 1998).

Major sources of folate include green vegetables and legumes. The absorption of food folate requires the conversion of polyglutamyl folates to monoglutamyl forms, which are then absorbed at physiological levels by a saturable transport process (Gregory, 2001). The bioavailability of food folate varies widely by food and may be influenced by processing of the food matrix (Castenmiller et al., 2000; Gregory, 2001). Folate absorption can be adversely effected by unidentified factors in food and by alcohol consumption. Overall, the bioavailability of food folate is estimated at about 50 percent (IOM, 1998). In contrast, synthetic folate in fortified foods is highly bioavailable (~85 percent [IOM, 1998]).

Rates of folate deficiency in developing countries are largely unknown and may be less common than deficiencies of many other micronutrients because of the relatively low cost of legumes and greens, which are major dietary sources.

TABLE 2-10 Recommended Dietary Allowances (RDAs) and Tolerable Upper Intake Levels (ULs) for Folate

Age (yr)	RDA (μg DFE[a]/d)	UL[b] (μg synthetic folate/d)	Synthetic Folate Intake[c] (μg/d)
1–3	150	300	265
4–8	200	400	452
9–13	300	600	525
14–18, boys	400	800	662
14–18, girls	400	800	599
Adults, men	400	1,000	725
Adults, women	400	1,000	611

[a] As dietary folate equivalents.
[b] As folate added to foods or in supplements only (IOM, 1998).
[c] Based on a maximum of 80 μg DFE/emergency relief food product bar (or 340 μg/1,000 kcal).
SOURCE: IOM (1998).

However, seasonality, local dietary traditions, or other health conditions may lead to observable rates of deficiency in some populations. In western Venezuela, 91 percent of individuals in a single Bari Indian community were assessed as folate deficient, whereas very little (5 percent) deficiency was observed in a second community (Diez-Ewald et al., 1997). In Malawi, 21 to 34 percent of anemic pregnant women were folate deficient (van den Broek and Letsky, 2000).

Vitamin B_{12} deficiency leads to functional folate deficiency because vitamin B_{12} acts as a cofactor in recycling folate (IOM, 1998). Early research also suggested an adverse effect of zinc deficiency on folate absorption. However, subsequent work has failed to replicate this result (Gregory, 2001).

Using the method previously outlined, the minimal nutrient density value for folate is based on the RDA of 400 μg/day (IOM, 1998) for girls 14 through 18 years of age (Table 2-4). The minimal nutrient density value is 207 μg of dietary folate equivalents (DFE)/1,000 kcal (IOM, 1998). To cover the potential for additional reduced absorption due to gastrointestinal problems, the baseline value is increased by 50 percent and the recommended level of folate for the EFP is 310 μg DFE/1,000 kcal (72 μg DFE/EFP bar).

The UL for folate is 1,000 μg/day for adults, and lower values for children are adjusted on a metabolic weight basis (IOM, 1998) (Table 2-10). The UL for folate is for folate added to foods or taken as supplements only. The UL for children ages 4 through 8 years (IOM, 1998) is 400 μg/day, which is less than the proposed level for the EFP. The UL is based on reports of adverse effects of high levels of folate masking the irreversible neurological damage seen in cases of vitamin B_{12} deficiency. Since some of the folate in the total amount per food

bar may be contributed by food sources, it is assumed that this level will not be exceeded. However, given a concern for inadequate mixing, the maximum content is 340 μg DFE/1,000 kcal (specified content + 10 percent). If synthetic folate is used, these values should be divided by 1.6.

Vitamin B_{12}

Vitamin B_{12} is required for methyl transfer to folate; for conversion of homocysteine to methionine; and for synthesis of succinyl CoA, the Krebs cycle intermediate, from L-methylmalonyl CoA. Vitamin B_{12} deficiency can lead to megaloblastic anemia and neuropathy. Neuropsychiatric symptoms can include irritability, fatigue, apathy, and emotional instability (IOM, 1998). Cognitive and neuropsychiatric complications can precede anemia by a considerable period of time. As many as 90 percent of individuals with clinically observable vitamin B_{12} deficiency present neurological complications (IOM, 1998).

Nearly all naturally occurring vitamin B_{12} must be obtained by consumption of animal products, although vitamin B_{12} may be present in small amounts in some plant products via contamination by microorganisms (IOM, 1998). In much of the developing world, animal products are not routinely consumed due to poverty. Some individuals do not consume meat for religious and cultural reasons.

Vitamin B_{12} is efficiently stored in the liver, and losses are minimized in the healthy individual through enterohepatic recirculation. However, because vitamin B_{12} is secreted in the bile as a part of normal digestion, individuals can become deficient due to poor resorption (and absorption) of the vitamin (Stopeck, 2000). *Helicobacter pylori* infection of the gastrointestinal tract may be an important cause of adult vitamin B_{12} deficiency, and treatment of atopic gastritis with antibiotics can be an effective means of reversing B_{12} malabsorption (Kaptan et al., 2000; Suter et al., 1991). In the United States, an estimated 10 to 15 percent of persons aged 60 or older suffer from vitamin B_{12} deficiency, mostly due to poor absorption (Baik and Russell, 1999).

When initial vitamin B_{12} stores are abundant, deficiency due to malabsorption or a vegetarian diet can take years to manifest. However, when low stores are combined with low intake or malabsorption, deficiency occurs more rapidly. In rural Mexico, where consumption of animal products is limited, increased incidence of low levels of vitamin B_{12} in human milk and plasma and decreased holotranscobalamin II have been noted (Allen et al., 1995; Black et al., 1994). High rates of deficiency among children were attributed to maternal malnutrition. In an urban Mexican population, 12 percent of nonpregnant, nonlactating women had low plasma B_{12} values (Casanueva et al., 2000). In Guatemala, 47 percent of lactating women had low plasma B_{12} values, 31 percent of breast milk values were low, and 32 percent of mothers had low holotranscobalamin II

values (Casterline et al., 1997). In Malawi, 16 percent of anemic pregnant women were vitamin B_{12} deficient (van den Broek and Letsky, 2000). In Kenya, the vitamin B_{12} content of human milk was very low (Neumann and Harrison, 1994). Epidemiological research in Zimbabwe also suggests that vitamin B_{12} deficiency may be a public health problem in that country (Savage et al., 1994).

The significance of adequate vitamin B_{12} stores is indicated by research on Dutch children who were raised on a macrobiotic diet during the first 6 years of life (Louwman et al., 2000; van Dusseldorp et al., 1999). Subsequently, these children consumed lacto-ovovegetarian or omnivorous diets. However, when assessed during adolescence, vitamin B_{12} status remained low and cognitive function was shown to be impaired. The authors speculated that poor vitamin B_{12} status was the combined effect of low stores from the macrobiotic period and somewhat low subsequent intakes (van Dusseldorp et al., 1999). However, other nutrients were also deficient during the macrobiotic dietary period.

In summary, limited consumption of animal products as a result of poverty concomitant with high rates of diarrhea and gastrointestinal infection due to parasitic and other enteric diseases will likely predispose populations in developing countries to vitamin B_{12} deficiency, and certainly to a lack of stores of the vitamin.

Little information is available on consumption of high levels of vitamin B_{12} from either food or supplements and associated adverse effects; therefore data were inadequate to establish a UL for this vitamin (IOM, 1998).

The minimal nutrient density value for vitamin B_{12} is based on the RDA for girls 14 to 18 years of age (2.4 µg/day [IOM, 1998]), and is 1.2 µg/1,000 kcal (see Table 2-4). However, given the concerns about lack of stores and the probability of a high level of vitamin B_{12} deficiency, higher levels are indicated to ensure that an adequate amount of the nutrient is absorbed and that stores are replenished to the extent possible. Vitamin B_{12} is very stable in foods. Therefore, it is recommended that the baseline value be increased by a factor of 10 to 12 µg/1,000 kcal (2.8 µg/EFP bar). The maximum content is 14.4 µg/1,000 kcal (specified content + 20 percent).

Pantothenic Acid

Pantothenic acid is required for the synthesis of coenzyme A (CoA), which functions in a broad range of enzymatic processes, many of which involve lipid metabolism (IOM, 1998). CoA is ubiquitously distributed in cells, is required by most forms of life, and is hydrolyzed in the gut to pantothenic acid. Therefore, pantothenic acid can be obtained from a wide range of foods, and pantothenic acid deficiency is thought to be unusual.

Epidemics of deficiency, however, have occurred when food choice was severely restricted. During World War II, prisoners of war in Asia suffered symptoms that were attributed to pantothenic acid deficiency (Plesofsky-Vig, 1999).

More recently, Afghan refugees who were provided white wheat flour without other supplemental food suffered similar symptoms (Golden, 2001). Deficiency can lead to headache, irritability, fatigue, insomnia, nausea and vomiting, hypoglycemia, and paresthesia of the extremities (IOM, 1998).

Absorption of pantothenic acid occurs by active transport at low concentrations, is saturable at higher concentrations, and is passive (Fenstermacher and Rose, 1986). The effects of diarrhea on absorption of pantothenic acid are unknown.

The minimal nutrient density value for pantothenic acid is based on the AI for girls 14 to 18 years of age (5 mg/day [IOM, 1998]) and is 2.6 mg/1,000 kcal (see Table 2-4). The importance of pantothenic acid in energy metabolism, the potential for decreased absorption due to gastrointestinal symptoms or disease, and the lack of reported adverse effects of chronic consumption at higher than recommended levels (no UL has been established) suggest that the content of the EFP can safely be increased by 50 percent to 3.9 mg/1,000 kcal (0.9 mg/EFP bar). The maximum content is 4.7 mg/1,000 kcal (specified content + 20 percent).

Biotin

Biotin is a cofactor for four adenosine triphosphate-dependent carboxylases (IOM, 1998). This nutrient is necessary for normal cell growth, glucose homeostasis, and DNA synthesis. Biotin deficiency has been shown to be teratogenic in a variety of mammalian species (Mock et al., 1997).

Severe biotin deficiency is rare in the industrialized countries; it occurs due to unusual conditions such as metabolic abnormalities, heavy and sustained intake of avidin from raw egg white, and total parenteral nutrition without biotin supplementation (IOM, 1998). In developing countries, severe protein–energy malnutrition may be accompanied by poor biotin status and impaired carboxylase activity (Velázquez, 1997; Velázquez et al., 1995). Marginal biotin deficiency may be fairly common; a substantial proportion of pregnant women in Iowa exhibited evidence of biotin depletion as pregnancy progressed (Mock et al., 1997). The prevalence of biotin deficiency in both industrialized and developing countries is unknown. A study in rat models has documented an adverse effect of biotin deficiency on *n*-6 PUFA metabolism (Mock, 1990).

Biotin is present in a range of animal and plant foods, but for most foods the precise biotin content and its bioavailability are poorly understood (Said, 1999). Free biotin is nearly 100 percent bioavailable (Zempleni and Mock, 1999). However, much of the biotin in foods is protein-bound and bioavailability is not known (IOM, 1998).

The minimal nutrient density value for biotin is based on the estimated needs of women 51 years of age and older. Based on an AI of 30 μg/day for this group, the baseline value would be 16 μg/1,000 kcal (IOM, 1998). To cover the

potential for reduced absorption due to gastrointestinal problems, the minimal nutrient density value is increased by 50 percent to the recommended level for the EFP of 24 µg/1,000 kcal (5.6 µg/EFP bar). The maximum content is 28.8 µg/1,000 kcal (specified content + 20 percent). No UL has been established for biotin; concern about excess intake is unwarranted in this situation.

Choline

Choline is involved in the synthesis and release of acetylcholine (a neurotransmitter), and is a precursor of phospholipids and sphingomyelin (important constituents in cell membranes), and also synthesis of the methyl donor, betaine (IOM, 1998). The human body has a limited capacity for de novo choline synthesis and rates of synthesis may not be sufficient to meet the needs of at least some individuals (Zeisel, 2000).

Large amounts of choline are transferred from mother to fetus during pregnancy, and considerable amounts are delivered later to the child via human milk (IOM, 1998). Therefore, adequate maternal intakes of choline are needed to protect the mother against deficiency and to provide the infant with the choline required for normal development. Animal models have demonstrated the importance of adequate choline intake for normal brain development (Blusztajn, 1998). In the rat, choline deficiency adversely influences brain development at two times during growth: during late gestation (12 to 17 days) and postpartum (6 to 30 days) (Jones et al., 1999; Zeisel, 2000). Prenatal effects appear to be permanent (Blusztajn, 1998).

The choline content of foods is poorly characterized, and the bioavailability of many choline-containing compounds in foods is unknown (Zeisel, 2000). However, eggs contain significant amounts of choline, as do liver and peanuts (IOM, 1998).

Free choline is absorbed from the small intestine (Le Kim and Betzing, 1976). The influence of diarrhea, bacterial overgrowth, and parasitic infection on choline absorption is unknown, but it can be assumed to be adverse. No research has been done on the prevalence of choline deficiency in either industrialized or developing countries.

The minimal nutrient density value for choline is based on the AI for the subgroup of men over 50 years of age (550 mg/day [IOM, 1998]), and is 244 mg/1,000 kcal of choline (see Table 2-4). As with other water-soluble vitamins, to cover the potential for reduced absorption due to gastrointestinal problems, the minimal nutrient density value is increased by 50 percent. Therefore, the minimum content is 366 mg/1,000 kcal (85 mg/EFP bar). The maximum content is 439 mg/1,000 kcal (specified content + 20 percent).

Conclusion

The recommendations for the nutrient content and energy sources contained in this chapter meet the goal of the report: to develop a high-energy, nutrient-dense food product that would be nutritionally adequate for all people 7 months of age and older. The recommendations are designed to provide all known nutrients in quantities to satisfy the needs of the most vulnerable life stage and gender group. In addition, levels of nutrients were frequently increased above the minimal nutrient densities to compensate for poor bioavailability, processing and storage losses, and reduced absorption due to mild diarrhea, infections, or parasites. However, as was described in the beginning of this chapter, the nutritional content is not the highest priority in the design of the ration—in terms of importance, it comes after safety, palatability, ease of delivery, and ease of use.

An additional characteristic that may also be critical is cost. Since this product is intended to be an emergency stop-gap to be used no longer than 15 days while a more permanent food supply line is put in place, if cost is a consideration, it is recommended that food ingredients be analyzed for nutrient content and supplemented only as necessary. It should be assumed that the recommended amounts and sources are considered optimal, but other factors take precedence in the final formulation.

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3

Processing and Packaging of the Emergency Food Product

Developing energy-dense nutritional foods that can be packaged and stored for extended periods of time in environments that vary from arctic to tropical presents a challenge to the processor. In an emergency situation these products must also meet the nutritional needs of all age groups from infants to adults, and be sufficiently palatable to be consumed for up to two weeks as the sole food. Nutrient profiles for an emergency food product (EFP) can and have been developed (see Chapter 2), but the required useful life of the product will be met only through careful consideration and selection of ingredients, processing techniques, and packaging materials. Key considerations include microbiological and chemical safety, dispersability, and ease of use.

DESIRABLE CHARACTERISTICS OF AN EMERGENCY RELIEF FOOD PRODUCT

The use of a few nutrient-dense products in a variety of emergencies by relief organizations such as the United Nations High Commissioner for Refugees, the World Food Programme of the United Nations, and the International Committee of the Red Cross and Red Crescent, has resulted in anecdotal information about the desirable characteristics of such foods. These characteristics should be taken into consideration during prototype development in order to develop a superior EFP. Historically, some of the most important emergency relief food products available in Europe, particularly the most successful one—the Norwegian BP-5—were not developed with food relief in mind. They were intended to

be rations stowed in lifeboats for use in the event of passengers and crews having to abandon ship. Nevertheless, their use in the field during diverse emergencies, such as the Ethiopia and Eastern Sudan famine of 1985 to 1986 and the more recent Balkans conflicts, have permitted an evaluation of their efficacy from the standpoint of nutrition, acceptability, ease of delivery, and some practical aspects such as potential for diversion seldom discussed in refereed publications. The following sections provide some aspects that representatives from various relief organizations urged be considered in developing specifications for the EFP.

Packaging the EFP for Airdrop or Surface Delivery

Considering that the EFP is for use at the onset of emergencies, when infrastructure destruction and security considerations make it impossible to run feeding centers, the EFP should be available in a packaging modality amenable to low-altitude airdrop as well as delivery on land. There have been attempts to configure EFPs in ways that facilitate air delivery without damaging the product upon impact on the ground or hurting the intended recipients. Such packaging must also allow for dissemination of the product over a wide area so that it may reach many people. (Past experience indicates that concentrating the drop in the form of parachuted pallets, for example, contributed to hoarding, thus defeating the primary objective of ample distribution of the food relief, and also contributed to its diversion to unintended uses).

Packaging the EFP to Discourage Diversion

Information provided by relief organizations indicate that the high energy content of some EFPs, the density of nutrients in them, and the ease with which they may be carried has resulted in these products being collected by military combatants in emergency situations involving armed conflict. Biscuit-type EFPs are easily diverted to become military rations in emergencies involving armed conflict to the detriment of and even at a risk to the intended civilian recipients. The diversion is facilitated when the shape and size of the unit makes it easy to fit into the side pockets of military wear; rectangular, thin presentations seem to be best suited for this purpose. In addition, the use of eye-catching, glittery, space-age packaging materials encourages such diversion. It has been, therefore, the consensus among representatives of several relief agencies that the shape and size of the outside package of a successful EFP should be uncomfortable to carry in military pockets and should be made of nonlustrous materials. Furthermore, separation of the ration into smaller portions that cannot easily be rewrapped after opening also discourages diversion while aiding in apportioning the ration among children and adults.

Packaging to Facilitate Distribution and Consumption of the EFP and Reuse of its Secondary Package

Based on information from relief organizations, other anecdotal considerations for a superior EFP are the size of the unit and the potential for reuse of the secondary package. It is important that the size of the total unit and its breakdown into meal portions are designed so that adults can apportion it to individual sittings. Meal-size portions should be scored to facilitate partitioning them for children.

It is also important that the primary and secondary packages be able to serve additional uses in emergency situations. For example, a combustible primary package for emergency rations has found use in various emergencies as fuel for cooking. The secondary package may also be put to good use by recipients. For example, tin cans used to package emergency rations have been used as containers for water, as storage boxes, and even as metal shingles for building roofs after being pounded flat. In addition, from the technical standpoint, this type of secondary packaging might be very helpful in maintaining the integrity of the EFP against impact and pressure damage, insect and rodent attack, and other environmental challenges during transport, storage, and delivery. Therefore, the secondary package for the EFP should be designed such that it could afford secondary uses to the recipients.

Characteristics of Similar Ration Products

Conventional and novel technologies were considered for manufacturing the EFP. Combining some of these technologies may be the best approach to optimize the stability of the product and preserve its nutritional and sensory qualities. Dehydration, infusion, compression, and cold extrusion are some examples of processing technologies to be considered. These processes have been tested by the U.S. Army to obtain calorie-dense rations (Briggs et al., 1986; Schulz et al., 1992). A caloric density of 1.1 kcal/cc can be obtained using dehydration and compression. Higher caloric densities (up to 5 to 6 kcal/cc) are also possible using extrusion. The U.S. Air Force General Purpose (GP) Survival Packet ration for aircraft and life rafts, in turn, includes a variety of compressed bars such as a shortbread bar, a chocolate chip bar, a granola bar, and a corn flake cereal bar. The GP is designed to be consumed for periods of less than 5 consecutive days and contains approximately 100 g of carbohydrate and a low protein level (< 8 percent of calories) to counteract the effects of starvation and to conserve body water. This ration provides 1,447 kcal with 18 g of protein (5 percent of calories), 202 g of carbohydrate (56 percent of calories), and 64 g of fat (39 percent of calories). Its storage requirement is 5 years at 80° F and 1 month at 140° F (SBCOMM, 2001a).

The Meal Ready-to-Eat, Individual (MRE) is the standard military ration developed to support the individual soldier in all the U.S. Armed Forces (Army, Air Force, Navy, and Marine Corps). The MRE replaced the C Ration in the early 1980s and has since been continuously updated. It is designed to serve as the sole source of food for up to 10 days in a field environment, until group rations are available. Its use has in many situations been for longer—up to 145 days were reported during the Gulf War in 1993. Feedback from Operations Desert Shield and Desert Storm suggested that soldiers would consume more if their preferences were taken into account (IOM, 1993).

Improvements have focused on revising items to make the rations more acceptable and to expand variety (SBCOMM, 2001b). For example, the MRE bread is a pouch bread (Natick Research, Development, and Engineering Center, 1993) that contains glycerol, sucrose esters, lipids, and sorbic acid to extend shelf life up to 3 years, and has received high hedonic ratings (Hallberg and Chinachoti, 1992). This is now in every MRE ration. The average equilibrium pH and water activity of this bread are 5.0 and 0.86, respectively. The bread is further preserved by controlling oxygen content and initial microbial load (Hallberg et al., 1990; Powers and Berkowitz, 1990).

In an investigation to develop a high-energy biscuit for use as an EFP in disaster relief, low-moisture (3.5 percent) biscuits were prepared using a traditional baking method, with formulation and processing strategies as the means to control caloric density and sensory quality (Young et al., 1985). The products were highly acceptable to sensory panels made up of children both in England and India. This shows that traditional processing methods—perhaps in combination with some of the novel MRE technologies described above—can be used to produce baked EFPs such as biscuits having desirable sensory and nutritional qualities and long shelf life.

Role of Water Activity, Water Mobility, and Water Content in Packaged Food Products

Three aspects of water are important to consider in describing a food system: water activity (a_w), water mobility, and water content. Water activity is defined as the ratio of partial pressure of water in the product over that of pure water at the same temperature. The concept of a_w was first put forward in the early 1950s, as a means of explaining the availability of water for chemical and biological reactions. It has been a useful tool in the food industry for many years and it is particularly useful when dealing with intermediate and high moisture biological systems (Ruan and Chen, 1998; Taoukis et al., 1988). The rate-limiting step in a chemical reaction is frequently associated with the mobility of water and its ability to participate in those reactions. At low a_w, the binding of water (monolayer moisture) to components of the system makes it unavailable as a solvent. As a_w increases, water exists in multilayers and is more mobile.

Solvation and reactant mobility increase, so biological and chemical changes occur. This classic general relationship between moisture content, a_w, and reaction rate was characterized over 30 years ago (Labuza, 1971).

Water activity is used to predict the stability of food systems and quality changes likely to occur. However, in the past 10 years there have been numerous papers pointing out the limitations of the concept (Frank, 1991; Ruan and Chen, 1998; Slade and Levine, 1991). There are practical and theoretical concerns because a_w measurement assumes that the food system is at equilibrium, a condition where the partial vapor pressure above the food system is the same as that of the water within it (Ruan and Chen, 1998). Since most food systems are not in equilibrium, this frequently does not hold true.

Water mobility, as measured by nuclear magnetic resonance, is thought to be a more accurate way of determining the "availability" of water. Slade and Levine (1992) proposed the "polymer" approach to describe the role of water in food systems as a plasticizer that affects the glass transition temperature, which, in turn, could help explain the relationship between moisture and reaction rates (Nelson and Labuza, 1994).

In practice, the use of a_w as a means to predict product stability remains important, while the polymer science approach can be viewed as a more generalized theoretical explanation (Reid, 1995). Water activity is a better indicator of food product susceptibility to spoilage than is water content. Dried foods normally contain 2 to 20 percent moisture, corresponding to a_w in the range 0.20 to 0.60. In contrast, intermediate moisture foods (IMFs) normally contain 15 to 40 percent total moisture and have an a_w of 0.60 to 0.85 (Jayaraman, 1995; Karel, 1973; Sloan et al., 1976).

PROCESSING CONSIDERATIONS

Moisture control, mostly by dehydration, to lower the a_w of the product is considered critical to attaining the required shelf life of the EFP of 2 to 3 years. The basic principle underlying drying and IMF technologies is the premise that water—the universal solvent—can become a limiting factor for spoilage and pathogenic microbial growth in foods when it is adequately reduced to low enough levels (Bone, 1973; Davies and Birch, 1976; Erickson, 1982; Gould, 1985; Rahman and Labuza, 1999). This reduction in moisture content and a_w is sometimes accompanied by the use of other preservation factors such as chemical preservatives (e.g., antimicrobial agents, antioxidants, or antibrowning compounds), reduction of oxygen by vacuum and/or gas flushing techniques with maintenance through means of oxygen barrier packaging and/or oxygen-absorbent materials (oxygen scavengers), pH adjustment, and selection of packaging designs that protect the food from light, moisture, and environmental contamination. In the case of the EFP, moisture plays a critical role in determining microbial, sensory, chemical, and physical stability.

Extrusion

High-temperature, short-time extrusion cooking has been extensively applied in IMF and dried food production. Basic phenomena in extrusion cooking have been described by many (Harper, 1978, 1979, 1988; Linko et al., 1981; Rossen and Miller, 1973; Smith, 1982). In an extruder, the raw food material is subjected simultaneously to heat, pressure, and shear within a short time. Desirable product functional characteristics are typically controlled by altering the feed composition and extrusion process parameters. Water is always an integral part of physicochemical processes (e.g., gelatinization of starch and protein denaturation and plasticization) that determine the final textural characteristics of an extruded product.

Extrusion can be applied to produce foods having various moisture levels, from dry IMF products (e.g., puffed snacks and ready-to-eat breakfast cereals) to soft, moist ones. Production of modern IMFs can belong to either one of three categories: (1) moist infusion, in which solid food pieces are soaked and/or cooked in a solution having low a_w, (2) dry infusion, where initial dehydration is followed by soaking the food in a solution having low a_w, and (3) blending, in which the components are weighed, blended, cooked, and extruded (Erickson, 1982). Extruded IMF products are also considered thermally processed as high-temperature, short-time (HTST). This not only helps to further preserve the product from potential microbial growth and adverse enzymatic action, but also can help reduce the amount of preservatives that would be necessary otherwise. HTST processes are rapid by definition, so little destruction of vitamins or loss of protein quality are expected. According to Harper (1988), heat-stable B vitamins and pantothenic acid are stable under extrusion conditions. However, oxidation of ascorbic acid or carotenoids could occur, particularly in puffed products, so puffed processing is not recommended for the EFP.

It may be possible to hot extrude some combination of the ingredients, such as a protein and carbohydrate mixture, and then combine it with other ingredients (e.g., fat) in a compressed bar. Microencapsulation might be used for some nutrients and flavors that are mixed into a compressed bar formulation, given that most encapsulation materials are not intended for heat-processed foods. Spray coating of some ingredients after heat processing might also provide ways of incorporating heat labile ingredients during manufacturing of the EFP, as is done in breakfast cereals (Caldwell et al., 2000). Thus, the more stable vitamins might be included in the extrusion mix and others incorporated later (e.g., ascorbic acid and thiamin).

Maillard Browning Reaction

The Maillard reaction leads to brown color and to the appearance of new odors and flavors. The reaction involves reducing sugars and amino acids. It is a

series of reactions that start with the formation of Amadori compounds from aldose or hexose carbonyl compounds condensing with free amino groups of amino acids or protein. The condensing product is a Schiffs base that later becomes aldosylamine, and this, in turn, is converted into ketosamines in the Amadori rearrangement. The final step involves formation of melanoidins, which are brown nitrogenous polymers or copolymers. Due to the complexity of Maillard reactions and their dependence on multiple factors (e.g., pH, temperature, composition of the medium, and moisture), it is difficult to predict the extent of browning. Sugars with different degrees of reducing power greatly influence the reaction kinetics. Water also affects it in a variety of ways. For example, a concentration of solids increases the reaction rate because of a reactant concentration effect; further concentration of solids leads to a reduced rate as the reactant mobility is decreased. In highly concentrated systems, the Maillard reaction is inhibited or retarded until, at some point, caramelization is more likely to occur than Maillard.

Generally, the activation energy of the Maillard reaction increases with decreasing moisture content, suggesting that mobility retardation may be the rate-limiting factor (Labuza and Saltmarch, 1981). There is an a_w range where maximum Maillard reaction occurs that depends on: (a) the extent of the dilution effect at the high-moisture end, and (b) the limited mobility of reactants at the low-moisture end. For instance, the maximum a_w range in apple is 0.53 to 0.55, whereas in dried anchovy it is 0.93 (Labuza, 1980). Unfortunately, most of the data available on reaction kinetics of the Maillard reaction is limited to a_w values higher than 0.3 (Eichner and Karel, 1972; Warmbier et al., 1976). This suggests that if the EFP had an a_w below 0.3, it would have an extended shelf life. Additionally, not much information is available on very high moisture systems that are believed to have slower reaction rates. From an equilibrium consideration, a Maillard reaction is not favored at high moisture because the advanced reaction and the early formation of a Schiff base involve removal of water (Hodge and Osman, 1976).

One of the nutritional implications of this reaction is a possible decreased digestibility and the loss of reactive amino acids, such as lysine (Kaanane and Labuza, 1989; Labuza 1994; Saltmarch and Labuza, 1982). This has been related to the cross linking of proteins, as demonstrated in freeze-dried meat (Barnett and Kim, 1997). In related work using an MRE chicken-a-la-king stored for 3 years between 4° and 30° C, Barnett and Kim (1997) reported that textural and sensory deterioration occurred much before the observed decrease in nutritive value. The Q_{10} (i.e., the increase in the rate constant as temperature is increased by 10° C) in military MREs has been reported as 3 to 4, suggesting that under abusive storage conditions, a decrease in nutritive value in terms of reduced digestibility and loss of lysine can occur.

From the lysine-loss data, an estimation of the loss in nutritive value of the proteins in chicken meat heated at 73° C for 8 days in a high concentration of reducing sugar has been calculated to be about 13 percent (Barnett and Kim, 1997). If the above Q_{10} is assumed for the browning reaction, heating for 8 days

at 73° C would correspond to a storage for 22 years at an ambient temperature of 23° C, which exceeds the military shelf-life requirement of 3 years (ambient). Unfortunately, this information applies to chicken protein, but the EFP would contain only vegetable proteins. Therefore, the validity of this effect would need to be tested using EFP prototypes and conditions of storage and use simulating those expected during actual use of the EFP. Nevertheless, the key implication of this issue is that although the sensory quality may decrease and the nutritive value, to a lesser extent, may also be reduced because of the Maillard reaction, proper selection of ingredients for the EFP can help minimize sensory deterioration (e.g., appearance of brown color and firmer texture) and keep its nutritional quality from being adversely compromised.

Microencapsulation

Microencapsulation provides a physical barrier to oxygen, metal catalysts, and other pro-oxidants. This type of technology has been used in the food industry for many years, but a wide range of patented processes have been developed in recent years (Brazel, 1999; Risch and Reineccius, 1995). The protection of nutrients and other unstable additives is made possible by microencapsulation formulations that can allow controlled release of the nutrient during digestion as well as preserve it during storage (Deasy, 1984; Kondo, 1979). By using microencapsulation, flavor, color, and texture can be improved, thus making the product more acceptable.

The selection of shell material for microencapsulated nutrients will depend on the material being protected, processing needs, and storage stability concerns (Brazel, 1999). Capsule shell-wall materials are food additives by definition, and include polysaccharides (e.g., alginates, agarose), proteins (e.g., caseinates, zein), and fats. The water or oil solubility of the component to be protected will dictate the shell material composition (Brazel, 1999).

Diffusion of oxygen and catalysts in the aqueous matrix of a food is dependent on the amorphous or crystalline nature of the aqueous phase (Shimada et al., 1991), and it has been proposed that the *glassy-rubbery* transition temperature (T_g—the temperature at which a rigid, amorphous, glassy material becomes molten and rubbery) plays a key role in governing oxidation of lipids embedded in the matrix (Roos and Karel, 1991). The free volume theory implies that gas diffusion through intermolecular spaces in the barrier (i.e., the continuous matrix in a dried micro-emulsion containing the oil droplets) depends on the glassy or rubbery state. As a glassy amorphous material undergoes a glass transition it gains a greater intermolecular freedom that can be described as the increase in molar free volume. A crystalline solid is a perfect barrier to diffusion and thus diffusion rate would depend on the intricacy of the barrier. In a system where water and oxygen diffusion occur simultaneously, the penetration front of water into a glassy, hydrophilic region would result in a decrease in T_g, with possible swelling or other structural change (e.g., collapse) at the hydration front

as the polymer relaxes, transforming into a rubbery material depending on the time frame of the relaxation process with respect to diffusion time. This is expected to have a strong influence on oxygen diffusion (Chinachoti, 1998).

Therefore, migration of oxygen and other small molecules depends on polymer chain flexibility that can "flip-flop" according to local chain mobility, which creates openings or holes for small molecules to travel through. This mobility depends on the state of hydration.

The microstructure of microencapsulated oil has been reported to be a critical factor (Hardas et al., 2000, 2002; Ponginebbi et al., 2000). Oxidation rates of surface and encapsulated lipids have been shown to follow various mechanisms depending on the physical integrity and mobility of the matrix. Hence, the effect of moisture on oxidation of surface and encapsulated lipid fractions can vary widely (Hardas et al., 2002).

Vitamins and minerals are often encapsulated to prevent unpleasant flavors and to prevent oxidation. Labile components such as fat-soluble vitamins are blended with lipids in emulsion droplets as part of the encapsulation process. Proper selection of surfactants that are antioxidants is advised, and care must be taken to ensure emulsion stability. To prevent easy moisture penetration, the encapsulation matrices should not have a low T_g. However, for enhanced bioavailability, they should disintegrate upon rehydration in the mouth or upon adding water. Capsule materials that ensure release of the nutrient during digestion are usually hydrophobic fats or waxes, but some cellulose and protein derivatives can be used (Brazel, 1999).

Microencapsulation has been shown to greatly retard the oxidation of some oils that are rich in unsaturated and polyunsaturated fatty acids (Lin et al., 1995; Velasco et al., 2000). Typically, the oil is homogenized in water with the aid of an emulsifier and the resultant mixture is rapidly dried—most often in a spray drier—to yield a powdered, encapsulated product. Numerous encapsulation formulas have been tried; those that result in the highest amount of oil in the core of the particle have the best stability. Combination of antioxidants such as Δ-tocopherol (Han et al., 1991) and ascorbic acid or α-tocopherol and ascorbyl palmitate (Kaitaranta, 1992) may be used for additional protection. However, very few investigations have focused on the effect of storage and antioxidants on the oxidation of surface (free) and encapsulated lipids in microencapsulated fish oil (Velasco et al., 2000). In addition, the physical changes from amorphous to crystalline discussed above are factors that remain to be further investigated in order to improve product stability.

Coating or encapsulation is routinely done in the manufacture of fortification nutrients and flavors, but because the techniques are proprietary, it is difficult to find specific studies in the scientific literature. The primary reasons for encapsulation are to prevent interactions with other nutrients, prevent losses due to oxidation or moisture, and to minimize undesirable flavors from vitamins or minerals. The type of coating or capsule used is dependent on the compound, and to a lesser extent, the matrix (Meyers, 1998). Vitamin C (ascorbic acid) is

most often coated with fat or Ethocel to provide stability against oxidation. Vitamin K encapsulated in gum acacia is available on the market.

Stable Nutrient Forms

Stable nutrient forms, other than encapsulated, may also include metal chelates such as sodium iron EDTA (NaFe EDTA), which has been shown to be effective in reducing anemia, particularly in diets high in phytates, without adversely affecting other minerals (Davidsson et al., 1994, 1998; Hurrell et al., 2000). Iron chelates have been tested in various feeding situations and found to enhance absorption of soluble iron fortificants, such as ferrous sulfate or ferrous fumarate. For example, both NaFeEDTA and Na_2EDTA were effective enhancers of iron absorption from cereal foods (Davidsson et al., 2001a, 2001b; Hurrell et al., 2000). Ascorbic acid had a similar effect in high-phytate foods (Davidsson et al., 2001a, 2001b; Hurrell et al., 2000).

MICROBIOLOGICAL CONSIDERATIONS

The addition of small amounts of solutes and dehydration are two main methods of decreasing a_w and of increasing osmotic pressure in a food system to inhibit microbial growth. Reduced availability of water contributes to impaired microbial growth, and hence it has been used widely as a microbiological safety parameter (Beuchat, 1987; Gould, 1985; Lenovich, 1987; Troller, 1987; Troller and Christian, 1978). However, a_w is not a universal parameter but rather an empirical one (Franks, 1982). The efficacy of manipulating a_w is limited to certain types of microorganisms and is affected by food composition and environmental conditions (Andrews and Pitt, 1987; Corry, 1978; Vaamonde et al., 1982), and by the presence of microbial inhibitors (Leistner, 1995). Hence, there is no single minimum a_w for inhibiting microbial growth that can be applied to all foods and all microorganisms.

It is generally accepted that bacteria are more susceptible to osmotic effects than are molds and yeasts (with some exceptions). For IMF products, the main pathogenic bacterium of concern is *Staphylococcus aureus*, which can produce serious food poisoning if a significant amount of its enterotoxin is ingested. *S. aureus*, implicated in 20 to 40 percent of all foodborne illness outbreaks in the United States (Lavoie et al., 1997), is able to grow at an a_w as low as 0.85. Additionally, yeasts and molds, particularly the xerophilic kind (those that prefer dry ambient conditions), survive and grow in moisture-limited environments. The lowest a_w values at which mold growth may occur, albeit very slowly, are 0.61 to 0.62 (Pitt and Christian, 1968), whereas mold sporulation does not take place at a_w less than 0.75 (Pitt, 1975).

In addition to a_w, factors influencing microbial survival and growth have been investigated with respect to water mobility, the translational or rotational

motion of water molecules (Lavoie et al., 1997; Pham et al., 1999). It has been demonstrated that water mobility may influence transport of nutrients to microbial cells and hence growth. Under conditions of limited moisture, mold spore germination and mycelial growth strongly correlate with water mobility (Pham et al., 1999). For the EFP, the type and composition of ingredients used will influence the interaction of solids with water, thereby affecting water mobility and a_w, and thus the survival and potential growth of pathogenic microorganisms. More importantly, should spores or vegetative cells of microorganisms able to withstand dry conditions survive the processing, they could germinate and grow during storage if moisture is not properly controlled in the product and other provisions, such as addition of preservatives, are not made to inhibit microbial growth. To minimize the risk of biological hazards, a multiple hurdle approach is highly recommended (Leistner, 1995). In this approach, also called the combined methods approach, several factors are used together to inhibit microbial growth, such as thermal processing, plus a_w, storage temperature, preservatives, and packaging. For the EFP, it can be expected that there will be little, if any, opportunity to control storage temperature and ambient humidity. On the other hand, the cost of production and materials (including packaging) that would be incurred in making an IMF-type EFP might be too high, and there would also be a price to pay in terms of product shelf life and safety. As pointed out earlier, dehydration and IMF technologies can only stop microorganisms from growing but do not necessarily inactivate them. Consequently, and although an EFP having IMF characteristics should not be ruled out as an option, ***the optimal approach to the microbiological stability of the EFP would be a product design having an a_w value lower than those in the IMF range (e.g., 0.4) and to add some preservatives.***

CHEMICAL STABILITY CONSIDERATIONS

Lipid Oxidation

Auto-oxidation of lipids occurs in foods largely via a self-propagating free radical mechanism. Since direct reaction of unsaturated linkages in lipids with oxygen is energetically difficult, production of the first few radicals needed to start the propagation reaction must occur through some catalytic mechanism (Nawar, 1996). It has been proposed that the initiation step may take place by decomposition to free radicals of preformed hydroperoxides via metal catalysis or heat, by exposure to light, by direct reaction of metals with oxidizable substrates, or by mechanisms where singlet oxygen is the active species involved (Nawar, 1996).

Upon formation of sufficient free radicals, a chain reaction is initiated by the abstraction of hydrogen atoms at positions alpha to double bonds followed by oxygen attack at these locations. The result is production of peroxy radicals,

ROO•, which in turn abstract hydrogen from α-methylenic groups or other molecules, RH, to form hydroperoxides, ROOH, and yield R• groups that react with oxygen, and so on. Due to resonance stabilization of the R• species, the reaction is usually accompanied by shifting in the position of double bonds resulting in the formation of isomeric hydroperoxides that often contain conjugated diene groups.

Lipid oxidation gives rise to formation of a number of breakdown products, some of which are responsible for various off-flavors known as rancidity (Nawar, 1996). Even if only a single type of substrate is involved (e.g., one unsaturated fatty acid), the rate and pathway of its oxidation will depend on many factors that include its molecular structure (i.e., the number and location of double bonds), concentration, type of oxidant, oxygen tension, temperature, surface area, pH, time, physical state, and pro- and antioxidants present (Nawar, 1996).

Numerous antioxidant compounds have been studied, including α-tocopherol, α-tocopherol acetate, ascorbyl palmitate, butylated hydroxytoluene, butylated hydroxyanisole, di-t-butylhydroquinone, green tea catechins, and flavonoids, with mixed results (Lindsay, 1996). Briefly, it appears that the degree of oxidation inhibition apparently attained with antioxidants is affected by the method used to measure it and on the system studied.

Effect of Moisture on Lipid Oxidation

Although moisture reduction may discourage or inhibit microorganisms from growing in a food during storage, the moisture that remains may promote some chemical reactions such as nonenzymatic browning and enzymatic reactions. Depending on the system, these reactions are normally slowed down at low a_w values, and, in general, at a_w < BET[1], the rates can be very slow and the product may remain in good condition through extended storage if it is properly formulated, processed, and packaged.

There is one exception, however, with respect to oxidative deterioration of lipids and fat-soluble nutrients. It has been shown that lipid oxidation can be increasingly high at moisture levels below a "critical a_w" (Nelson and Labuza, 1992a, 1992b). This critical a_w value is reached when a reduction in the moisture content is accompanied by a decrease in the oxidation rate up to a minimum. At moisture levels below this point, oxidation may rise again. Thus, there is a line of demarcation for lowering a_w: in the a_w range of 0.2 to 0.3, lipid oxidation is likely to be accelerated, whereas at a_w between 0.3 and 0.6, lipid oxidation and other deteriorative reactions are minimized. There are a number of proposed explanations for this effect that implicate the state of hydration of catalysts (e.g.,

[1] Brunauer-Emmett-Teller value, normally 4 to 5 percent moisture (Brunauer et al., 1938).

metals) and hydroperoxides, phase transition, mobilization of pro- and antioxidants, and diffusion-related phenomena (Fritsch, 1994).

In the case of the EFP, oxidative changes in the lipid phase would be of concern when unsaturated lipids and minerals are present in significant amounts, for not only could they lead to adverse changes in flavor and acceptability, but also to production of toxic by-products and destruction of fat-soluble vitamins (Gregory, 1996). Therefore, although products at an intermediate moisture range may be more appealing in sensory quality, they may also be more prone to spoilage, browning, and other reactions. On the other hand, lowering the water content of the product to a dry state (< 5 percent moisture) may promote lipid oxidation. A solution to this dilemma would be to develop a dry product (< 5 percent moisture) in which lipids and pro-oxidants are kept separate by means of physical barriers, such as in encapsulation. Use of antioxidants also may be necessary depending on the level of saturation of the lipids. Additionally, the packaging method and materials used would play an important role in the oxidative stability of the EFP (Burke, 1990). The advantages of a dry product must be weighed against the fact that, for a thirsty recipient, eating it may be an unpleasant experience.

It should be noted that in the event the product is amenable to hydration before consumption, its microbiological safety should be evaluated. Potential growth of pathogenic microorganisms after rehydration—particularly if the product is not immediately consumed—could pose serious health risks, especially for recipients having impaired immune systems and vulnerable subgroups such as young children and the elderly.

Because of the above considerations, it is advisable that the lipids and pro-oxidants (e.g,. added mineral ingredients) in the EFP be kept physically separated within the product during manufacturing and subsequent storage by encapsulation of the minerals. Careful design of the encapsulation materials will be required so that they cover the intended ingredients efficiently, hold their integrity under the selected processing conditions, and disintegrate upon consumption so that nutrients are made physiologically available. Further protection against oxidation of unsaturated fats and vitamins in the EFP may be accomplished through a combination of microencapsulation, use of suitable antioxidants, development of stable emulsion prior to drying, and appropriate packaging.

Nutrient Stability During Processing and Storage

Experimental data on vitamin stability and degradation kinetics have been extensively reviewed (Karmas and Harris, 1988; Kirk, 1981; Villota and Hawkes, 1992). The nutritional quality of dehydrated foods is a function of temperature, light, oxygen, moisture, and the physicochemical state of the water (Bluestein and Labuza, 1988). The various chemical forms of added nutrients

are subjected to degradation differently (Gregory, 1996). The description below reflects some major aspects of the degradation kinetics of nutrients related to the effect of moisture.

Experiments have been conducted on the effects of long-term storage at 4.4°, 21.1°, and 37.8° C, nutritional quality, oxidative and browning reactions, and sensory quality of fruit cake and chocolate brownies (Salunkhe et al., 1979). When stored in retort pouches at 37.8° C , the approximate half-life for thiamin was 30 months (fruit cake) or 15 months (chocolate brownies); for riboflavin and niacin, the half-life was less than 30 months in both products. However, the products were unacceptable due to off-flavor, dryness, and rancidity at about the half-life time for thiamin (at this point, rancidity had doubled). This indicates that the shelf life of this type of product could be less than 6 months when stored in a hot environment (e.g., 37° C), and that additional deterrents such as dehydration, reduction of a_w, and others might be necessary to provide vitamin stability.

Fat-soluble vitamins, particularly vitamins A and E, exhibit stability similar to unsaturated fat. Their degradation rates significantly increase with increasing a_w values from very low (~0) to 0.4. Temperature can also greatly influence their destruction; their activation energy is in the range 10 to 25 kcal/mol and decreases with increasing a_w. In the presence of metal catalysts, the degradation kinetics of vitamin A are not affected if the a_w is kept adequately low so that the catalyst is immobilized (Kirk, 1981; Labuza, 1971).

In the case of water-soluble vitamins, their degradation is dependent on the state of the water (free to act as a solvent for reactants and catalysts or bound) and the a_w in the system. Degradation of thiamin seems to be enhanced when a Maillard-type browning is observed, which is to say, when reactants are mobile. A study by Kirk (1981) indicated that when thiamin, vitamin A, and riboflavin are used in fortification of dehydrated foods, very little degradation (< 2 percent loss) takes place at an a_w in the range 0.1 to 0.4 and storage in paperboard boxes at 30° C. However, at a higher temperature (37° C), a significant decrease in vitamin retention was observed with an increasing a_w over the same range. Ascorbic acid degradation studies in a dry model food system indicated that the rate of degradation of this nutrient increased with increasing relative humidity of storage or increasing initial moisture content (Purwadaria et al., 1979).

Therefore, based on the information available, some conclusions may be advanced regarding retention of nutrients that will need to be confirmed when the exact prototypes of the EFP are developed. ***First, to ensure nutrient retention, a_w may need to be kept lower than 0.4; the lower the a_w, the more stable some of the nutrients would be.*** This is more critical in tropical and arid areas, where storage of the EFP at elevated temperatures could accelerate the degradation process. In addition, this low a_w would be in agreement with that necessary to provide protection against microbial growth in the EFP. ***A higher a_w (e.g., up to 0.6) may be used if there are compelling reasons to do so and its***

influence on stability and shelf life of the EFP are determined. Second, it might be possible to apply microencapsulation technology to add additional oxygen barriers to labile components such as fat-soluble vitamins, always keeping a low a_w (< 0.4) in the product. Third, for water-soluble vitamins, it is most critical that water mobility be kept low again by keeping a_w adequately low (< 0.4), and that minerals are encapsulated. Minerals are unlikely to be influenced by a_w, since they are stable during most processing and storage regimens.

Testing of EFPs must be conducted throughout the expected shelf life of the EFP and under conditions of delivery and storage simulating actual use, to ascertain the initial content and stability of nutrients. Standard methodologies for determining vitamin and mineral content are well described in the literature, and appropriate procedures, such as those used for nutritional labeling, can be applied to the EFP. Determining bioavailability of micronutrients from the EFP is not a feasible outcome of its development and manufacture, given the complex issue of such testing (Van Campen and Glahn, 1999).

ACCEPTABILITY CONSIDERATIONS

The characteristics of a food product (i.e., appearance, flavor, and texture), the conditions under which it is consumed, and the appeal that it has for a specific consumer determine its acceptance. Measurement of liking, described below, is used during product development to predict consumer response before investments are made in equipment, production, and distribution (Stone and Sidel, 1993).

Measurement of Liking

Preference and liking are generally thought to be almost the same, and techniques used for their measurement are often similar (Peryam, 1998). However, preference implies a choice between products, without considering how well liked each one is. Therefore, measurement of the degree of liking, or "hedonic value," is a means of determining not only whether one food is preferred to another, but how acceptable or well liked it is.

In the 1950s, considerable work was done at the U.S. Army Quartermaster Food and Container Institute to establish methodology for predicting soldiers' food choices (Peryam and Girardot, 1952). The relevance and reliability of the hedonic scale method, based on known rating scale methods used in psychology, were established through extensive field testing of army rations of all types (Peryam and Pilgrim, 1957). Since the development of the technique, the nine-point hedonic scale has been used extensively and validated by numerous studies of food products. Although there are still issues regarding its use, it

remains one of the most useful tools for determining consumer acceptance (Lawless and Heymann, 1999; Meilgaard et al., 1999; Stone and Sidel, 1993).

In hedonic rating, testers are presented with a continuous or discrete scale with nine marked points, where 1 is "dislike extremely," 5 is "neither like or dislike," and 9 is "like extremely" (Peryam and Pilgrim, 1957). Other points are like or dislike "very much," "moderately," or "slightly." Testers are asked to respond to the food product on this scale and express their honest opinion of liking. They are reassured that there is no correct answer. The data are then interpreted numerically and analyzed statistically.

Interpretation of hedonic testing results is open to debate. At what value on the nine-point scale does a product become unacceptable, and when is it an excellent product? According to Peryam (Peryam and Girardot, 1952; Peryam and Pilgrim, 1957), a hedonic rating less than 4.5 is unacceptable, while an ordinary staple food would range between 6.25 and 7.25. Interpretation is based on the food product; some items, such as candy and ice cream, would be expected to achieve averages higher than 7.25 or be poor prospects.

Prediction of food consumption is an area of continuing research in both food and behavioral sciences. Cardello and colleagues (2000) pointed out that food preference and acceptability testing may not be a successful indication of consumer behavior towards consumption. However, affective tests of liking remain an integral part of food product development and marketing. Cardello and coworkers (2000) found that predicting consumer behavior toward foods in real-life situations is difficult and that standard methods of determining liking in controlled situations may not be reliable. In the case of the EFP, it will not be possible to test the product in a real-life situation. However, ***testing under conditions similar to those used by the U.S. Army for GP Survival Packets and MREs is recommended***.

Shelf-Life Testing

The length of time that a product is acceptable and meets consumer expectations of its quality is considered to be its shelf life (Labuza, 1982). Procedures for determining shelf life comprise microbiological, chemical, and sensory testing to give an objective point for stating that the product does not meet expected quality. In general, microbiological and sensory endpoints are used. Criteria for determining shelf life must be determined prior to starting the process. However, moisture content, a_w, lipid oxidation, and vitamin losses can be correlated with sensory changes and serve as indices of stability (Giese, 2000).

A standard guide for shelf-life determination by sensory methods is being considered by the ASTM E-18 Committee (1997), which describes criteria and experimental design considerations for real-time and accelerated shelf-life testing. For products expected to have an extended storage time, such as the

EFP, accelerated testing is needed (Labuza and Schmidl, 1985). The concept behind these tests is that subjecting foods to a controlled environment in which temperature or humidity, for example, is higher than normal causes an increased deterioration rate. At least one characteristic (e.g., sensory quality, vitamin content, or oxidative rancidity) must be measured analytically so that a prediction model can be built (Labuza and Schmidl, 1988; Ragnarsson and Labuza, 1977). Based on the accelerated testing, a prediction of the storage stability of the product can be made. Other models are available: Nelson and Labuza (1994) examined two models for determining the effects of a_w on shelf life, while others (Cardelli and Labuza, 2001; Duyvestyen et al., 2001; Gacula, 1975a, 1975b; Gacula and Singh, 1984) have evaluated the Weibull Hazard Analysis proposed for use in shelf-life testing by Gacula in 1975.

The critical issue for the EFP is maintenance of eating and nutritional quality. Shelf-life testing for the product, therefore, should be based on both of these criteria, as well as on microbiological safety for an at-risk population. The suggestion made by the U.S. Army to use its facilities at various overseas locations to test the EFP among local populations, so that its acceptability by populations having diverse ethnic and cultural backgrounds can be evaluated, seems to be a realistic method to evaluate the prototypes.

PACKAGING CONSIDERATIONS

EFP Configuration and Packaging

The EFP will be used in environments that exhibit a wide range of temperature and humidity conditions, including extreme environments, often characterized by a lack of a delivery infrastructure. Therefore, all packaging components must be capable of withstanding a wide range of temperatures (Riordan, 1970) and physical abuse. In addition, these food items will be delivered by various modes of transportation, including airdrop. Separate packaging, or more likely, additional packaging, may be necessary for EFP airdrop operations.

The first step in defining packaging requirements is to define a product configuration for product delivery and the use and protection requirements for each component. The starting point in the configuration for the EFP is a daily ration required to provide 2,100 kcal along with proteins, lipids, vitamins, and minerals to maintain nutritional status. This unit must be protected for a 3-year shelf life, and because of the product's dual susceptibility to moisture and oxygen, moisture and oxygen must be removed from the product environment before or during packaging and essentially excluded throughout its storage life. The ration is likely to be a low-moisture product (< 5 percent) that achieves microbiological stability through limited a_w (< 0.4), and includes polyunsaturated fatty acids, which are prone to oxidation. Moisture should be restricted

through the initial formulation. Oxygen, on the other hand, must be removed during the packaging operation by drawing a vacuum, flushing with nitrogen, or both. A high barrier to both moisture and oxygen transmission is also essential to protect the product post-packaging.

Oxidation of food components is curtailed in low-oxygen environments, as described earlier. Oxygen levels below 1 percent have been found to reduce oxidation sufficiently to provide stability for unsaturated fatty acids (Brody, 1989). Some molds can grow at oxygen levels as low as 0.1 percent and produce mycotoxins (Nielsen et al., 1989). Oxygen levels of 0.2 to 0.5 percent (2,000 to 5,000 ppm) can be achieved using vacuum and vacuum plus gas flush technologies for solid products. Initial oxygen concentrations at these levels can be obtained for porous products as well, but degassing of these products (i.e., gas losses by the product itself) may quickly raise the initial oxygen concentrations into the 1 to 2 percent range. Salame (1974) suggested that dried foods required protection to restrict oxygen gain to a maximum of 5 to 15 ppm and could tolerate a maximum moisture gain of 1 percent over their shelf life.

Acceptably low oxygen levels can be maintained only with packaging materials having sufficient barrier properties. The initial oxygen level and the upper limit of oxygen to be permitted in the package must be set in specifying actual barrier requirements. The necessary oxygen barrier to limit oxygen influx to 10 ppm over 3 years for the EFP would require an essentially perfect barrier to oxygen. More realistically, ***to maintain an oxygen level below 2 percent for the expected 3-year shelf life of the EFP at 23° C (70° F) in a 10×10×5-cm configuration, which yields 500 cc (450 cc for a product having an approximate density of 1.0 and a 50-cc allowance for primary wraps), and assuming a pouch surface area of 400 cm^2, for example, and an initial oxygen concentration of 0.1 percent, sufficient barrier is achieved with a maximum oxygen transmission of 0.00088 cc/100 in^2/day***.

Such a barrier can be achieved using glass, metal, or thick films of high-barrier polymers. Glass packaging would be inappropriate for the EFP because of excessive weight, field disposal, and fragility. Rigid metal or plastic containers are contraindicated for similar reasons. This leaves flexible materials: aluminum foil, high-barrier polymers, and metalized films. ***The optimal choice for barrier and cost reasons is an aluminum foil laminate*** (Lampi, 1977; Szczeblowski, 1971).

To be sufficiently thick, high-barrier polymers such as ethylene vinyl alcohol or polyvinylidene chloride would be too costly and too bulky. "Metalized" films, in turn, can be prepared with excellent barrier properties if—and only if—the metalization completely covers the substrate. Typically, these films would be able to provide moderate, but not sufficient, barrier for use in the EFP. Therefore, aluminum foil would be the choice material.

Aluminum foils range in thickness from 4.3 μm (0.00017 in) to 150 μm (0.0059 in). By industry definition, rolled aluminum becomes foil at a thickness below 152.4 μm (0.006 in). Foils exhibit pinholes as a function of thickness.

When foils are rolled to gauges below 10 μm, the incidence of pinholes increases exponentially (Anderson, 1988). Studies conducted in 1961 and 1985 showed that improvements in rolling techniques reduced the incidence of pinholes for thin foils (Anderson, 1988). For example, approximately 200 pinholes were observed per square meter with 9-μm foils in 1961, whereas a similar performance was obtained with 8-μm foils in 1985.

Foils are considered impermeable at a thickness of 25.4 μm (0.001 in) and above. At 8.9 μm (0.00035 in), the water vapor transmission rate (WVTR) is equal to or below 0.065 cc/m^2 (0.02 cc/100 in^2)/day at 37.8° C (100° F) (Brody and Marsh, 1997). These values drop if foil is laminated to appropriate polymeric materials. ***The thickness for the foil layer, therefore, could be within the range 8.8 to 18.0 μm (0.00035 to 0.0007 in), which provides the needed barrier at the lowest thickness, and therefore, at the lowest cost.*** Within this thickness range, foils still exhibit minor pinholes (Anderson, 1988), but lamination with polyolefin provides sufficient protection from influx of oxygen. At a thickness of 0.00035 in, aluminum foil was reported to present pinholes of approximately 0.00004 in^2/100 in^2. Marsh (1996) calculated that a 1-mil polypropylene coating applied to a foil substrate with an effective surface area for permeation of 0.00004 in^2/100 in^2 of film would exhibit reduced transmission values of 0.0076 cc/100 in^2/day/atm for oxygen and 0.00002 cc/100 in^2/day/atm for water vapor. These transmission rates are expected to be sufficient to maintain an oxygen level below 2 percent and to provide acceptable protection against moisture influx for 3 years at 23° C.

Aluminum foil is fragile and prone to tearing unless it is protected. A tough polymer—toughness being defined as the area under the stress strain curve (Marin and Sauer, 1954)—can provide both puncture and tear protection to aluminum foil. Two applicable polymers are polyethylene terephthalate (polyester) and polyamides (nylon). According to Lampi (1977), a 0.0005-in thick film laminated to the outside of the foil via extrusion or adhesives would provide sufficient protection: an O_2 transmission rate below 1 cc/100 in^2/atm/day (15.5 cc/m^2/day) and a WVTR below 0.05 cc/100 in^2/day. Compared to the numbers given in the paragraph above, and by current standards, these specifications for barrier properties appear to be high. However, the apparent discrepancy is easily resolved after considering that Lampi's values represented an untested level. The verification of low water and oxygen transmission was through sensory testing rather than permeation testing (Szczeblowski, 1971). Additionally, the limit of permeation detectability in the 1970s was lower than today. In 1970, the level of oxygen transmission detectability was 0.003 cc/100 in^2/day (0.0456 cc/m^2/day); in 2001 the level of detectability was 0.00003 cc/100 in^2/day (0.0005 cc/m^2/day) (personal communication, MOCON, 2001). Foil laminates, therefore, would have measured below the limits of detectability in the earlier work.

As a result of the above discussion, ***a trilaminate structure that has been extensively tested for long shelf-life food applications is recommended for the EFP*** (Lampi, 1977; Szczeblowski, 1971). Because lamination costs are related to quantity, primarily because short-run set-up charges can render lamination costs prohibitive, the trilaminate currently used by the military is recommended (from inside to outside: 0.003- to 0.004-in thick polyolefin/0.00035- to 0.00078-in thick aluminum foil/0.0005-in thick polyester [Natick Research, Development, and Engineering Center, 1993]). Other laminations with the recommended properties are also applicable, including the enhanced laminate currently recommended by the military that uses both polyester and nylon for additional protection against distribution (mechanical) forces. ***The package should be nitrogen flushed, and residual oxygen must not exceed 0.5 percent.***

A notch in the package seal must be provided to facilitate opening by EFP recipients, who would likely have no scissors or other tools at their disposal.

Individual EFP Bars

Although the EFP has been designed to provide 2,100 kcal/day to recipients as a daily ration of approximately 450 g, it should be divided into smaller units for a number of reasons. First, considering that most people would normally eat more than one meal during the course of the day, dividing the daily ration into smaller portions provides for multiple meals. Second, it facilitates feeding children who need smaller amounts to meet energy needs. Third, it helps prevent the entire ration from being exposed when only part of it is going to be consumed. Therefore, ***it is suggested that the ration be divided into equal portions having the shape of bars***. To facilitate packaging, ***nine bars would be appropriate***. To further facilitate division of portions for young children, ***each individual bar should be centrally scored across the width of the bar to provide two 116-cal portions upon breaking it.***

Each one of the nine bars should be wrapped in a primary packaging—defined as the package that is in intimate contact with the product (Saroka, 1999)—that does not need to be a barrier material. (Lampi [1977] defined the pouch and carton used for retort pouches as the "immediate container" in the EFP application, the immediate container would include this primary wrap, the barrier container for each daily ration, and the bundling bag for multiple days or people.) It is recommended that this primary wrap be pulp-based and have a moisture-barrier coating. Individual bars could be wrapped in polyethylene or wax-coated paper (Fennema and Kester, 1991) as an unsealed wrapping similar to the inner wrapping of a candy bar. This wrap would provide a minor moisture barrier for individual bars after the EFP trilaminate is opened. More importantly, it would separate individual bars for easier access and would prevent microbial contamination from insects, handling, and surroundings. In

addition to separating individual bars, the wrappings could be used by refugees or victims of emergencies to start fires for cooking or providing heat.

The secondary packaging is defined as that which bundles the primary packages (Saroka, 1999). ***A daily supply of nine bars should be packaged together under a nitrogen flush or vacuum into a barrier package*** to provide the barrier against oxygen and moisture needed for extended shelf life. The secondary packaging could be a pouch having a similar construction to that of the trilaminate pouch utilized by the military for long shelf-life rations, which consists, from the inside out, of polyolefin, aluminum foil, and polyester or nylon (defined above). This daily supply is considered a "unit."

Five-Day EFP Package for Distribution

Anecdotal evidence provided by relief agencies indicates the convenience of grouping five daily rations into a single bundle. The rationale for this selection is that it permits 5 days worth of food for a single individual or 1 day of feeding a five-member family to be distributed as a unit. In addition, it is desirable to have a distribution-bundled package that is not easily carried by soldiers or personnel for which the EFP is not intended. Therefore, it is recommended that ***five EFP daily rations be bundled into a monoaxially- or biaxially-oriented polyolefin bag. The bag should be notched to facilitate opening***.

A rigid container could be used for the bundling instead of the polymeric bag. This could be a rigid plastic or metal container. Although such containers would add weight, they offer additional protection against rodents and may also serve additional purposes after consumption of the EFPs, such as carrying water. This option becomes especially attractive if such use (e.g., as a water carrier) replaces or precludes a separate delivery of such items.

Shipping Containers

Eight bundles of five EFPs each will be placed into a shipping container to constitute a case. The shippers could be of corrugated construction, sufficient to pass distribution protocols of ASTM International or the International Safe Transit Association. Their dimensions will depend upon the actual shape of the bars, which is not specified in this performance-based recommendation. It is anticipated that single-wall, C-flute corrugate would suffice. Each shipper will weigh in the vicinity of 40 lb. Shipping containers could also be constructed of metal so that they can be recycled for use as storage or water containers as suggested by anecdotal evidence.

Pallets

Shippers will be assembled onto a pallet for transport. ***Pallets will be of construction and dimensions to provide efficient transport, with overhang and underhang restricted to a maximum of 2 in***. Approximately 50 cases will be placed on a pallet. Pallets may be unitized using stretch wrap, banding, adhesive, or other means.

Airdrop

Naked Rations

Depending upon the ultimate shape and density of the EFP, it should be possible to airdrop individual EFP packs in ways similar to MREs, using the Triad (tri-wall aerial distribution system) that was used to airdrop food in Bosnia (Roos, 1993). Individual MREs were found to fall with a terminal velocity of 58 mph, which was suitable for delivery. The tri-wall distribution container was used to transport the MREs, but was not included in the drop. This method may be applicable if the terminal velocity of the EFP is found to be sufficiently low to allow for safe delivery. However, given the caloric density requirements of the EFP, it is anticipated that it will be a heavy product such that additional packaging protection will be required for air delivery in this manner.

Flutter Packs

The World Food Programme developed a plastic film tube package with unequal amounts of food product sealed into each end. The length of tube between the product catches air during free fall and slows the descent. The unequal product weights cause a precessing (whirling) motion that absorbs energy during the free fall, thus the package name. However, because the weight of product delivered using this system is less than that of the EFP, its applicability for EFP delivery must be tested.

Wing Packs

Alternate configurations to the Flutter Pack may be developed that provide sufficient wind resistance to slow descent to a safe level.

Bubble Packs

Bubble packs or suitable cushioning material may be layered such that impact is attenuated as successive layers absorb impact and rupture. This would constitute an individual pack adaptation of the airbag approach that has been developed for bulk delivery (see below). Dimensions of bubbles, pressure of

enclosed gas (which would change with altitude), and strength of substrate that will rupture must all be determined to prove efficacy for specific ration configurations (single or multiple) and specified drop heights. Drop height may be extended if terminal velocity is acceptable.

Cushion Packs

Additional cushioning materials may be employed to attenuate impact to acceptable levels for EFP delivery. These cushions could be composed of a variety of materials (thermoset or thermoplastic foams, rubber, cellulosic), composites, or constructions (such as paperboard honeycomb, mentioned below). Two considerations are required for suitability, however: the integrity of the EFP and the safety of the delivery. Unless remote delivery is assured, the airdrop must not present a hazard to the intended recipients.

Bulk Drops

Cushion

The steady descent velocity experienced with parachute airdrops is about 28 ft/sec (Lee, 1992). Ground impact at this velocity requires an energy absorber to dissipate the impact energy. An evaluation of cushioning materials by Ellis and coworkers (1961) concluded that paperboard honeycomb was the most cost-effective, all-around, airdrop, energy-dissipating material. A majority of U.S. Army airdrops are delivered by the Container Delivery System, which has a 2,000-lb payload and uses honeycomb protection. Other cushioning materials may also prove adequate, but ***any material will require evaluation to assure proper loading and effectiveness under the environmental conditions that may be expected during the airdrops.***

Foam

The U.S. Army evaluated alternatives to cushions because of specific shortcomings. Cushioning materials take up substantial warehouse space, are labor-intensive to use (primarily for equipment loads that require assembly, but would be less of a concern with uniform loads for items such as the EFP), and may degrade in high humidity (especially the paperboard honeycomb). Foams offers an alternative that overcomes these difficulties (Goldberg, 1990). As mentioned above, testing would be necessary to determine loading and use conditions.

Air Bag

Another option for airdrop impact reduction is air bags. This option utilizes the restricted venting of the air bag to reduce impact forces. Complex air bags

using vent control and/or gas injection, and augmented air bags using paperboard honeycomb or other cushioning, have been found to improve the performance of simple air bags by decreasing peak gravity forces (Lee, 1992). Such systems offer further alternatives for bulk air drops of the EFP that could be evaluated when prototypes are prepared.

COST CONSIDERATIONS

The goal of this report is to develop an EFP that has an optimal nutritional profile and could meet the most severe environmental, storage, and logistic conditions. However, it is recognized that the requirements to produce such a sophisticated product are substantial. If funds are limited, a high unit cost can dramatically reduce the quantity of rations available to a needy population. Given this concern, the technical specifications recommended in this report should be considered optimal; however, the sponsoring agencies may choose to consider developing EFPs prepared and packaged to less stringent specifications if cost becomes a primary consideration. Under these circumstances, an EFP packaged in airtight foil bags inside a water-repellent paperboard box, for example, would allow greater quantities of products to be procured for a fixed cost and would be adequate in many relief situations, particularly for disaster relief. However, in this case, the long shelf-life objective and possibly also the goal of prepositioning supplies around the world would have to be modified by the agencies.

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4
Performance Specifications

ITEM DESCRIPTION

This product—the high-energy, nutrient-dense emergency relief food product (EFP)—is intended to provide a compact, self-contained, high-energy, nutrient-dense emergency food for refugees and victims of disasters for a short duration at the initial stages of an emergency. The expected use period is 3 to 7 days, with a maximum use of up to 15 days. The product may be used in climatic extremes from arctic to tropical. The EFP is expected to be the sole source of food during the period of use and to provide adequate energy, protein, fat, vitamins, and minerals to promote survivability. The product may be consumed directly or crumbled to make a gruel or porridge.

The EFP must be suitable for a wide age range—from infants over 6 months through older adults.

The EFP must be provided in a format acceptable to people from a wide range of ethnic, cultural, and religious backgrounds.

The EFP will be consumed under worldwide environmental extremes. It is essential that this item be produced in accordance with Good Manufacturing Practices applicable to ready-to-eat food products.

There are five characteristics critical to development of a successful EFP. These are listed in order of importance. The EFP must be:

1. Safe
2. Palatable
3. Easy to dispense
4. Easy to use
5. Nutritionally complete.

PERFORMANCE REQUIREMENTS

Nutrition

The EFP must provide a nutritional profile as described in Table 4-1. Each unit (nine bars) will provide 2,100 kcal.

Shelf Life

The packaged EFP shall meet the minimum shelf-life requirement of 36 months at 21° C (70° F).

Appearance

1. **Exterior** – The EFP will be a bar of a rectangular shape that promotes efficient packing. The color of the bar will depend on the ingredients and processing methods used. Artificial colors are not recommended, and it is required that the product not be white or cream-colored. The product, if dispersed in water, must not resemble milk.
2. **Interior** – The EFP shall be a compressed, cold-extruded, or baked product of essentially uniform composition.
3. **General** – The packaged EFP shall be free from foreign material such as, but not limited to, dirt, insect parts, hair, wood, glass, or metal. The product shall show no evidence of excessive heating (materially darkened or scorched).

Odor and Flavor

1. The EFP shall be slightly sweet with blended cereal flavor from the base ingredients, and no distinct flavor notes attributable to the protein source or vitamin and mineral additions may be present. Flavorings may be used, but should not be strong or unusual (i.e., not targeted for a specific population).
2. The EFP shall be free from foreign odors and flavors such as, but not limited to, burnt, scorched, rancid, sour, or stale.

Texture

The texture of the bars will depend on the ingredients and processing methods used. When crumbled, particle size should be large enough to make a porridge-like product when dispersed in water, and not small enough to resemble milk. The EFP shall be sufficiently firm and resilient to withstand delivery via various modes of transportation (air, land, and sea) including low-altitude airdrop. It must maintain structural integrity through short periods of extreme temperatures.

Size

The EFP dimensions shall be such that a unit will deliver 2,100 kcal and be divided into nine bars, each bar scored to yield two equal portions. Each portion will contain approximately 116 kcal. The total net weight of the unit (2,100 kcal/EFP) shall be approximately 450 g (~50 g/EFP bar).

Acceptability

A prototype of the finished product shall be tested by the procuring agency and must receive a hedonic score of 6.0 or better on a 1.0- to 9.0-point scale, where 9.0 represents "like extremely" when assessed by likely target populations under conditions simulating field use (e.g., during 3 to 7 days' consumption).

Nutrient Content

The nutrient content is described in Table 4-1. The overall description is:

- **Energy content** – The EFP shall be designed as a 2,100-kcal unit.
- **Moisture content** – The moisture content shall not be greater than 9.5 percent. Water activity shall not be greater than 0.6.
- **Protein content** – The protein content shall be not less than 63 or greater than 80 g/2,100-kcal unit (8 to 9 g/EFP bar). The protein must have a minimum Protein Digestibility-Corrected Amino Acid Score of 1.0.
- **Lipids** – The lipids must be 22 percent by weight minimum (approximately 40 percent of kcal, 82 to 105 g/2,100 kcal unit, or 9 to 12 g/EFP bar). The source of lipids must not be lard, tallow, other animal fats, or similar animal-based products. The ratio of linoleic acid to α-linolenic acid shall be 5:1 to 10:1.
- **Carbohydrates** – The remaining calories will come from carbohydrates as specified in Table 4-1.
- **Vitamins and minerals** – As specified in Table 4-1.

Note: Vitamin E must be encapsulated (or stabilized) for stability. Separate encapsulation is also necessary for ascorbic acid and for metals: iron (as NaFe EDTA), chromium, copper, manganese, selenium, and zinc.

- **Caloric density** must be between 233 and 250 kcal/50 g bar (2,100 kcal/unit).

Additives

Additives must be consistent with guidelines of both the U.S. Food and Drug Administration (FDA) and Codex Alimentarius, and comply with the

TABLE 4-1 Nutrient Specifications for a High-Energy, Nutrient-Dense, Emergency Relief Food Product (EFP)[a]

Nutrient	Minimum Required Nutrient Density/EFP Bar (50 g)	Maximum Required Nutrient Density/EFP Bar (50 g)	Minimum Required Nutrient Density/1,000 kcal
Energy	233 kcal	250 kcal	
Fat	9.1 g (35% of calories)	11.7 g (45% of calories)	39 g (35% of calories)
Protein[b]	7.9 g (13.5% of calories)	8.9 g (15% of calories)	34 g (13.5% of calories)
Total carbohydrates			100–125 g (40–50% of calories)
Total sugars	7–11.7 g (12–20% of calories)	14.7 g (25% of calories)	30–50 g (12–20% of calories)
Glucose	2 g		8.5 g
Lactose		4 g	
Monosaccharides		5.8 g	
Sodium	0.30 g	0.33 g	1.3 g
Potassium	0.40 g	0.47 g	1.7 g
Chloride	0.47 g	0.51 g	2.0 g
Calcium	180 mg	207 mg	768 mg
Phosphorus	172 mg	206 mg	740 mg
Magnesium	44 mg	54 mg	190 mg
Chromium	3.0 μg		13 μg
Copper	131 μg	156 μg	560 μg
Iodine	24.5 μg	53.6 μg	105 μg

Maximum Allowed Nutrient Density/1,000 kcal	Minimum Required Nutrient Density/2,100 kcal	Maximum Allowed Nutrient Density/2,100 kcal	Comments
	2,100 kcal		2,100–2,250 kcal/9 bars
50 g (45% of calories)	82 g (35% of calories)	105 g (45% of calories)	Saturated fat > 10% of calories; PUFA 7–10% of calories from vegetable oil; LA:LNA ratio of 5:1 to 10:1
38 g (15% of calories)	71 g (13.5% of calories)	80 g (15% of calories)	PDCAAS ≥ 1.00
	210–263 g		
63 g (25% of calories)	63–105 g (12–20% of calories)	131 g (25% of calories)	Palatability requires the use of sugar or high fructose corn syrup
	18 g		6 g/g of Na; from maltodextrins
17 g		36 g	Should be present only if milk solids are used
25 g		53 g	< 25% by weight of carbohydrates
1.4 g	2.7 g	3.0 g	EFP should not taste salty
2.0 g	3.5 g	4.2 g	EFP should not taste bitter
2.2 g	4.2 g	4.6 g	Equimolar to sodium
885 mg	1,620 mg	1,865 mg	Phosphate, citrate, or carbonate salt forms
890 mg	1,555 mg	1,865 mg	Nonphytate form
230 mg (< 167 mg as supplement)	400 mg	480 mg (< 350 mg as supplement)	Only supplemental Mg contributes to UL
	27 µg		
670 µg	1,180 µg	1,410 µg	
230 µg	220 µg	480 µg	

continued

TABLE 4-1 Continued

Nutrient	Minimum Required Nutrient Density/EFP Bar (50 g)	Maximum Required Nutrient Density/EFP Bar (50 g)	Minimum Required Nutrient Density/1,000 kcal
Iron[c]	3.7 mg	4.2 mg	16 mg
Manganese	0.33 mg	0.40 mg	1.4 mg
Selenium	6.5 µg	7.9 µg	28 µg
Zinc	2.4 mg	2.7 mg	10.4 mg
Vitamin A (preformed)	117 µg	233 µg	500 µg
Vitamin D	1.2 µg	1.4 µg	5.2 µg
Vitamin E	2.2 mg		16 mg
Vitamin K	14 µg		60 µg
Vitamin C	23.3 mg	46.6 mg	100 mg
Thiamin	0.28 mg	0.33 mg	1.2 mg
Riboflavin	0.28 mg	0.33 mg	1.2 mg
Niacin	2.6 mg	2.9 mg	11.2 mg
Vitamin B_6	0.28 mg	0.33 mg	1.2 mg
Folate[d]	45.2 µg	49.7 µg	194 µg
Vitamin B_{12}	2.8 µg	3.4 µg	12 µg
Pantothenic Acid	0.9 mg	1.1 mg	3.9 mg
Biotin	5.6 µg	6.7 µg	24 µg
Choline	85.3 mg	102.3 mg	366 mg

[a] The energy content of the EFP is specified as 4.5 to 5 kcal/g, which provides a range of 2,100 to 2,250 kcal per 9-bar ration (EFP). It is important to note that calculation of nutrient density for all other nutrients is based on the minimum energy requirement for the EFP of 2,100 kcal (IOM, 1995). Calculations based on information in the text may differ slightly from the numbers presented in the table due to rounding.

[b] Protein digestibility-corrected amino acid score (PDCAAS) is a method described by FAO/WHO (1989) for protein evaluation that is based on the essential amino acid requirements of the 2- to 5-year-old child. The use of this method of protein evaluation by U.S. food manufacturers has Food and Drug Administration approval.

Maximum Allowed Nutrient Density/1,000 kcal	Minimum Required Nutrient Density/2,100 kcal	Maximum Allowed Nutrient Density/2,100 kcal	Comments
18 mg	34 mg	38 mg	Encapsulated as NaFeEDTA suggested
1.7 mg	2.9 mg	3.5 mg	
34 µg	60 µg	72 µg	Selenomethionine form
11.4 mg	22 mg	24 mg	Sulfate or acetate; molar ratio of Zn:phytate < 15
1,000 µg	1,050 µg	2,100 µg	Does not include carotene
5.8 µg	11 µg	12 µg	Cholecalciferol form
	34 mg		0.6 mg/g PUFA
	120 µg		
200 mg	210 mg	420 mg	Encapsulation required
1.4 mg	2.5 mg	3.0 mg	
1.4 mg	2.5 mg	3.0 mg	
12.4 mg	23.6 mg	26.0 mg	Maximum only refers to added nicotinic acid
1.4 mg	2.5 mg	3.0 mg	
213 µg	406 µg	447 µg	Maximum only refers to added folate
14.4 µg	25.2 µg	30.2 µg	
4.7 mg	8.2 mg	9.8 mg	
28.8 µg	50.4 µg	60.5 µg	
439 mg	769 mg	923 mg	Choline could be provided as lecithin

[c] Iron requirements based on FAO/WHO (2000) for adolescent girls, which assumes 10% bioavailability.

[d] Assumes that folate provided will be as a food fortificant and thus will be synthetic folate, which is 1.6 times more available than naturally occurring food folate.

specifications set forth in the Food Chemicals Codex (National Academy Press, Washington, D.C.).

PROHIBITIONS

The EFP shall contain no sensitive ingredients that would limit its intended use for diverse populations. No alcohol shall be incorporated in it, nor any meat products used.

PROCESSING REQUIREMENTS

Bars shall be prepared through extrusion, compression technology, or baked.

Units will be prepared consisting of nine bars of approximately 233 kcal each, with central scores that allow easy division to 116-kcal portions.

It is desirable that the EFP be amenable to being made into a gruel by crumbling the bar and mixing with water.

PACKAGING REQUIREMENTS

The EFP will be subjected to environments that exhibit a wide range, including extremes, of temperature and humidity, and to delivery conditions that will often be characterized by lack of infrastructure. Therefore, all packaging components must be capable of withstanding temperature and physical abuse. In addition, the EFP will be delivered using all modes of transportation, including airdrop. Separate packaging, or more likely, additional packaging, may be employed for airdrop requirements.

Primary Packaging

Each 2,100-kcal daily EFP unit will be prepared as nine equal-sized bars, each centrally scored to allow breaking into two segments. The nine bars will be individually wrapped to facilitate handling of individual bars, while reducing contamination to additional bars, through human, insect, animal, or microbial intervention. The primary wrap need not be a barrier material, and it is recommended that it be pulp-based, with a moisture-barrier coating. The coating may be polyolefin or wax-based. This primary package, after use, may also serve as an energy source through combustion. Polyethylene- or wax-coated paper both provide similar heating value as comparable weights of fuel oils. The package will be nitrogen flushed. Residual oxygen must not exceed 3 percent (2 percent if feasible).

Secondary Packaging

A daily supply of nine bars will be packaged under a nitrogen flush or a vacuum, into a barrier package, to enhance product shelf life. The secondary packaging will be a pouch construction similar to the trilaminate construction utilized by the military for long shelf-life rations: from inside to outside, 0.003- to 0.004-in thick polyolefin, 0.00035- to 0.0007-in thick aluminum foil, and 0.0005-in thick polyester or nylon. The pouch material shall be FDA-approved for food use and shall show no evidence of delamination or degradation when heat sealed or fabricated into pouches. Pouches that contain the nine bars may be preformed or formed on line. The pouches will have an inside dimension sufficient to hold the nine individually wrapped bars. The pouch shall be made by heat-sealing three edges (two sides and bottom) with 3/8-in- (+/- 1/8 in) wide seals. The heat seals shall be made in a manner that will ensure hermetic seals. The pouch shall maintain its integrity and air tightness of the side and bottom seals when tested by appropriate methods. The side and bottom seals shall have an average seal strength of not less than 6.0 lb/in, and no individual specimen shall have a seal strength of less than 5.0 lb/in. A V-, C-, or U-shaped tear notch at least 1/32-in deep, located 3/4 to 1 in from the top edge of the pouch (excluding the lip) shall be made on one or both side seals. The distance between the inside edge of the tear notch and the inside edge of the seal shall be no less than 1/8 in. One side of the open end of the pouch may be provided with an extended or fold-over lip, extended not more than 1/8 in (+/- 1/16 in) to facilitate opening and filling. In order to discourage diversion of the product, the pouch must be of a neutral color (e.g., off-white, tan); no bright, attractive colors or shine may be used.

A multiple set of bars, sufficient for a 5-day supply of nine bars per day (i.e., five pouches containing nine bars each) will be packaged together and constitute the distribution unit called a "bundle." The five trilaminate pouches will be bundled into a low-density polyethylene bag to provide either a 5-day individual EFP supply or a daily ration for a family of five members. The film used to prepare the bundle will be monoaxially or biaxially oriented, with machine direction oriented across the pouch. Filling will therefore be accomplished on a horizontal wrapping machine. A V-, C-, or U-shaped tear notch at least 1/32-in deep, located 3/4 to 1 in from the top edge of the pouch (excluding the lip) shall be made on one or both side seals. The notch will allow easy opening by propagating the notch tear across the bundle bag.

As an alternative, the outer package may be a reusable, semi-rigid polyolefin container which could be used for storage and/or water transport.

A third option is to utilize a metal outer package, such as a tinplate box with a cover. The cover shall be easily removable. This container may also have multiple uses, such as storage and/or water carrier.

Tertiary Packaging

Rations will be available in two formats: Ground delivery and airdrop.

- **Ground delivery** – Eight bundles consisting of five pouches each (each pouch contains five daily units) will be placed in a 4×2 configuration in a corrugated shipping container that constitutes 1 case. Approximately 50 cases will be placed on a pallet. The shipper will be sufficient to contain the rations and allow stacking to five pallets high in similar environmental temperature and relative humidity extremes as experienced in the Guam, Italy, and Maryland storage facilities used by the U.S. Agency for International Development.
- **Airdrop** – Pouches (five units each) or bundles (five pouches each) may be packaged for low-altitude airdrop using appropriate package protection to simultaneously provide impact protection for the EFP and reduce the terminal velocity to a level that prevents injury to recipients on the ground. Testing must be conducted to verify adequate protection.

Labeling

The secondary and tertiary packaging shall carry simple, graphic instructions on how to open the package and on alternative ways to consume the product (i.e., directly or as a porridge). A disclosure of the energy nutrient (fat, carbohydrate, protein) content by weight, in metric units, must be made on the basis of 1 day's ration (2,100 kcal). In addition, each pouch shall carry a complete list of ingredients, the net weight of the unit, in grams, and any other information required by the purchasing agency.

MISCELLANEOUS INFORMATION

Ingredients may be determined by bid from potential manufacturers to provide the nutritional profile and other characteristics defined above. The product will be distributed among multiple ethnic and cultural groups. Therefore, alcohol or animal products other than milk may not be used. Foods containing known allergens, such as peanuts, should be avoided.

The vitamin and mineral mix must be encapsulated to provide required product shelf life and avoid objectionable odors or flavors.

Recommended ingredients:

- **Cereal base**: wheat flour, corn, oat flakes or flour, rice flour
- **Protein**: soy products, such as concentrates, isolates, or TVP; milk solids, casein, or derivatives
- **Lipid sources**: partially hydrogenated soybean or cottonseed oil, flaxseed oil (source of omega-3 fatty acids), canola oil, sunflower oil
- **Sugars**: sucrose, glucose, high-fructose corn syrup, maltodextrins

- **Baking and leavening agents**, if needed
- **Vitamin and mineral premix** as specified in the nutrient profile (see Table 4-1).

The product must be prepared using Good Manufacturing Practices and maintain suitability as a food for the shelf life of the product.

Note: This performance specification is written to facilitate innovation from suppliers. It is recommended that off-the-shelf ingredients and materials be utilized where possible.

Appendix
Biographical Sketches of Subcommittee Members

Barry L. Zoumas, Ph.D. (*chair*) is the Alan R. Warehime Professor of Agribusiness in the Department of Agricultural Economics and Rural Sociology at The Pennsylvania State University. Dr. Zoumas' academic training is in human nutrition and chemistry. He has direct expertise in development of the type of food product under study. He was Director of Research and Development and later Corporate Vice-President of science and technology at Hershey Foods Corporation, where he was in charge of development of the Hershey bars for the space program before joining Penn State. He is coauthor of a book on candy bars. He also has ample international experience as a visiting scientist in nutrition programs for developing countries with the Food and Agriculture Organization of the United Nations and as an advisor on agricultural development to the U.S. Agency for International Development for the Caribbean and other Latin American countries. He has served as Chairman of the American Institute of Nutrition and several industrial associations, and is a member of the Institute of Food Technologists, the American Association for the Advancement of Science, the American Chemical Society, the American Society for Nutritional Sciences, and the International Food and Agribusiness Management Association.

Lawrence E. Armstrong, Ph.D. is an Associate Professor in the Departments of Physiology, Neurobiology and Exercise Science and of Sport, Leisure, and Exercise Sciences, University of Connecticut. He has a doctorate in human bioenergetics and specializes in physiological responses involving exercise, dietary intervention, heat tolerance, temperature regulation, and acclimatization to heat. His research deals with laboratory and field studies of metabolic, ventilatory, cardiovascular, fluid-electrolyte, and strength perturbations, viewed in light

of physical training, cardiorespiratory fitness, and hydration status. He teaches courses in exercise physiology and metabolism. Dr. Armstrong is a former member of the Committee on Military Nutrition Research. He is also a member of the American College of Sports Medicine, American Physiological Society, and the Aerospace Medical Association.

Jeffrey R. Backstrand, Ph.D. is an Associate Professor in the joint Ph.D. Program on Urban Systems at the University of Medicine and Dentistry of New Jersey. Previous to this position he was Assistant Professor and Director of the Public Health Nutrition Program at the New York University Department of Nutrition and Food Studies. His academic training is in nutritional anthropology. His research interests are sociocultural determinants of food intake and food sources of micronutrients in developing countries. He has conducted research and has supervised several Ph.D. dissertations and M.S. theses dealing with various aspects of food preferences in Korea, Niger, and Mexico. He has written papers on fortification and growth in children in developing countries, with an emphasis on Mexico. He is Chair of the Public Nutrition Research Interest Section, American Society for Nutritional Sciences and a fellow of the Society for Applied Anthropology. He is also a member of the American Anthropological Association, the American Public Health Association, the Association for the Study of Food and Society, and the Council on Nutritional Anthropology

Wanda L. Chenoweth, Ph.D. is Professor of Nutritional Sciences in the Department of Food Science and Human Nutrition, Michigan State University. Her area of expertise is clinical dietetics, and her research interests are in mineral bioavailability and clinical nutrition. She has conducted research also on the effects of processing on the nutritional quality of various food ingredients. Dr. Chenoweth is a member of the American Society for Nutritional Sciences, American Dietetic Association, and the Institute of Food Technologists. She is a member of the Committee on Military Nutrition Research.

Pavinee Chinachoti, Ph.D. is Professor of Food Physico-chemistry, Department of Food Science and Nutrition, University of Massachusetts, Amherst. Dr. Chinachoti teaches food processing at the graduate and undergraduate levels. Her research focuses on characterization of phase transitions and the role of water on food quality and stability, particularly in cereal polymers and products. It includes staling mechanisms, moisture migration, recrystallization of sugars and other carbohydrates, and water activity in relation to microbial spoilage and pathogenic microorganisms, applied to various foods and intermediate moisture foods. She has conducted joint research with the U. S. Army Natick technical group and is a member of the Institute of Food Technologists, Sigma Xi Scientific Research Society, Gamma Sigma Delta Agricultural Honorary Society, Phi Kappa Phi Honorary Society, and Phi Tau Sigma Honorary Society.

Barbara P. Klein, Ph.D. is Emeritus Professor of Foods and Nutrition in the Department of Food Science and Human Nutrition, University of Illinois. Her areas of expertise are sensory perception and evaluation and development of high soy protein foods such as snacks, cereals, and dairy analogs. Her research is in the areas of formula and sensory optimization of food products manufactured

with high protein ingredients, vitamin C content of various foods, nutrient conservation in processed food products, sensory science in general, and in alterations in food quality that occur during storage, processing, and preparation for consumption. Dr. Klein is a member of the Institute of Food Technologists, American Institute of Nutrition, American Chemical Society, American Dietetic Association, and American Society for Testing Materials.

Helen W. Lane, Ph.D., R.D. is Chief Nutritionist and Program Manager for Advanced Human Support Technology at the NASA Johnson Space Center. Dr. Lane has extensive experience on the relationship of diet and exercise and on diet formulation. Her research has focused on nutritional aspects of space flight, nutrient availability, body composition, and nutritional requirements for energy, water, electrolytes, protein, calcium, and iron. Her work as NASA's Advanced Human Support Technologies Program includes innovative work in food science and technologies for extended-duration space flight. She is a member of numerous societies, among them the American Dietetic Association, American Society for Nutritional Sciences, American Society of Clinical Nutrition, Institute of Food Technologists, and Sigma Xi Scientific Research Society.

Kenneth S. Marsh, Ph.D. is President/Research Director of Kenneth S. Marsh & Associates, Ltd. While with the Packaging Science Department at Clemson University, Dr. Marsh held the CRYOVAC Chair, the first endowed position in Packaging Science in the United States. Dr. Marsh's academic background is in food science, with specialization in food packaging. His research focuses on utilizing physical chemistry, food science, and packaging to extend the useable life of foods, reduce distribution damage, and improve the deliverable food supply in poor countries. He has 12 years of food industry experience (Quaker Oats Company, Ball Corporation, Pillsbury Company, and Thomas J. Lipton, Inc.), and 16 years of experience as a private consultant in food packaging. Dr. Marsh also has had extensive international exposure as a lecturer in various aspects of food packaging and has participated in packaging-related activities of Research & Development Associates. He is a member of the Institute of Food Technologists and the Institute of Packaging Professionals, and is a Fellow of the Institute of Packaging Professionals

Marjatta Tolvanen, Ph.D. is Project Officer in Nutrition in Emergencies with the United Nations Children's Fund, New York. She is an international nutritionist from Finland in charge of monitoring nutritional emergencies in various countries and coordinating emergency responses. Dr. Tolvanen also conducts assessments of the impact of using special feeding products for emergency feeding and oversees development of such products for UNICEF. She has international experience in Vietnam and Nepal.

Pennington Biomedical Research Center

June 2001 Program Review

INSTITUTE OF MEDICINE

Pennington Biomedical Research Center

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Subcommittee on Program Review of the Pennington Biomedical Research Center

Committee on Military Nutrition Research

Food and Nutrition Board

INSTITUTE OF MEDICINE

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NOTICE: The project that is the subject of this report was approved by the Governing Board of the National Research Council, whose members are drawn from the councils of the National Academy of Sciences, the National Academy of Engineering, and the Institute of Medicine. The members of the committee responsible for the report were chosen for their special competences and with regard for appropriate balance.

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The serpent has been a symbol of long life, healing, and knowledge among almost all cultures and religions since the beginning of recorded history. The serpent adopted as a logotype by the Institute of Medicine is a relief carving from ancient Greece, now held by the Staatliche Museen in Berlin.

"Knowing is not enough; we must apply.
Willing is not enough; we must do."
—Goethe



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The Committee on Military Nutrition Research's (CMNR) Subcommittee on Program Review of the Pennington Biomedical Research Center (PBRC) expresses its sincere appreciation to Donna H. Ryan, M.D., principal investigator of the PBRC military nutrition grant, and Claude Bouchard, Ph.D., director of PBRC, for their hospitality during the site visit, the excellent organization of the review procedures, and the background material provided prior to the visit. The subcommittee also thanks the PBRC scientists who presented summaries of their activities and proposed plans for the future, patiently answered questions, and were very forthcoming on requested information. Additionally, special thanks are extended to the PBRC staff who provided transportation to and from the hotel and made special arrangements for the subcommittee whenever requested.

The chair of the subcommittee wishes to acknowledge the excellent contribution of Food and Nutrition Board staff, especially Mary I. Poos, Ph.D., study director, and Tazima Davis, senior project assistant, for the organization of the review and handling of the many details necessary for synthesis and publication of the report. The chair also expresses deepest appreciation to the members of the subcommittee who participated extensively in the review, discussions, and preparation of the summary and recommendations in this report.

This report has been reviewed in draft form by individuals chosen for their diverse perspectives and technical expertise, in accordance with procedures approved by the National Research Council's Report Review Committee. The purpose of this independent review is to provide candid and critical comments that will assist the institution in making its published reports as sound as possible and to ensure that the report meets institutional standards of objectivity, evidence, and responsiveness to the study charge. The review comments and draft manuscript remain confidential to protect the integrity of the deliberative process. The subcommittee wishes to thank the following individuals for their review of this report:

Gary D. Foster, University of Pennsylvania
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Robert Neshiem, Berkley, California
Jerrold Olefsky, University of California at San Diego
Dale A. Schoeller, University of Wisconsin-Madison

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Although the reviewers listed above have provided many constructive comments and suggestions, they were not asked to endorse the conclusions or recommendations nor did they see the final draft of the report before its release. The review of this report was overseen by Judith S. Stern, Sc.D., University of California at Davis, appointed by the Institute of Medicine, who was responsible for making certain that an independent examination of this report was carried out in accordance with institutional procedures and that all review comments were carefully considered. Responsibility for the final content of this report rests entirely with the authoring committee and the institution.

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Executive Summary

The purpose of the site visit and program review that are the subject of this report was to assist the Military Nutrition Division (MND) of the U.S. Army Research Institute of Environmental Medicine (USARIEM) in its review of a proposed research program submitted by the Pennington Biomedical Research Center (PBRC) for military funding. The focus of the research proposal reviewed in this report is *Sustaining Performance and Healthy Weight in Career Military Personnel.*

FOCUS OF THE REPORT

This report focuses on the research program proposed by PBRC in support of the military nutrition research program at USARIEM. The Army staff at USARIEM, in consultation with the Institute of Medicine's (IOM) Committee on Military Nutrition Research (CMNR), periodically reviews and makes recommendations on research projects proposed by the PBRC.

This report and the site visit to PBRC is the fifth that CMNR has performed at the request of the U.S. Army Medical Research and Materiel Command (see Appendix B). Because the focus of this new proposal is on maintaining performance and healthy weight in career military personnel, a subcommittee with expertise in body composition, energy metabolism, physical activity, and eating behavior was appointed to conduct the site visit and proposal review.

The CMNR subcommittee was asked to provide recommendations to the Army concerning the new PBRC proposal with respect to the adequacy of staffing and facilities, as well as the appropriateness of the goal and design of each specific research task relative to the military's needs with respect to weight maintenance, physical activity, energy expenditures, and eating behavior of military personnel.

HISTORICAL BACKGROUND

Committee on Military Nutrition Research

The CMNR was established in October 1982 following a request by the Assistant Surgeon General of the Army that the National Academy of Sciences form a committee to advise the DoD

on the need for, and conduct of, nutrition research and related issues. The committee works under the guidance of IOM's Food and Nutrition Board.

As a standing committee, the membership of CMNR changes periodically, however the disciplines represented consistently have included human nutrition, nutritional biochemistry, performance physiology, food science, dietetics, psychology, and clinical medicine. For issues that require broader expertise than exists within the committee, CMNR has convened workshops, utilized consultants, or appointed subcommittees with expertise in the desired area to provide additional state-of-the art scientific knowledge and informed opinion to aid in deliberations.

The Pennington Biomedical Research Center

The PBRC was founded in 1980 when C.B. "Doc" Pennington and his wife, Irene, donated $125 million to Louisiana State University (LSU) for its establishment. At the time the gift was made, it was the largest single donation to an educational institution in the United States. The PBRC is a member of the LSU system, and as such is linked administratively to the LSU Medical Center, LSU Agricultural Center, and the LSU main campus in Baton Rouge. The PBRC's major sources of funding currently include state funds, federal grants, indirect costs from grants, and other (e.g., corporate, foundations, gifts). The three major sources of federal funding are DoD, the National Institutes of Health, and the U.S. Department of Agriculture.

Under the initial Congressional appropriation in 1988 of DoD funds for the PBRC, the U.S. Army awarded a grant entitled *The Effect of Food, Diet, and Nutrition on Military Readiness and Preparedness of Army Personnel and Dependents in a Peacetime Environment.* The total grant award was $3.8 million. A second DoD appropriation in 1992 awarded a grant entitled *Military Nutrition Research: Six Tasks to Address Medical Factors Limiting Soldier Effectiveness* to the PBRC for a total of $11.4 million. In 1997, the PBRC received another five-year grant award from the Army entitled *Military Nutrition Research: Eight Tasks to Address Medical Factors Limiting Soldier Effectiveness*. The total award for this grant was $16.7 million. The proposal on nutrition research activities reviewed in this report are to be conducted at PBRC with funds earmarked for military nutrition research activities at the PBRC in the 2001 Defense Appropriations Bill. The primary focus of the current proposal is on *Sustaining Performance and Healthy Weight in Career Military Personnel.*

THE PROPOSED RESEARCH

Research at the PBRC is focused on a single mission—promoting healthier lives through nutritional research and preventive medicine. The new 5-year proposal to the DoD focuses specifically on the rising trend of overweight in career military personnel, and methodology for remediation and reversal of this trend. This issue is of direct concern for the military, faced with shortfalls in recruitment goals and loss of trained personnel due to failure to meet body composition and fitness standards. The research as outlined in the preproposal provided to the subcommittee is summarized in the following sections. A total of seven specific tasks were developed by PBRC to meet DoD objectives.

Task 1: Sustaining Performance and Healthy Weight in Career Military Personnel

The purpose of this task is to develop a methodology that the military can use to help career personnel achieve weight-related fitness standards. This project has two phases. The goals of Phase 1 are to: (a) develop and evaluate a clinic-based weight management program for military personnel that is grounded in behavior theory and results of current lifestyle behavior modification research; and (b) develop an Internet-based alternative to the clinic-based program that is founded on the same behavior modification principles but designed to be similar to a home-study course. Phase 2 is to evaluate the relative efficacy of the clinic-based and Internet-based programs in a randomized controlled clinical trial. This trial will be conducted at Fort Bragg, North Carolina, which is home to approximately 50,000 military personnel with a base population that can be relatively stable. (See Appendix A for further description of this task.)

Task 2-A: Metabolic Understanding of Energy Balance: Variability in Response to Perturbations in Energy Balance

This task comes under the PBRC proposal's general task 2 on clinical studies in health and performance enhancement. The mission of task 2 overall is to better understand the interactive process between physiological functions, genetic makeup, and environmental conditions that favor weight gain; and to identify the most appropriate dietary regimen for achieving peak physical and cognitive performance while maintaining ideal body weight.

Task 2-A proposes to examine the variation among individuals in degree of body weight gain or body weight loss in response to overfeeding or negative energy balance. Task 2-A has three goals: (1) to determine the effect of three days of over- or underfeeding on 24-hour energy expenditure in obese-prone and obese-resistant individuals, (2) to relate the responses to autonomic nervous system activity, and (3) to identify differences in adipose tissue and skeletal muscle gene expression in response to these dietary perturbations. This task aims to identify genotypic correlates responsible for the phenotypic variations seen in individual responses to over and underfeeding.

Task 2-B: Influence of Dietary Fat on Training and Performance

This task proposes to examine the impact of dietary fat on training adaptations and exercise performance in both men and women. One study will examine the influence of two levels of dietary fat (20 percent and 35 percent) in combination with exercise training on aerobic power, endurance, and body composition outcomes of healthy but previously inactive individuals. A second study will evaluate the effect of a controlled, very low-fat (10 percent) and moderate-fat (35 percent) recovery diet on the quantity of intramuscular lipid and glycogen stores following a bout of prolonged moderate exercise, and the effect of depleted intramuscular fat on subsequent performance of trained endurance athletes.

Task 2-C: A School/Community-Based Intervention to Prevent Adult Obesity in Overweight Adolescents: A Three-Year Randomized and Controlled Trial

This task proposes to develop and evaluate a community/school-based intervention program for at-risk adolescents (i.e., overweight ninth-graders) to prevent the development of adult obesity (i.e., by twelfth grade). The volunteer subjects and family members will be required to attend

a 90-minute educational session once weekly after school for one year, and then once a month for the next two years. The control subjects will attend standardized health information classes once a month after school for three years. Annual assessments of weight, body composition, blood pressure, and fitness of subjects at both the intervention and control schools will evaluate the effects of the program.

Task 2-D: Functional Genomics

This task includes a range of genetic studies to examine the central (i.e., central nervous system) and peripheral (e.g., fat and muscle) regulation of food intake and energy expenditure. These studies will include the expression level of candidate genes in fat and muscle, and the impact of exercise and diet on gene expression to identify genetic pathways that influence energy homeostasis and contribute to the development of obesity. These goals will be pursued utilizing biopsy fat and muscle tissues samples collected as part of tasks 2-A and 2-B and application of state-of-the-art molecular genetic techniques.

Task 3: Nutrition, Stress, and Body Weight Regulation

Task 3 proposes to employ rodents as animal models in studies addressing the multiple mechanistic pathways that control food intake. The objectives are to (1) identify neurochemical and physiological mechanisms of stress, hunger, food reward, satiety, and spontaneous disruption of eating behaviors that are associated with maintenance of body weight and composition, and (2) identify nutritional interventions that modify or prevent weight gain or regain, and stress- and exercise-induced disruption of homeostasis and behavior in experimental animals. These objectives will be accomplished through a series of studies that include (1) identification of genes in brain tissue that change consistently with over- or under-feeding, (2) identification of neurochemical markers for glucose-sensing cells in the hind brain, (3) effects of different foods on dopamine neuron-containing circuits in the brain, (4) the role of cholecystokinin in generation of satiety signals in the brain, (5) identification of neuronal signaling molecules in the brain that mediate stress-induced eating behaviors, and (6) the effect of dietary interventions on stress-induced eating behaviors.

Task 4: Laboratory for Human and Food Samples

The Laboratory for Human and Food Samples provides the analytical support for tasks 1, 2, and 7, and equally important, also supports studies conducted by USARIEM. Types of support provided by this lab include: (1) more than 70 different tests for nutrients, indicators of nutrition status, and measures of biochemical and physiological function, (2) provision of PBRC personnel on-site for military field studies to collect, log, and process samples, (3) development of testing protocols, development and validation of specially requested analyses, intensive quality control efforts on analytical methodologies, and (4) compilation of results in databases or spreadsheets, and assistance in writing protocols and results for publication.

Task 5: Stable Isotope Laboratory

There are three studies with USARIEM in the planning stages requiring the support of the Stable Isotope Laboratory: two focused on weight gain and energy expenditure, and one examining total daily energy expenditure of Marines to determine the length of time they can subsist on

the Meal, Cold Weather ration. Additional studies will use doubly labeled water to validate the respiratory chambers and other methods of determining energy expenditure. The laboratory proposes to expand its activities into the use of other stable isotopes to examine body tissue build-up and catabolism through monitoring the metabolism of protein, fat, and carbohydrate.

Task 6: Nutrient Database Laboratory

The Nutrient Database Laboratory, which includes Dietary Assessment and Counseling and Nutrient Data Systems, is designed to support USARIEM field studies and PBRC research. This laboratory provides computerized nutrient analyses of recipes, menus, and dietary intakes of soldiers. Laboratory personnel also assist in data collection at field sites for military studies. A major objective of this current proposal is to translate PBRC expertise on the nutrient database to in-house USARIEM personnel and assist USARIEM in identifying computer-support personnel. The laboratory will provide dietary intake assessment and dietary counseling services at Fort Bragg for Task 1.

Task 7: Metabolic Unit

The Metabolic Unit contains 14 beds and 2 indirect calorimeters. It is served by a metabolic kitchen capable of producing standardized controlled diets for up to 110 subjects per day, and is staffed by a seasoned clinical trial team. The Metabolic Unit will support Tasks 2-A and 2-B of this proposal although no specific in-patient projects have been proposed at this time.

CONCLUSIONS AND RECOMMENDATIONS

The major conclusions and recommendations of the subcommittee are highlighted here. More detailed and task-specific recommendations can be found in Chapter 2.

General Conclusions

Significant progress has been made in staffing the PBRC facility. The staff now includes 385 physicians, researchers, technicians, support personnel, and student workers. The laboratories are well equipped for the conduct and support of research in areas of interest to the military. The PBRC has also made significant progress in attracting other sources of research funding (both federal and corporate) in support of PBRC's research programs.

Recent additions to the PBRC staff will significantly enhance the new focus on weight management and energy metabolism. In addition, the PBRC has extensive experience in large multicenter weight-loss clinical trials, as well as Internet-based interventions. The PBRC indirect calorimetry chambers, stable isotope lab, metabolic kitchen, and food chemistry lab, as well as the fitness facility, should be able to provide important new data for the military as well as provide support to military studies on energy balance and weight management. The PBRC clinical research facilities are vital to the objectives of the MND at USARIEM.

An overriding concern about this proposal is the extent to which the work adequately addresses issues germane to the military population and its environment, as distinct from the civilian population. It is important that research tasks of the PBRC be reviewed and changed as needed to assure that military-funded research at the PBRC remains mission oriented.

Based on the very preliminary nature of the new five-year grant proposal that was ultimately submitted to the Subcommittee for review, and subsequent discussion with Army representatives

and the PBRC principal investigator for the proposed project, it is clear that a closer liaison between USARIEM and PBRC personnel would be helpful to all concerned.

General Recommendations

- ***A much closer and on-going interaction between the PBRC and the Military Nutrition Division is needed.*** It is of paramount importance that DoD-funded research at PBRC is reviewed regularly to assist them in maintaining a program that is relevant, focused, and mission-oriented with respect to the needs of the Armed Services. As a beginning, such an effort might be facilitated by a regular, more formal orientation by DoD that includes representatives of all branches of the Armed Services, to educate all PBRC investigators involved in military-funded research with respect to military needs and processes. The military should also consider the possible addition of a PBRC scientist to DoD's Nutrition Committee. The establishment of post-doctoral positions for military personnel at the PBRC would also help to strengthen the interface between the two organizations.
- ***Consideration should be given to widening the selection of subjects to include all military services instead of limiting selection to Army personnel.*** Although it can be reasoned that studies conducted using personnel from one service would be applicable to the other services, a case can be made that there are important differences between personnel from different military services that may have an impact on study results. These include the type and level of activity, eating environment, service culture, the emphasis placed on weight programs by the service, average age of personnel, and the level of education and training. These factors may not have a significant impact on metabolism but may influence eating behavior.
- ***Imaging methods, such as magnetic resonance imaging and/or computerized axial tomography should be incorporated into the core facilities of the PBRC as technologies central to completing the PBRC mission for the military.*** This would maximize the information obtained from the genotype–phenotype studies that will form the foundation of much of the PBRC weight management research.
- ***To facilitate implementation of research, a single Institutional Review Board (IRB) review by the local IRB should be considered adequate for the purposes of human research conducted on civilians under the direction of PBRC faculty.*** DoD should implement an annual review of the PBRC IRB's membership, procedures, and decisions on DoD-funded projects to insure the proper levels of protection are followed. Under federal law, DoD, as the granting agency, would retain certain rights in reference to monitoring proper constitution and conduct of the local IRB, so assurance and verification of adequate compliance with national policies concerning human safety would be retained.

Task-Specific Recommendations

Task 1: Sustaining Performance and Healthy Weight in Career Military Personnel

It is strongly recommended that an initial phase of this task should investigate the specific characteristics of the target military population. This should include: their levels of knowledge regarding nutrition, exercise, and weight control; what issues related to diet and physical activity are germane to them; the special characteristics of the military environment generally and the test military base specifically that may impact on weight and efforts to control it.

Phase 1 should be the development of military-oriented, nonclinical weight loss programs, one Internet-based and one non-Internet based. Phase 2 should be the comparison of these two programs. PBRC researchers should evaluate existing military and civilian Internet weight-loss programs and adapt them as needed rather than developing a totally new Internet program.

Task 2-A: Metabolic Understanding of Energy Balance

For this study to be of military relevance, it must include men as well as women between the ages of 18 and 45 as study subjects.

The inclusion of a bioinformatics specialist as part of the research team is essential for interpretation of the large amounts of genetic information generated in this and the following task.

Task 2-B: Influence of Dietary Fat on Training and Performance

The design of the studies in this task should be reconsidered to alter the dietary fat levels and to add a high-carbohydrate "wash-out" period. Addition of the high-carbohydrate period will equalize muscle glycogen content so it does not confound the impact of intramuscular lipids on physical performance.

An experienced exercise biochemist should be involved in this task from the beginning, rather than in Year 2.

Task 2-C: A School/Community-Based Intervention to Prevent Adult Obesity in Overweight Adolescents

Consideration should be given to increasing the sample size. A study requiring a three-year commitment of weekly and monthly sessions for an entire family is likely to have a high dropout rate.

Consideration should also be given to assessing a group of nonparticipants at each school to determine if there is a spillover effect to those in the same school or community as the test subjects.

Task 2-D: Functional Genomics

The participation of a statistical geneticist and a bioinformatics specialist is crucial for adequate analysis and interpretation of the large amounts of genetic data generated in this task.

The proposed list of candidate genes should be re-evaluated for applicability in population screening.

Task 3: Nutrition, Stress, and Body Weight Regulation

A rodent model of stress-induced eating should be included with the model of stress-induced anorexia and nonstressed controls. Inclusion of a model that has been previously researched (e.g., the tail-pinch model) would provide additional knowledge regarding neuropeptide changes that might be expected.

Task 4: Laboratory for Human and Food Samples

Accreditation of nutrient analytical techniques by an outside authoritative organization, similar to what has been done for human samples, would provide more assurance of the quality of this data.

Task 5: Stable Isotope Laboratory

Expertise in use of stable isotopes (other than doubly labeled water) should be expanded to strengthen work in isotope analysis of nutrient metabolism.

Task 6: Nutrient Database Laboratory

Efforts should be continued to improve methodologies for gathering accurate intake data.

Task 7: Metabolic Unit

As recommended in Task 1, a closer and ongoing relationship between PBRC staff and military researchers is essential to assure these outstanding facilities will be available when needed for military research studies.

1

Introduction and Background

This brief report, *Pennington Biomedical Research Center: June 2001 Program Review*, is the latest in a series of reports of the activities of the Committee on Military Nutrition Research (CMNR) and its various subcommittees. Under the guidance of the Food and Nutrition Board (FNB), Institute of Medicine (IOM), the National Academies, CMNR responds to the commander of the U.S. Army Medical Research and Materiel Command (USAMRMC) concerning issues brought to the committee through the Director of Research Area III at USAMRMC headquarters, and the Military Nutrition Division (MND) of the U.S. Army Research Institute of Environmental Medicine (USARIEM) at Natick, Massachusetts.

The purpose of this site visit and program review was to assist the MND in its review of a proposed research program submitted by the Pennington Biomedical Research Center (PBRC) as a follow-on to Grant No. DAMD 17-97-2-7013 awarded April 1, 1997, titled *Military Nutrition Research: Eight Tasks to Address Medical Factors Limiting Soldier Effectiveness*. The proposed nutrition research activities are to be conducted with funds earmarked for military nutrition research activities at PBRC in the 2001 Department of Defense (DoD) appropriations bill. The current proposal's primary focus is on sustaining performance and healthy weight in career military personnel.

PBRC is a member of the Louisiana State University (LSU) system and, as such, is linked administratively to the LSU Medical Center, LSU Agricultural Center, and LSU main campus in Baton Rouge. PBRC's major sources of funding (as of fiscal year 1999) include state funds (30 percent), federal grants (38 percent), indirect costs from grants (13 percent), and others (e.g., foundations, gifts—19 percent). The three major sources of federal funding are DoD, the National Institutes of Health, and the U.S. Department of Agriculture.

HISTORY OF THE COMMITTEE

CMNR was established in October 1982 following a request by the Assistant Surgeon General of the Army that the Board on Military Supplies of the National Academy of Sciences form a committee to advise the DoD on the need for, and conduct of, nutrition research and related issues. This newly formed committee was transferred to the oversight of FNB in 1983. The committee's tasks are to identify factors that may critically influence the physical and mental performance of military personnel under all environmental extremes, to identify knowledge gaps,

to recommend research that would remedy these deficiencies as well as approaches for studying the relationship of diet to physical and mental performance, and to review and advise on military feeding standards.

As a standing committee of IOM, the membership of CMNR changes periodically, however the disciplines represented consistently have included human nutrition, nutritional biochemistry, performance physiology, food science, dietetics, psychology, and clinical medicine. For issues that require broader expertise than exists within the committee, CMNR has convened workshops, utilized consultants, or appointed subcommittees with expertise in the desired area to provide additional state-of-the art scientific knowledge and informed opinion to aid in the deliberations.

FOCUS OF THE REPORT

This report focuses on the research program conducted at PBRC in support of the military nutrition research program at USARIEM. Under an initial congressional appropriation in 1988, the U.S. Army awarded Grant No. DAMD 17-88-Z-8023 entitled *The Effect of Food, Diet, and Nutrition on Military Readiness and Preparedness of Army Personnel and Dependents in a Peacetime Environment.* The total grant award was $3.8 million. A second appropriation in 1992 resulted in the awarding of U.S. Army Grant No. DAMD-92-V-2009 entitled *Military Nutrition Research: Six Tasks to Address Medical Factors Limiting Soldier Effectiveness* to PBRC for a total of $11.4 million. In 1997, PBRC received another five-year award from the Army, Grant No. DAMD-17-97-2-7013, entitled *Military Nutrition Research: Eight Tasks to Address Medical Factors Limiting Soldier Effectiveness*. The total award for this grant was $16.7 million.

The Army staff at USARIEM, in consultation with CMNR, periodically reviews and makes recommendations on research projects proposed by PBRC. In June 1989, CMNR was first asked by the Army to review research plans of PBRC funded through the initial DoD appropriations. A letter report with the CMNR's recommendations was submitted to the Army. In September 1991, as the initial three-year grant to PBRC was nearing conclusion, the Army requested CMNR to review the progress of PBRC during the three-year grant. This review was submitted to the Army in May 1992. Immediately following this, in June 1992, the Army asked CMNR to review new research plans proposed by PBRC for renewal of its Army funding. CMNR was to assist the Army in identifying research activities that fell within the mandate of the congressional appropriation. This second appropriation was for approximately $13 million over five years. In 1996, as the five-year contract was nearing its end, the Army again requested CMNR to review the progress of PBRC's military nutrition research program, as well as the research proposed by PBRC for the next five years.

In March 2001, LTC Karl Friedl, Ph.D., Director of the Military Operational Medicine Program and Grant Officer Representative for the USAMRMC for Grant No. DAMD17-99-1-9478 to the National Academies for support of CMNR, requested that the committee again conduct a site visit of PBRC and review the new five-year proposal, *Sustaining Performance and Healthy Weight in Career Military Personnel,* for Army funding. CMNR was asked to provide recommendations to the Army concerning the adequacy of staffing and facilities, and the appropriateness of the goal and design of each specific research task as it relates to military needs with respect to weight maintenance, physical activity, energy expenditures, and eating behavior of military personnel (see Appendix A).

This site visit to PBRC is thus the fourth that CMNR has performed at the request of USAMRMC. Because the focus of this new proposal is on maintaining performance and healthy weight in career military personnel, a subcommittee with expertise in body composition, energy

metabolism, physical activity, and eating behavior was appointed to conduct the site visit and proposal review.

SUBCOMMITTEE PROCEDURE

Prior to the site visit, the subcommittee reviewed (1) the preproposal requesting funding for continuation of the agreement between PBRC and USAMRMC for military nutrition studies at PBRC for an additional five years, beginning April 1, 2002, as well as other background materials; (2) the Pennington Biomedical Research Center Annual Report for 2000 submitted by the principal investigator Donna H. Ryan, M.D., to the Army; and (3) past CMNR reviews of PBRC activities in the form of reports transmitted to USAMRMC. The preproposal and portions of previous CMNR reviews of PBRC are included in the appendixes of this report.

Activities of the CMNR's Subcommittee on Program Review of the Pennington Biomedical Research Center during the site visit included (1) initial discussion of the preproposal in closed session prior to input from PBRC staff, (2) presentations by PBRC staff on details of the objectives and methods for each of the new tasks proposed, (3) discussion of the progress of previous grants and the new proposal with the Army sponsors, (4) discussion and evaluation of the proposed research in closed session, and (5) development of a brief report to the Army stating the subcommittee's conclusions and recommendations.

Subsequent to subcommittee approval of the final draft, the report was reviewed in confidence by a separate and anonymous scientific review group in accordance with National Academies guidelines. The subcommittee evaluated reviewer comments and incorporated their suggestions where appropriate. This report is thus a thoughtfully developed presentation that incorporates the scientific expertise and opinion of the Subcommittee on Program Review of the Pennington Center and CMNR.

The remainder of this report provides the subcommittee's evaluation of the proposed research presented to it and to Army personnel at the PBRC during the June 2001 site visit.

2

Project Review

This chapter presents general comments on the Pennington Biomedical Research Center (PBRC) program, followed by a review of the specific tasks included in the preproposal for a new five-year grant, and the subcommittee's critique of the proposed research.

GENERAL COMMENTS

The subcommittee is impressed with the excellence of the laboratory and clinical research facilities at PBRC. Significant progress has been made in staffing this facility, and the staff now includes 385 physicians, researchers, technicians, support personnel, and student workers. The laboratories are well equipped to conduct and support research in areas of interest to the military. PBRC has also made significant progress in attracting other sources of research funding (both federal and corporate) in support of its research programs.

Research at PBRC is focused on a single mission—promoting healthier lives through nutritional research and preventive medicine. The new five-year proposal to the Army focuses on the rising trend of overweight in career military personnel and a methodology for remediation and reversal of this trend. This issue is of direct concern to the military, faced with shortfalls in recruitment goals and loss of trained personnel due to failure to meet body composition and fitness standards.

Recent changes in administrative and research staff will have a significant positive impact on PBRC's ability to contribute to military needs in the area of weight management. In August 1999, Dr. Claude Bouchard was appointed as the new director of PBRC. Dr. Bouchard's research career has focused on the genetic and molecular basis of obesity, metabolic complications associated with obesity, and the genetic and molecular basis of the response to physical activity. Since his arrival, PBRC has generated a strategic plan for growth that includes a doubling of its operating budget by 2005, an increase in faculty and support personnel from the current 385 to 750, and the development of a postdoctoral training program. Construction of a new basic sciences building has already begun.

OVERVIEW OF PROJECT TASKS

As described in Chapter 1, PBRC is requesting funding for an additional five years to conduct research concerning issues of nutrition in weight control, weight management, and physical activity of relevance to the military. The research as outlined in the preproposal consists of the following:

- sustaining performance and healthy weight in career military personnel;
- health and performance enhancement;
- nutrition stress and body weight regulation; and
- providing laboratory support for field studies.

A total of seven specific tasks were developed by PBRC to meet military objectives. The specific tasks that the subcommittee was asked to review follow:

1. Sustaining performance and healthy weight in career military personnel
2. Clinical studies in health and performance enhancement
   A. Metabolic understanding of energy balance
   B. Influence of dietary fat on training and performance
   C. Prevention of obesity
   D. Functional genomics of energy balance and training
3. Nutrition, stress, and body weight regulation
4. Laboratory For Human and Food Samples
5. Stable Isotope Laboratory
6. Nutrient Database Laboratory
7. Metabolic Unit

Task 1: Sustaining Performance and Healthy Weight in Career Military Personnel

Project Summary

The principal investigator for this task is Donald Williamson, Ph.D. Dr. Williamson is a psychologist who has published extensively in the area of behavior modification and treatment of eating disorders. He is also director of the Psychological Services Center at Louisiana State University in conjunction with his research appointment at PBRC.

The overriding purpose of this task is to develop a methodology that the military can use to help career personnel achieve weight-related fitness standards. This project has two phases. The goals of Phase 1 are to (1) develop and evaluate a clinic-based weight management program for military personnel that is grounded in behavior theory and the results of current life-style behavior modification research, and (2) develop an Internet alternative to the clinic program that is based on the same behavior modification principles but designed to be similar to a home-study course. The goal of Phase 2 is to evaluate the relative efficacy of the clinic and Internet-based programs in a randomized controlled clinical trial. This trial will be conducted at Fort Bragg, North Carolina, which is home to approximately 50,000 military personnel with a base population that can be relatively stable. (See Appendix A for further description of this task.)

General Comments

Overweight and obesity appear to be on the rise in the armed services, and as the PBRC proposal notes, failure to meet weight and performance standards results in about 4,000 to 5,000 personnel separations each year across all branches of the Armed Services. Current practices for evaluation and remediation are clearly not as effective as desired. The problem will likely increase in years to come as population rates of obesity continue to increase, as duties become more sedentary, and presumably, as the military continues to induct significant numbers of recruits who are already overweight by accession standards in order to meet recruitment goals.

Dr. Williamson and PBRC staff have considerable experience and expertise in the assessment and treatment of obesity and overweight in the clinical setting. In particular, Dr. Williamson is a recognized authority in the cognitive–behavioral management of obesity. Further, through existing and prior Army grants, he and PBRC have experience in studying the weight status and dietary intake of military personnel. Access to large amounts of military intake data and the nutrient content of a wide range of military rations is available through the PBRC's Nutrient Database Laboratory. Staff assigned to this task also have experience in developing an Internet-based obesity prevention program for adolescents.

An overriding concern about the proposed work is the extent to which it takes into account the special nature of the targeted environment and population, as distinct from civilian clinical populations. The target population is younger, healthier, more fit, and nontreatment seeking, with a much higher proportion of males, compared to the clinical groups on whom most of the extant obesity treatment literature is based. All of these participant characteristics suggest that there should be ways in which the proposed interventions differ from those offered the typical participant in clinical trials. Further, military life poses different constraints and opportunities regarding weight control behaviors than does the civilian world. The staff has the knowledge to distill the most important elements from civilian clinical treatment programs and apply them to the military setting, and should be encouraged to conduct the necessary preliminary studies to determine how this can best be done. A second general concern is the need for project staff to avoid trying to develop, on their own, Internet resources and methods that have already been produced in other settings. Development and maintenance of Internet-based programs are time consuming and costly, and they require ongoing technical support as well as evaluation and updating on a regular basis. There are a number of interactive, Internet-based programs available commercially that should be evaluated for potential modification to meet military needs.

Specific Comments, Concerns, and Questions

The committee strongly recommends an initial phase to investigate the following: the specific characteristics of the target population; their levels of knowledge regarding nutrition, exercise, and weight control; what issues are germane to them; and the special characteristics concerning the general military environment and the specific military base to be tested that may impact on weight and efforts to control it. This initial assessment should also try to identify those at greatest risk, as has been proposed by the applicants—those who are overweight at the time of entry into the military. PBRC staff may profit from consulting with professionals who have experience in working with similar groups of participants, such as young males who are perhaps overweight and/or unfit but not obese, or even athletes. The committee suggests that in lieu of an uncontrolled assessment of a typical clinical weight-loss program, resources might be better utilized if these types of information were collected and integrated with the *essence* of the lessons

learned from clinical intervention trials, to develop and assess a program that has been designed from the ground up to be effective in the military setting.

Regarding the Internet program, the committee recommends appreciation of the labor-intensive nature of developing the supporting software and methodologies from scratch. The staff should be encouraged to identify existing resources from noncommercial and commercial organizations that could be adapted and utilized in the Internet-based intervention. For example, Blackboard and other teaching-focused software, publishing software, other military Internet-based education activities, and other sources may have products that are applicable.

The subcommittee believes that an Internet-based program could serve as a means of accomplishing modest weight loss and weight maintenance in the military setting. Several recent studies have compared Internet-based treatment to in-person treatment in civilian subjects (Harvey-Berino et al., 2002; Tate et al., 2001; Wylie-Rosset et al., 2001). Although one study found no differences in weight loss between the two approaches (Harvey-Berino et al., 2002), the other study found an Internet education treatment produced significantly less weight loss than an Internet-based program that was therapist-led (Tate et al., 2001). In a related vein, it will be important from a cost-effectiveness perspective to minimize the amount of counselor time spent on one-to-one Internet communication (Wylie-Rosset et al., 2001).

Recommendations

The committee recommends that Phase 1 of this project be reoriented as noted above and that Phase 2 be adjusted based on the results of Phase 1. Thus, ***Phase 1 should be focused on utilizing PBRC's and military data and resources to determine how to construct nonclinical, military-based weight loss programs for the typical military person in need of such a program, with one program designed for Internet application and another for non-Internet delivery.*** The subcommittee believes that the military would obtain far greater benefits from a non-clinical program than from a clinical program, especially since they most frequently are addressing nonobese overweight individuals, and are also targeting a highly mobile population.

The subcommittee supports the planned research on the use of the Internet for providing individuals in the military with assistance in weight control and weight reduction. ***It is recommended that PBRC investigators study carefully the existing information on civilian programs as well as information obtained by the Air Force, and the Navy with programs testing the use of the Internet for providing individuals with weight control information and counseling.*** These programs were used to help individuals at remote locations where dietitians or other medical personnel were not available and also to provide additional assistance to personnel assigned to a mandatory weight reduction program. Among the several programs that have used the Internet are the Air Force LEAN program, an Army study at Tripler Hospital, and a Navy program conducted at the Navy hospital in San Diego. Phase 2 could then focus on a direct comparison of the two approaches.

Additional recommendations include examination of the necessity for the high proposed staffing levels, coordination with any programs resulting from other CMNR recommendations with respect to basic training nutrition education, assessment of easily identifiable predictors of weight problems, and collection of low-cost data that may have relevance for health beyond the military (e.g., factors predicting weight gain once in the service and factors moderating the impact of weight status on health).

Task 2-A: Metabolic Understanding of Energy Balance: Variability in Response to Perturbations in Energy Balance

Project Summary

This task comes under PBRC proposal's general Task 2, *Clinical Studies in Health and Performance Enhancement.* The mission of Task 2 overall is to better understand the interactions between physiological functions, genetic makeup, and environmental conditions that favor weight gain. In addition, the overall task is designed to identify the most appropriate dietary regimen for achieving peak physical and cognitive performance while maintaining ideal body weight.

Task 2-A is designed to examine the variation among individuals in degree of body weight gain or body weight loss in response to acute overfeeding or negative energy balance. It has been established that individuals who can increase their metabolic rate and fat oxidation in response to overfeeding are less likely to become obese. However, mechanisms underlying this variability are unknown. Task 2-A has three goals: (1) to determine the effect of three days of over- or underfeeding on 24-hour energy expenditure in obese-prone and obese-resistant individuals, (2) to relate the responses to autonomic nervous system activity, and (3) to identify the changes in adipose tissue and skeletal muscle gene expression in response to these acute dietary perturbations. Specifically, then, the project aims to identify genotypic correlates responsible for the phenotypic variations seen in individual responses to acute over- and underfeeding. Once these are identified, they will be further evaluated for their role in long-term changes in weight. Appropriately, Dr. Ravussin, the principal investigator for this task, has extensive experience in studying the energy expenditure responses to over- and underfeeding.

General Comments

Eric Ravussin, Ph.D., heads this task with the assistance of Lilian de Jonge, Ph.D. Dr. Ravussin is an internationally known and generally acknowledged expert in the field of measuring energy expenditure. More importantly, he is known for his ability to test appropriate hypotheses about the regulation of energy expenditure in man using calorimeter chambers as a tool. His recruitment to PBRC is a major asset to the work proposed. Dr. de Jonge is a new investigator with excellent training, good research experience, and publications in the field of energy expenditure. She, too, is an asset to this project.

Specific Comments, Concerns, and Questions

The subcommittee concluded that these are important studies to the military in two respects. First, the underfeeding studies will relate directly to the calorically challenged regimens consumed by Rangers, SEALS, and other service men and women in active combat circumstances. Thus, for example, the study may ultimately be able to provide prospective markers to identify those individuals who will require specific additional rations during calorie-deficient training circumstances. Second, the overfeeding studies will have direct relationship to the circumstances that occur in military personnel after completion of basic training and during other types of military duty where rations are plentiful but activity is diminished. In this context, however, ***the use of only female subjects is a significant detriment to military applicability since most military personnel are men. Therefore, men should be included in the study groups.*** No rationale is given for the power calculation (i.e., only an 80 percent chance of detecting a difference of 100

kcal) and subsequent choice of 20 subjects per group. Thus, it is not clear whether the inclusion of men will increase the total number of subjects significantly or whether the aims can be accomplished with little or no increase in total subject number.

The subcommittee was also not convinced that the studies of autonomic activity were sophisticated enough to pick up subtle, but physiologically relevant, differences in autonomic activity (e.g., catecholamine recycling at the nerve terminal). Nor was it clear how differences, if measured, could be interpreted unequivocally as either a cause or an effect of the feeding perturbations.

An additional concern is the proposed length of time for the over-and underfeeding. There is a metabolic adaptation to over and under-feeding, although there are questions surrounding duration of the required intervention. Specifically, can "short-term" evaluations be used to predict "long-term" effects? The question is, what duration represents "short-term"? With acute over- or underfeeding the "early" intervention phase is associated with metabolic adaptations that are different from those observed later. For example, the early starvation studies by Cahill (1998) and others showed an early transition phase followed by a longer adaptive phase.

The term "early" is defined differently by investigators, but the preponderance of over- and underfeeding literature describes early as the first few weeks of dietary perturbation and "late" weeks or months into the intervention. For example, Katzeff et al. (1986) invoked a several week change in energy balance to study the metabolic effects of norepinephrine. Danforth and coworkers (1979), defined short and long-term overfeeding as several weeks and months, respectively.

Ukkola and colleagues (2001) at PBRC investigated the role of insulin-like growth factor (IGF)1, IGF2, IGF binding protein 1 (IGFBP1), and IGFBP3 gene variants on the metabolic changes observed in response to a 100-day overfeeding protocol conducted with 12 pairs of monozygotic twins. According to the investigators, genetic variation at the IGF2 and IGFBP1 loci may be among the factors responsible for the inter-individual differences observed in the response to "long-term" alterations in energy balance.

There is no question that brief periods of over- or under-feeding induce metabolic changes in humans and animals. Seematter et al. (2002) assessed the "short-term "consequences of carbohydrate or fat overfeeding or of food restriction on the metabolic effects of mental stress in healthy lean women after a 3-day overfeeding with 40 percent excess calories as either carbohydrate or fat. Subjects were also evaluated after a 3-day period of underfeeding with a protein sparing modified fast. Underfeeding reduced the stimulation of energy expenditure and enhanced lipolysis during sympathetic activation. The investigators suggest that these adaptations may be involved in mobilization of endogenous fat while limiting weight loss. In contrast, they report that short-term overfeeding failed to alter the sympathetic control of energy expenditure and lipolysis.

Use of a rodent model demonstrated rapid induction of insulin and leptin resistance by "short-term" overfeeding. After 3 and 7 days on an assigned diet regimen, rats were tested for their biological responses to acute elevations in plasma insulin and leptin concentrations. Severe resistance to the metabolic effects of both leptin and insulin ensued after 3 days of overfeeding (Wang et al., 2001).

McDevitt and coworkers (2001) hypothesized that de novo lipogenesis would increase during 96 hours of overfeeding, would vary depending on the type of carbohydrate consumed, and would be greater in obese than in lean women. De novo lipogenesis increased after 96 hours of

overfeeding with glucose and sucrose to the same extent in lean and obese women but did not contribute greatly to total fat balance.

Although these studies show that short-term—several days—of energy imbalance induce metabolic effects in humans and animals, a number of studies support the view that the early phase of imbalance lasts longer, perhaps 5 to 7 days, depending on the degree of caloric restriction or overfeeding. It seems likely that the effects of several days of energy imbalance, will be conditioned by prior diet and exercise effects and less representative of long-term weight related phenomenon. Full activation of lipolytic and lipogenic pathways likely requires adaptation.

Finally, the data analysis and interpretation requirements of genotype–phenotype mapping outlined in this task will be substantial, if not overwhelming.

Recommendations

This task has to include male subjects as well as female subjects or it will have very limited relevance to the military, since the military population is approximately 80 percent male. If the need to restrict the study to a single sex is to try and reduce between-individual variation, the study would be more militarily relevant if the subjects were men rather than women.

PBRC should reconsider their approach to evaluating the effects of acute dietary perturbations on the autonomic nervous system. The subcommittee does not believe that the proposed parameters (e.g., urinary catecholamine excretion) will be sensitive enough to pick up subtle but important changes in catecholamine at the nerve ending.

The subcommittee's view is that the study would be improved and the likelihood of success greater if the 3 days of over- and underfeeding were extended within a reasonable time frame to 5-7 days or even longer.

Because the primary endpoints will deal with the responses of a very large number of genes (or groups of related genes) to over- and underfeeding, ***the subcommittee also recommends very strongly that a statistical geneticist and a bioinformatics expert be recruited for this and subsequent tasks.***

Task 2-B: Influence of Dietary Fat on Training and Performance

Project Summary

This task proposes to examine the impact of dietary fat on training adaptations and exercise performance in both men and women. Two studies are proposed. The first will examine the influence of two levels of dietary fat (20 percent and 35 percent) in combination with exercise training on aerobic power, endurance, and body composition outcomes of healthy but previously inactive individuals. The second study will evaluate the effect of a controlled very low-fat (10 percent) and moderate-fat (35 percent) recovery diet on the quantity of intramuscular lipid and glycogen stores following a bout of prolonged moderate exercise, as well as the effect of depleted intramuscular fat on subsequent performance of trained endurance athletes. In both studies the influence of the diet–training interaction and the composition of the recovery diet on gene expression will be measured, along with key metabolic enzymes, energy expenditure, fat oxidation, and substrate utilization using stable isotopes.

General Comments

This task is headed by Enette Larson-Meyer, Ph.D., R.D., head of the Nutrition and Exercise Laboratory, with advice and oversight by Dr. Ravussin. Dr. Larson-Meyer, although new to the research faculty of PBRC, has conducted previous clinical trials and has extensive experience conducting studies in female populations. She also has a number of publications on intramuscular lipid fractions.

There is limited research on the impact of diet composition and exercise on substrate utilization and performance in women. Inclusion of women in these studies is commendable and increases the applicability of the research to active soldiers in the military, particularly as it may apply to military women in basic training and may provide insights into increasing physical fitness while reducing the high incidence of injury that occurs in women during basic training (IOM, 1998b). Technical support for these studies from the various core labs at Pennington (biochemical assessment; indirect calorimetry chambers; metabolic kitchen; stable isotope lab) will be critical for this task.

Specific Comments, Concerns, and Questions

Information was lacking on the expertise of the investigators in techniques of using stable isotopes, other than doubly labeled water, for the examination of substrate utilization during exercise. Discussions with the investigators provided little additional documentation.

The addition of a senior-level exercise physiologist trained in nutrition, metabolism of various dietary energy substrates, and substrate utilization during exercise would greatly enhance the ability of the Pennington group to do more sophisticated exercise training and performance research studies. This in turn would enhance its ability to make more specific dietary recommendations for active military personnel under various environmental conditions. It is noted that recruitment of such a person is proposed for year 2 of this task, but having this person in place at the initiation of the study would be more appropriate.

It is unclear why different levels of dietary fat were chosen for the two studies included under this task. It would seem more appropriate to use similar levels in both studies so that differences in performance and substrate utilization between the trained and untrained individuals are more clearly delineated.

It is suggested that the proposed levels of dietary fat (20 percent and 35 percent) be reexamined. Since the goal of the first study is to determine the effects of dietary fat on intramuscular lipids in untrained individuals, a low-fat diet of 15 percent fat and a moderate-fat diet of 35 percent would be reasonable. In the second study, examining the effects of the fat content of a recovery diet in trained endurance athletes, the controlled very low-fat diet of 10 percent seems extreme, and a 15 percent fat diet as in the first study would be more appropriate. The moderate-fat diet of 35 percent fat seems appropriate and would be representative of the fat content of field rations.

In the proposed design of study 2, following depletion of intramuscular lipid, the low-fat diet and the moderate-fat diet are to be fed for three days prior to the performance test. It is suggested that one day be added to the experimental design during which both groups are fed the low-fat, high-carbohydrate diet the day before the performance test is done. In this way, both groups will have similar muscle glycogen levels, but different intramuscular lipid levels (i.e., glycogen levels will not be a confounding factor). This alteration of the experimental design will allow the researchers to test whether differences in intramuscular lipids impact performance when glycogen

levels are similar. In the current design, both muscle glycogen and lipid levels vary between the groups, which makes it more difficult to determine which factor (glycogen or intramuscular lipids) is impacting performance.

Recommendations

It is recommended that the addition of a senior-level exercise physiologist with training in nutrition and substrate metabolism and utilization be added at the beginning of this study rather than in year 2 as indicated in the proposal. Such an individual would greatly enhance the capability of PBRC to do more sophisticated exercise training and performance research studies. This in turn would enhance its ability to make more specific dietary recommendations for active military personnel under various environmental conditions.

The committee recommends that the design of the studies in this task be reconsidered to alter the levels of dietary fat to be tested and to add an additional day in which the low-fat diet is offered to both groups in study 2 to equalize glycogen content between the low- and moderate-fat diets. It would seem beneficial to use the same low-fat and moderate-fat content diets in the two studies to clarify the differences in dietary fat content on the performance of trained as compared to untrained individuals. The 24 hours of carbohydrate loading should equalize the muscle glycogen content so that differences in performance may be more appropriately attributed to differences in intramuscular lipids.

Task 2-C: A School/Community-Based Intervention to Prevent Adult Obesity in Overweight Adolescents: A Three-Year Randomized and Controlled Trial

Project Summary

This task proposes to develop and evaluate a community- and school-based intervention program for at-risk (i.e., overweight) ninth graders to prevent the development of later (i.e., by twelfth grade) obesity. Two of the three high schools in the Ascension Parish (county) public school system will be used and randomly selected as experimental and control schools, with 60 overweight students recruited in each school. An intervention program attempting to employ school and community resources and family involvement will be developed based on the existing PBRC "Committed to Kids" clinical weight-management program. This program has not previously been evaluated in a nonmedical setting. The volunteer subjects and family members will be required to attend a 90-minute educational session once weekly after school for one year and then once a month for the next two years. The control subjects will attend standardized health information classes once a month after school for three years. Effects of the program will be evaluated by annual assessments of weight, body composition, blood pressure, and fitness for subjects at both the intervention and the control schools. Dr. Melinda Sothern is the project leader for this task.

General Comments

The escalating rates of childhood and adolescent obesity are undeniable and contribute to increasing rates of adult obesity, as adolescent obesity is a harbinger of adult obesity. Therefore, the public health importance of the question is undeniable. The task also has relevance to the

military's mission in that the increase in adolescent obesity directly impacts the military's pool of new recruits. In addition, even though the Army has a more lenient entry standard than retention standard, overweight individuals have a decreased exercise tolerance and increased risk of injury to the joints and lower extremities during basic training and in times of physical stress (IOM, 1998b). This study should be relatively inexpensive to conduct since the intervention involves only education and physical fitness testing. Furthermore, it will certainly be conducted with participants who reflect the population of future military recruits.

Dr. Sothern has considerable experience, documented in peer-reviewed research, in developing, implementing, and evaluating pediatric weight control programs. The importance of the entire ecology of the adolescent (i.e., school, home, community) is acknowledged in the design of the intervention. The measures are all appropriate and easily obtained by PBRC staff. The targeted group, 13- to15-year-olds (ninth graders) who are already overweight (defined as between the 85th and 95th percentile age-adjusted body mass index [BMI]) are at a critical stage of development, thus increasing the opportunity to achieve significant results.

The weekly 90-minute educational intervention for the treatment group will include topics on nutrition, behavior, and exercise, while the monthly 90-minute educational sessions for the control group will contain standardized health information such as smoking prevention, fire safety, and prevention of sexually transmitted diseases.

Specific Comments, Concerns, and Questions

Subject numbers and characteristics are important issues of concern. The proposed sample size of 120 students (60 control and 60 treated) may not be sufficient when dropouts and geographic transfers over a three-year period are considered. Further, the proposed study size may not be adequate to test for gender and race interactions with the intervention, although this is not an expressed component of the program objectives.

Subjects will be expected to commit to a rather extensive schedule of meetings over the three years (if assigned to the treatment group): weekly meetings for one year and monthly meetings for the next two years. The same is expected of their families. These requirements may result in a sample that is somewhat nonrepresentative of the population of likely recruits to the military, many of whom may come from homes where such involvement is absent or are unlikely to make such ambitious commitments in their early teen years. Further, if consent is obtained after the school is randomized, the extent of commitment expected of the control school participants will be far less than that of the intervention school participants, introducing a potentially confounding variable.

The nature of the community aspects in the intervention is not specified, other than the statement that family-based educational sessions will utilize school, health, and community centers, yet this is described as a defining characteristic of the intervention. Therefore, it is conceivable that there may be contagion effects of the intervention from nonparticipants in the intervention school if they are from the same community or parish. Data on a sample of nonparticipants (i.e., subjects at each school who do not volunteer to participate in anything other than assessments) would address this issue.

Recommendations

The committee strongly recommends that consideration be given to increasing the sample size of the proposed study. The level of commitment that will be required, especially from the intervention group, will likely result in a high drop-out rate over the three-year period.

It is also recommended that assessment of a group of nonparticipants at each school be included. This would permit assesment of any contagion effects that may occur due to control students residing in the same communities as intervention students. Issues noted above, regarding subject selection, should be addressed.

Task 2-D: Functional Genomics

Project Summary

This task includes a range of genetic studies to examine the central (i.e., central nervous system) and peripheral (e.g., fat and muscle) regulation of food intake and energy expenditure. This study will investigate the expression level of candidate genes in fat and muscle and will examine the impact of exercise and diet on gene expression in an effort to identify novel genetic pathways that influence energy homeostasis and contribute to the development of obesity. The proposal seeks to address this important issue by looking at differential gene expression in tissue samples collected before and after a variety of environmental challenges (e.g., dietary intervention, exercise). These goals will be pursued utilizing biopsy fat and muscle tissue samples collected as part of Tasks 2-A and 2-B and application of state-of-the-art molecular genetic techniques. Because the samples to be analyzed will come from Tasks 2-A and 2-B, it will be possible to compare gene functional data with physiological measurements. This task will also examine the effects of energy intake and exercise on genes that are specifically involved in appetite control, insulin production, and energy production or consumption.

General Comments

This task will be supervised by George Argyropolous, Ph.D., director of the Functional Genomics Laboratory. Oversight is provided by Dr. Eric Ravussin. Dr. Argyropolous has demonstrated abilities in the molecular genetic techniques that are being proposed in this study. By focusing on the cellular and molecular genetic levels, this work offers the opportunity to explore how changes in gene expression may directly impact a variety of important health- and performance-related phenotypes such as insulin sensitivity, lipid metabolism, and adiposity. While the use of quantitative gene expression assays is not new, their application to the study of energy metabolism and obesity-related perturbations remains quite novel. Such work will help to define a new class of more proximal phenotypes for use in future studies on energy metabolism and obesity.

The resources provided by the institutions (PBRC and U.S. Army, Fort Bragg) involved seem well suited to meet the technical requirements of the proposed research.

Specific Comments, Concerns, and Questions

This is an innovative proposal that makes strong use of a unique resource provided by the work being conducted in Tasks 2-A and 2-B. Although the current study design is less than optimal for genetic studies to find previously unsuspected genes impacting the phenotypes being

collected (obesity prone and obesity resistant), it should provide adequate numbers of observations to address questions regarding the function of specific genes. This would include their level of expression under conditions of fasting, overfeeding, high-fat diets, low-fat diets, and exercise under trained and untrained conditions. As a result, this study provides the opportunity to help define a new class of proximal phenotypes that could be of tremendous utility in future gene discovery projects. However, while the technical aspects of this project are very strong, the analytical components could be strengthened. Although the subcommittee was impressed with the Functional Genomics Laboratory, based on the number of personnel assigned to this task there appears to be a far greater capability to generate genetic information than to analyze it statistically and relate it to phenotypic parameters.

There is also some concern that the list of candidate genes may need re-evaluation since some of those mentioned are not relevant for population screening. The subcommittee recognizes that it is somewhat questionable to pursue even positional candidate genes in populations other than those in which the original linkage was actually detected. However, in this case, the researchers should at least select their candidate genes based on significant linkage results for phenotypes relevant to those they are proposing to examine.

Recommendations

It is strongly recommended that PBRC at the very least, consult with a statistical geneticist in the hypothesis development stage of this task as indicated in the recommendations for Task 2-A. The ultimate interpretation of the proposed research will be statistical, and while the individuals named in this project are well qualified for the laboratory portion of the work being proposed, no one appears to be formally trained in the statistical analyses. In addition, there is a bioinfomatics issue inherent in this project that will benefit by the contribution of an individual experienced in the analysis and management of large biological/genetic data sets. Ideally, this should be more than someone functioning at a technician level. While it is difficult at present to recruit qualified individuals in the area of statistical genetics, it should be possible to identify a qualified individual to act as a collaborator or consultant.

Since the genetic analyses are being done on samples collected in Tasks 2-A and 2-B, it is essential that both genders be included in the studies proposed under those tasks.

Task 3: Nutrition, Stress, and Body Weight Regulation

Project Summary

This task will employ rodents as animal models to study the neurochemical and physiologic mechanisms that underlie weight gain and loss. It will look at the regulation, under a variety of conditions, of signaling molecules generated in gut, adipose tissue, and brain that regulate appetite and body weight. One set of studies will focus on rats that have been over- or underfed for one to three weeks by gastric gavage and will look for genes in the brain that change consistently with respect to these manipulations (candidate genes are not specified). A second set of studies will examine the possibility that glucose-sensing cells in the rat hindbrain are sensitive to streptozotocin. Such cells are posited to express the GLUT-2 transporter, and the hypothesis is that streptozotocin will destroy such brain cells, because the drug's toxicity depends on the presence of the GLUT-2 transporter on target cells. A polymerase marker of cell death will be used to identify neurons that are killed by streptozotocin (polyadenosinediphosphate ribose polymerase).

Specific markers for pancreatic alpha and beta cells will be applied to determine if killed neurons contain markers that might target them for the neurotoxin (i.e., by bearing chemical similarities to pancreatic cells). A number of other neurochemical markers previously linked to body weight and appetite control will be examined to assess whether they are influenced by this treatment. A third set of studies will examine whether the ingestion of palatable foods by rats activates dopamine neuron-containing circuits in brain. This is based on the premise that such dopaminergic circuitry may constitute reward pathways stimulated by the ingestion of favored foods. However, few details are given regarding the design of this third set of studies, and for this reason, they cannot be evaluated. A fourth series of studies will examine the role of cholecystokinin in the generation of satiety signals to the brain.

Additional studies will focus on stress-induced changes in food intake, looking in particular at a model for stress-induced *suppression* of food intake in rats. This paradigm is viewed as a model to study the reduction in caloric intake seen in soldiers under stressful combat and field situations. The goal is to identify neuronal signaling molecules that may mediate this effect in brain, with the ultimate goal of designing measures to improve food intake under stressful situations. The signaling molecule that will initially be examined in detail through the use of a genetically modified mouse that over-expresses it is the agouti protein, a melanocortin receptor antagonist. This mouse shows enhanced loss of weight following exposure to stress (relative to control mice), which may be mediated via the protein's known ability to block melanocortin receptors. Another series of experiments will be conducted in a rat model that produces a life-threatening reduction in food intake and body weight. The model is food restriction (limited to 2-hour ad lib access to food each day) combined with 24-hour access to exercise (running wheels), which causes animals to exercise themselves to death. This model will be used to identify candidate genes (proteins) in brain that may participate as signaling molecules in brain circuits mediating this unusual behavior effect.

Finally, the animal models described above will be subjected to a number of dietary strategies to assess if they can prevent or promote treatment-induced alterations in food intake and body weight.

General Comments

Studies on the impact of physical and psychological stress on hunger and satiety mechanisms and body weight regulation should be strongly encouraged, since they bear on important issues of health for military personnel under combat and field conditions. The subcommittee was pleased to note the continued participation of Dr. Ruth Harris in these studies as a subcontractor, since the Committee on Military Nutrition Research has been impressed by her work at PBRC during previous site visits. Drs. Martin and Harris are both seasoned investigators in this research area, and interesting results are likely to be derived from the proposed studies since the models are reasonable and virtually any outcomes will point to productive avenues of investigation.

Specific Comments, Concerns, and Questions

Current knowledge should allow several predictions to be made about neuropeptide changes that might be expected. Results with known genes, if consistent with prediction, would allow a "positive control" base from which to evaluate other changes observed in gene array assays. Although there is extensive discussion of the streptozotocin studies, the logic behind them is not clearly described. If these studies indicate that streptozotocin-sensitive cells show colocalization

with markers for alpha and beta cells, what are the implications of this? No details are provided on the design of or logic behind the dopamine reward studies, making it difficult to provide an evaluation.

In addition, no model of stress-induced eating has been proposed. While reduced food intake under combat conditions might motivate the studies of stress-induced anorexia, another military problem that is not combat related, (e.g. overweight) may derive in part from stress-induced eating. Hence, there is a rationale for pursuing both models in the military context. Interesting differences might appear in the brains of rats experiencing stress-induced eating versus stress-induced anorexia, which would not be as clear in comparisons with nonstressed controls.

Recommendations

A model of stress-induced eating should be added to this task. Interesting differences might appear in the brains of rats experiencing stress-induced eating versus stress-induced anorexia that would not be as clear in comparisons with nonstressed controls. One model that has been studied fairly extensively is that of the simple tail-pinch model of stress-induced eating in rats. Some of the physiological mechanisms that have been found to be involved in this model that could also be examined for changes in the stress-induced anorexia model include cholecystokinin, bombesin, thyrotropin-releasing hormone and the prostaglandins, all of which suppress the tail-pinch induced eating behavior. Neuropeptides that may facilitate the ingestive response to tail-pinching include the opioid peptides, dopamine, and beta-endorphins (Antelman and Szechtman, 1975; Antelman et al., 1975; Morley et al., 1983; Rowland and Antelman, 1976).

In the over- and underfeeding studies, candidate genes should be specified. The inclusion of the model suggested above may provide additional insights into candidate genes to be evaluated.

Task 4: Laboratory for Human and Food Samples

Project Summary

The Laboratory for Human and Food Samples provides the analytical support for Tasks 1, 2, and 7 and, equally important, also supports studies conducted by the U.S. Army Research Institute for Environmental Medicine (USARIEM). Under the direction of Drs. Jennifer C. Rood and Richard T. Tulley, the Laboratory for Human and Food Samples has developed broad expertise in the analyses of biological samples collected from humans and animals. These analyses include more than 70 different tests for nutrients, indicators of nutrition status, and measures of biochemical and physiological function. Almost 30 of these analyses were developed specifically for the military. The lab proposes to continue providing support for USARIEM studies and military-supported studies at PBRC. Four military studies are currently supported, and at least three additional military studies are expected to utilize the laboratory's services during the new five-year grant. Support provided by the laboratory includes on-site personnel for military field studies to collect, log, and process samples, development of testing protocols, development and validation of specially requested analyses, intensive quality control efforts on analytical methodologies, compilation of results in a database or spreadsheet, and assistance in writing protocols and results for publication. In addition to Drs. Rood and Tulley, the laboratory is staffed with one food scientist, eight medical technologists, four phlebotomists-accessioners, and three student workers.

General Comments

The laboratory is equipped with a wide array of state-of-the art equipment. Evidence of staff expertise and the capacity of the laboratory is the support provided to a large series of studies conducted by USARIEM and PBRC. Accreditation of the clinical laboratory by the College of American Pathologists, Centers for Disease Control and Prevention Lipid Standardization Program, and Department of Health and Human Services substantiates that good laboratory procedures are being followed. The assurance that certified standards are used routinely attested to the fact that data obtained from the laboratory analyses provide an acceptable level of accuracy and repeatability. The entry of laboratory data into a computerized database provides the opportunity for management and PIs to review data on a continuing basis. A flag system provides the ability to check for errors due to procedure, and verify entry of data in a timely manner.

Specific Comments, Concerns, and Questions

The food analysis laboratory appears to be less broadly developed than the clinical laboratory. The capability of this laboratory appears to be limited to energy-yielding dietary components (protein, fat, carbohydrates), fatty acids, cholesterol, total dietary fiber, vitamins A and E, and some elements.

Recommendations

Although the site visit team was assured that the same level of laboratory quality control was practiced in the food laboratory as that practiced for clinical analyses, ***accreditation by an outside authoritative organization, similar to what has been done for human samples, would provide more assurance of the quality of the laboratory's data.***

It is also recommended that nutrient analyses of foods should include various specific carbohydrate and fiber fractions, particularly with the military's ongoing interest in the role of carbohydrates in physical performance and endurance.

With a number of new units of activity for certain nutrients specified by the Institute of Medicine reports on dietary reference intakes (IOM, 1997, 1998a, 2000, 2001) the laboratory will need to update its analytical procedures to incorporate the new retinol activity equivalents which will require analyses for β-carotene and total carotenoids. Analyses specific for α-tocopherol will also be necessary, as well as analyses for synthetic folate separate from food folate to determine dietary folate equivalents.

Task 5: Stable Isotope Laboratory

Project Summary

The focus of the Stable Isotope Laboratory to date has been on the analyses of deuterium (^{2}H) and oxygen-18 (^{18}O) from military studies using the doubly labeled water (DLW) technique for determining energy expenditure, body fat composition, and total body water turnover. Over the previous grant period, the laboratory supported eight USARIEM studies. For the current proposal, there are three studies in the planning stages with USARIEM—two focused on weight gain and energy expenditure and one that will examine total daily energy expenditure to determine the length of time that Marines can subsist on the Meal, Cold Weather ration. Another study will use DLW to validate the indirect calorimetry chambers and other methods of

determining energy expenditure. Finally, the laboratory proposes to expand its activities into the use of other stable isotopes to examine body tissue buildup and catabolism through monitoring the metabolism of protein, fat, and carbohydrate.

General Comments

The Stable Isotope Laboratory is directed by James DeLany, Ph.D., who has extensive experience in isotope ratio mass spectrometry, principally measuring ^{18}O and ^{2}H for application of the DLW method to measure energy expenditure. A new addition to the laboratory is Laurie Byerley, Ph.D., who will serve as the assistant director. She has broad experience in studies of nutritional metabolism, including stable isotope tracer studies employing gas chromatography–mass spectroscopy (GC-MS) analytical approaches. Her expertise adds a new breadth to the laboratory.

The laboratory is well equipped for measuring energy expenditure using $^{2}H_2{}^{18}O$ and has amply demonstrated that it can provide data of direct and immediate applicability to problems of military significance. The lab sample throughput has been prodigious. There is no question that this laboratory can continue to supply the military with information relevant to the energy expenditure of servicemen and servicewomen under various training, combat, and noncombat conditions.

The laboratory has also discussed expansion into more traditional isotope tracer experiments of substrate fuels using GC-MS. Dr. Byerley's presence will enhance these applications, but the subcommittee cannot comment on these studies or their potential applicability to military nutrition research because they were not described in any detail.

Task 6: Nutrient Database Laboratory

Project Summary

The Nutrient Database Laboratory, which includes Dietary Assessment and Counseling and Nutrient Data Systems, is designed to support USARIEM field studies and PBRC research being conducted under Tasks 1, 2, and 7. The laboratory provides computerized nutrient analyses of recipes, menus, and dietary intakes of soldiers critical to the assessment of soldiers' nutrient needs. Laboratory personnel also assist in data collection at field sites for USARIEM studies. A special objective of this current proposal is to translate PBRC expertise on the nutrient database to in-house USARIEM personnel and assist USARIEM in identifying computer support personnel. The laboratory will also provide dietary intake assessment and dietary counseling services at Fort Bragg for Task 1.

General Comments

The team assembled to accomplish these efforts is considered very knowledgeable and use state-of-the-art techniques for collection of intake data. At the same time, it is recognized that even with the best quality control, the nutrient intake data generated will have a high standard deviation due to exchange of field ration components among military personnel and variation in nutrient composition data.

Recommendations

It is recommended that PBRC continue efforts to improve the methodology for gathering intake data. In addition, with changes in conversion factors for carotene to vitamin A and the discounting of δ-tocopherols in total vitamin E activity, for example, Nutrient Data Systems should maintain an overlap of nutrient composition data from both the new and the old procedures so as to provide a bridge by which new food intake studies can be compared with previous field studies.

Task 7: Metabolic Unit Project

Project Summary

The PBRC Metabolic Unit contains 14 beds and houses 2 indirect calorimeters. The unit is served by a metabolic kitchen capable of producing standardized controlled diets for up to 110 subjects per day. The unit is staffed by a seasoned clinical trial team. The availability of these facilities and trained personnel is an asset to enhance the USARIEM research portfolio. No specific inpatient projects have been planned with USARIEM at this time, although the Metabolic Unit will support Tasks 2-A and 2-B of this proposal. The unit is offered to USARIEM on an ad hoc basis for additional inpatient studies.

General Comments

The Metabolic Unit was found to be state of the art. It is operated efficiently and where possible, studies are planned for completion within a five-day week to lower cost. There is adequate capacity in the facilities and sufficient staffing to meet planned USARIEM research needs. The associated food preparation kitchens were organized in a manner to provide excellent accuracy and quality control. The associated metabolic chambers are well designed and utilize state-of-the-art measuring equipment.

Recommendations

There were no specific projects identified under this task, thus no recommendations are offered. However, the subcommittee urges strongly that PBRC remain aware of any military plans requiring use of the metabolic unit so that adequate time in the unit is available for military studies.

GENERAL CONCLUSIONS

Overall, the subcommittee felt that the lack of detail in the preproposal on the approaches to be used in accomplishing the seven tasks seriously hampered its ability to provide a thorough evaluation. However, presentations and discussions with the principal investigators were helpful in clarifying some of the issues raised by the limited information provided.

Recent additions to PBRC staff will significantly enhance the new focus on weight management and energy metabolism. In addition, PBRC has extensive experience in large, multicenter, weight-loss clinical trials, including 12 pharmaceutical-sponsored weight loss trials; the LookAHEAD trials, STOP I and II; and Internet-based interventions such as the MOMS and HipTeens programs. PBRC indirect calorimetry chambers, stable isotope laboratory, metabolic kitchen, and

food chemistry laboratory, as well as the fitness facility should be able to provide important new data for the military as well as support to military studies on energy balance and weight management.

An overriding concern of the subcommittee is the extent to which the proposed work adequately addresses issues germane to the target population and its environment, as distinct from the civilian population. Task 1 in particular appears to replicate work already in the literature and the marketplace. An additional important concern is that the technical capabilities of PBRC in human genomics have exceeded its data analysis capabilities. There has been a lack of staff expertise in stable isotope analysis other than in the use of DLW. However, the addition of Dr. Laurie Byerly to the staff of the Stable Isotope Laboratory should improve this capability substantially.

OVERARCHING RECOMMENDATIONS

This report was prepared before, but submitted after September 11, 2001. It addressed topics identified as needs by the Army prior to that date. These needs may have changed dramatically due to operational realities. It is important that the research tasks of PBRC be reviewed and changed as needed to ensure that military-funded research at PBRC remains mission oriented.

Based on the very preliminary nature of the new five-year grant proposal that was ultimately submitted to the subcommittee for review and subsequent discussion with Army representatives and the PBRC principal investigator for the proposed project, it is clear that a closer liaison between USARIEM and PBRC personnel would be helpful to all concerned.

- ***A much closer and on-going interaction between PBRC and the Military Nutrition Division is needed.*** It is of paramount importance that DoD-funded research at PBRC is reviewed regularly to assist them in maintaining a program that is relevant, focused, and mission-oriented with respect to the needs of the Armed Services. As a beginning, such an effort might be facilitated by a regular, more formal orientation by DoD that includes representatives of all branches of the Armed Services, to educate all PBRC investigators involved in military-funded research with respect to military needs and processes. The military should also consider the possible addition of a PBRC scientist to the DoD's Nutrition Committee. The establishment of post-doctoral positions for military personnel at PBRC would also help to strengthen the interface between the two organizations.

- ***Consideration should be given to widening the selection of subjects to include all military services instead of limiting selection to Army personnel.*** Although it can be reasoned that studies conducted using personnel from one service would be applicable to the other services, a case can be made that there are important differences between personnel from different military services that may have an impact on study results. These include the type and level of activity, eating environment, service culture, the emphasis placed on weight programs by the service, average age of personnel, and the level of education and training. These factors may not have a significant impact on metabolism but may influence eating behavior.

- ***Imaging methods, such as magnetic resonance imaging (MRI) and/or computerized axial tomography should be incorporated into the core facilities of PBRC as technologies central to completing the PBRC mission for the military.*** A major and intrinsically important goal of the work to be carried out by the PBRC research team over the next several years deals with precision mapping of genotype–phenotype relationships with respect to energy metabolism and body composition. This information is important since it has the potential to identify service

personnel at risk for development of obesity and its consequences and the potential impact on health maintenance and military readiness.

Clearly, the ability to achieve the goal of genotype–phenotype mapping is affected both by the level of genetic details that can be uncovered and by the refinement of phenotypic details. Genotype details will be well probed using dramatic new advances in gene array chip technology. Similarly, PBRC has many of the sophisticated techniques necessary to refine phenotype assessment, such as the metabolic chamber facility and dual-energy x-ray absorptiometry measurements of total body lean, fat, and bone masses.

There is evidence indicating that the regional distribution of body fat is genetically determined, and instrumentation currently available at PBRC is not capable of measuring regional body composition. This issue is not merely of academic importance, but is of critical value to both the military and the civilian population since it is now evident that regional fat distribution is an important determinant of the biological consequences of obesity, including insulin resistance and hypertension.

The subcommittee recommends strongly that imaging methods, such as magnetic resonance imaging and/or computerized axial tomography be incorporated into the core facilities of PBRC as technologies central to completing its mission for the military. This would maximize the information obtained from the genotype–phenotype studies that will form the foundation of much of the PBMC weight management research.

- ***To facilitate implementation of research, a single Institutional Review Board (IRB) review by the local IRB should be considered adequate for the purposes of human research conducted on civilians under the direction of PBRC faculty***. It was noted in the proposed tasks involving the use of human subjects that reviews by two different IRBs were required prior to initiation of the studies. In discussions with PBRC investigators and military personnel present during the site visit, it was indicated that DoD currently requires all DoD-funded studies involving the use of civilian human subjects to be reviewed both by the investigator's local IRB and by the Army's IRB located in Maryland. It is not clear to the subcommittee why a dual review is necessary before human studies at PBRC involving civilian subjects can be implemented.

In essentially all other areas of human research, the federal government has delegated human study safety issues to local IRBs, which must meet the requirements of the U.S. Department of Health and Human Services. PBRC has such a legally constituted IRB. Secondary review by a distant IRB seems unnecessary and delays efficient implementation of the proposed research studies. The subcommittee recommends very strongly that a single review by the local IRB be considered adequate for the purposes of human research conducted on civilians under the direction of PBRC faculty. DoD should implement an annual review of the PBRC IRB's membership, procedures, and decisions on DoD-funded projects to ensure that the proper levels of protection are followed. Under federal law, DoD, as the granting agency, would retain certain rights in reference to monitoring proper constitution and conduct of the local IRB, so assurance and verification of adequate compliance with national policies concerning human safety would be retained.

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Preproposal from the Pennington Biomedical Research Center

to the

U.S. Army Medical Research and Materiel Command

June 2001

PREPROPOSAL
FOR MILITARY NUTRITION STUDIES AT THE
PENNINGTON BIOMEDICAL RESEARCH CENTER (PBRC)

Background

The PBRC has a 12-year history of collaborative research with the Department of Defense (DOD). A series of specially funded cooperative agreements between the PBRC and the U.S. Army Medical Research and Materiel Command (USAMRMC) has provided high-quality analytical laboratory, nutrition database, and metabolic unit support for DOD nutrition-related research programs. The program currently supports the RDT&E-funded Military Nutrition Research Programs at the U.S. Army Soldier Systems Center (Natick, Massachusetts) and the U.S. Army Research Institute of Environmental Medicine (USARIEM) laboratories, as well as the Ration Sustainment Testing program. PBRC personnel frequently travel to DOD field studies to collect samples, which are returned to the PBRC for laboratory analyses. Additionally, the PBRC conducts research that complements and extends USARIEM's intramural program in areas of nutritional neuroscience, stress, physical, and mental performance, and garrison feeding. The PBRC program is periodically peer-reviewed by an external panel from the Committee on Military Nutrition Research (CMNR), Institute of Medicine (1988, 1990, and 1996). This effort has led to significant improvements of operational rations, better understanding of warfighter energy and nutritional requirements, and modifications in garrison feeding.

This proposal is aimed at continuing a research program that has been in place since 1988. The proposal is for the fourth specially funded cooperative agreement series that began in 1988. The previous cooperative agreements are listed below.

Dates of Award	Title	Funding
7/1/97-3/31/02	Military Nutrition Research: Eight Tasks to Address Medical Factors Limiting Soldier Effectiveness	$16,710,748
4/1/92-3/30/98	Military Nutrition Research: Six Tasks to Address Medical Factors Limiting Soldier Effectiveness	$11,340,567
8/1/88-7/31/92	Effect of Food, Diet and Nutrition on Military Readiness and Preparedness of Army Personnel and Dependents in a Peacetime Environment	$3,845,000

In each of the three previous cooperative agreements, the CMNR of the National Academy of Sciences provided peer review prior to program implementation. Additionally, USARIEM approved all projects and provided consultation on research design. Any modifications to the original research plan were approved by USARIEM prior to implementation.

The first two cooperative agreements did not allow for equipment purchase and the PBRC used other funding sources to provide over $5.4 million for equipment to support these research projects. Two neuroscience laboratories (a clinical sleep laboratory and a basic laboratory) were devoted almost entirely to military nutrition research. The Stable Isotope Laboratory, Clinical Chemistry Laboratory and Food Analysis Laboratory at the PBRC each devote approximately 50 percent of their activities to military nutrition support. About 20 percent of the PBRC Metabolic Unit activities in the past have related to military nutrition research, and we anticipate that 60 percent of metabolic unit activities will be devoted to military nutrition research in the next cycle. The equipment investment in these areas is detailed below.

Stable Isotope Laboratory	$1.8 million
Clinical Chemistry Laboratory	$1.7 million
Food Analysis Laboratory	$0.5 million
Basic Neuroscience Laboratory	$0.5 million
Metabolic Unit	$0.9 million

The PBRC research projects aimed at military nutrition issues have been completed on or ahead of schedule with high quality control and are detailed extensively in quarterly, annual and final reports.

The PBRC developed the following projects to meet Army objectives published in these grants:

- Stable Isotope Laboratory
- Laboratory for Human and Food Samples
- Menu Modification Project, modified to Enhancing Military Diets, 7/1/97
- Fort Polk Heart Smart Project, completed 6/30/91
- Nutritional Neuroscience Basic Laboratory Studies, modified to Stress, Nutrition and Mental Performance, 7/1/97, to be called Nutrition, Stress and Body Weight Regulation, 11/1/01
- Nutrition Neuroscience Clinical Sleep Studies, terminated 7/1/97
- Metabolic Unit Project
- Military Neuroscience Symposium, completed 5/20-21/96
- Stress, Nutrition and Work Performance, to be called Clinical Studies in Health and Performance Enhancement, 11/1/01
- Stress, Nutrition and Immune Function, terminated 7/1/00

Hypothesis: Aspects of body weight regulation of importance to American armed forces at home and abroad can be addressed, to a large degree, through strategies that focus on nutrition and physical activity. The factors of importance to the military to be addressed through nutritional and behavioral strategies include:

- Recruit readiness
- Weight gain and fitness decline in career soldiers
- Stress-induced alterations in food intake and body weight regulation
- Interactive effects of diet on physical performance

The strategies to address these factors will include a comprehensive program of basic research using animal models, human physiologic studies and clinical interventions.

The goal of this research is to assess, maintain, or improve a soldier's physical/ physiological/psychological capability to function effectively and to minimize adverse effects of medical problems on health, safety and performance. As detailed in the Army's Broad Agency Announcement 99-1, the research areas include:

1) Military health behavior promotional interventions;
2) Environmental physiology and metabolic intervention, such as thermal physiology and injury prevention, nonfreezing cold injury protection, sustainment in mountainous terrain, metabolic regulators to optimize performance in adverse environments, nutritional optimization of soldier mental status, optimization of physical performance and musculoskeletal injury prevention; and
3) Improve medical skills proficiency in improved home station training for distributed forces (active and reserve).

Technical Objectives

This proposal requests funding to continue the research relationships between the PBRC and USARIEM for five additional years, beginning November 1, 2001 through October 31, 2006. This proposal requests funding for collaborative and independent functions, as described below.

1) This cooperative agreement supports a collaborative effort between the PBRC and military scientists. This collaboration is based on a clinical project at Womack Army Medical Center, Fort Bragg, North Carolina. This task, **Sustaining Performance and Healthy Weight in Career Military Personnel (Task 1)**, is the cornerstone of the grant.

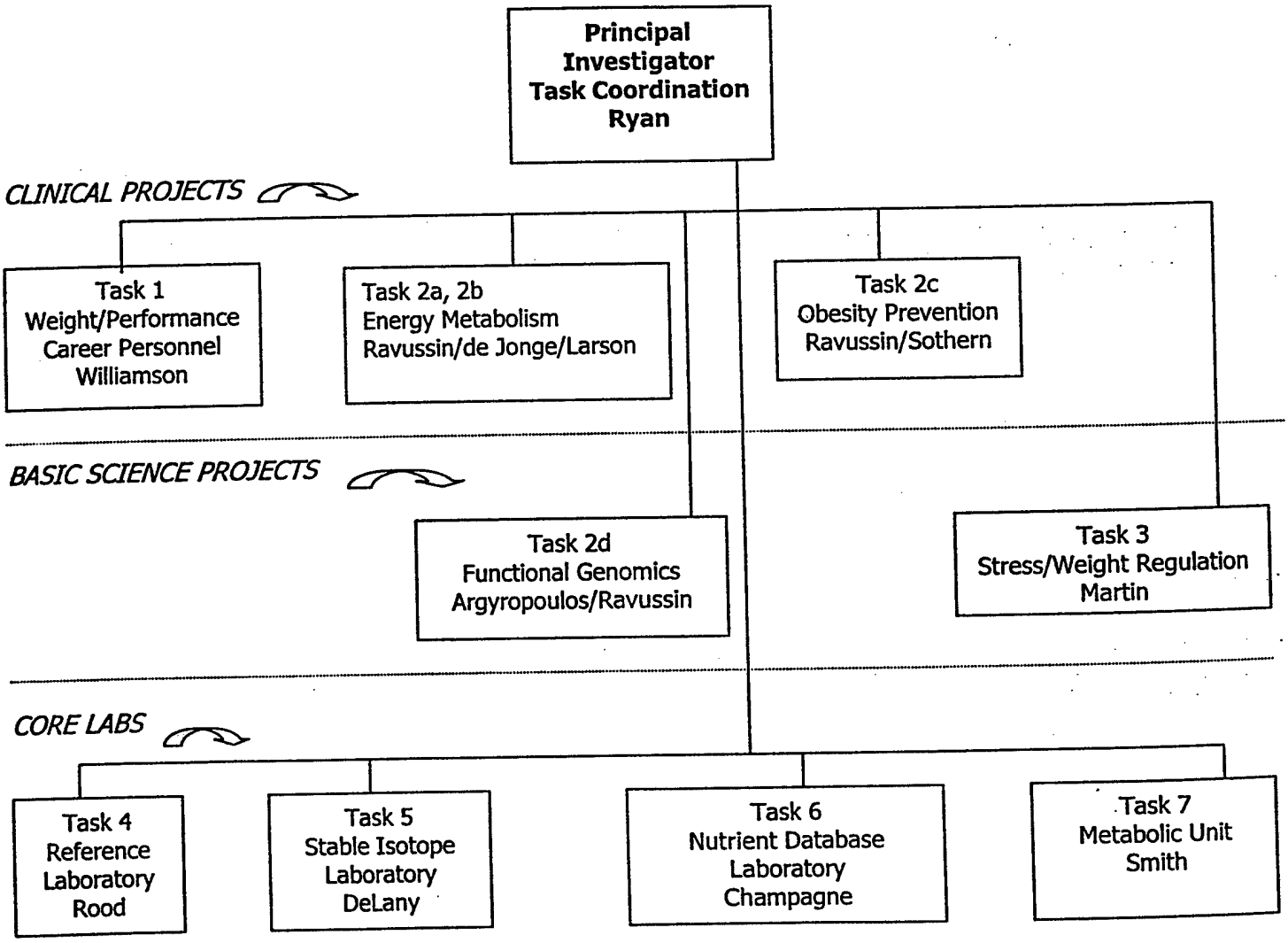
2) The proposed cooperative agreement would also allow for the continuation of the support laboratory functions where laboratories located at the PBRC serve USARIEM research protocols, as well as protocols based at the PBRC and sponsored by this grant. Those support laboratories include the following:

- **Laboratory for Human and Food Samples (Task 4)**
- **Stable Isotope Laboratory (Task 5)**
- **Nutrient Database Laboratory (Task 6)**

3) Last, and of equal importance, the grant supports PBRC-based projects which expand and amplify the objectives of **Task 1.** We propose to continue the following three PBRC-based projects:

- **Clinical Studies in Health and Performance Enhancement (Task 2)**
- **Nutritional Stress and Body Weight Regulation (formerly "Stress, Nutrition and Mental Performance" (Task 3)**
- **Metabolic Unit Project (Task 7)**

The schematic below displays the seven tasks.



Methods

Methodology for all projects have been detailed in quarterly and annual reports of Army Grants DAMD17-88-Z-8023, DAMD 17-92-V-2009, and DAMD-97-2-7013.

Animal and Human Use

Animals will be employed for basic research in Task 3-Nutrition, Stress and Body Weight Regulation. All protocols will be submitted to the PBRC Institutional Animal Care and Use Committee (IACUC) for approval. USARIEM personnel will approve all experiments in outline form prior to their performance.

The PBRC's Institutional Review Board (IRB) and the U.S. Army Office of the Surgeon General's Human Subjects Research Review Board (HSRRB) will approve all clinical protocols prior to implementation. The tasks that will require these reviews are as follows: Task 1-Sustaining Performance and Healthy Weight in Career Personnel, Task 2-Clinical Studies in Health and Performance Enhancement, and Task 7-Metabolic Unit Project.

Identification of Project Leaders

Principal Investigator (PI): Donna H. Ryan, M.D.
Project Leaders:

Task 1: Donald Williamson, Ph.D.
Task 2: Eric Ravussin, Ph.D.
Task 3: Roy Martin, Ph.D.
Task 4: Richard Tulley, Ph.D.
Task 5: James DeLany, Ph.D.
Task 6: Catherine Champagne, Ph.D.
Task 7: Steven Smith, M.D.

We queried all personnel who are involved in the project. There are no anticipated sabbatical or other anticipated leave requests expected. Donald Williamson, Ph.D. was a member of the Army and completed basic combat training. He served a two-year tour of duty as a PFC with clerical duties from 1970-1972.

Investigators' Qualifications

Curriculum Vitae (two to three pages) of all professionals affiliated with the project are attached. **Dr. Donna Ryan is the PI** for the project. She has served as PI for the prior three DOD grants and has led PBRC's military nutrition research since 1988. Dr. Ryan's qualifications for the post are her extensive scientific administration experience and research experience in obesity. She has served as Associate Executive Director at the PBRC since 1988 and formerly held administrative positions at LSU Medical Center, including Vice-Chancellor and Acting Chancellor. Her research experience in weight loss interventions includes serving as an Investigator on the NIH SHOW Trial – Study of Health Outcomes of Weight Loss. She has participated in a number of pharmaceutical weight loss trials. Dr. Ryan has a demonstrated track record of successfully leading independent research of military relevance at the PBRC, as well as successfully leading research collaborations with USARIEM.

The proposal is divided into seven tasks, each described in the following sections:

Task 1. Sustaining Performance and Healthy Weight in Career Military Personnel (Donald Williamson, Ph.D., Task Leader)

Task 2. Clinical Studies in Health and Performance Enhancement (Eric Ravussin, Ph.D., Task Leader)

Project 2A. Metabolic Understanding of Energy Balance: Mechanisms of Energy Metabolism Variability in

Response to Perturbations in Energy Balance (Lilian de Jonge, Ph.D. and Eric Ravussin, Ph.D.)

Project 2B. Influence of Dietary Fat on Training and Performance (Enette Larson-Meyer, Ph.D., R.D. and Eric Ravussin, Ph.D.)

Project 2C. Prevention of Obesity (Melinda Sothern, Ph.D. and Eric Ravussin, Ph.D.)

Project 2D. Functional Genomics of Energy Balance and Training (George Argyropolous, Ph.D. and Eric Ravussin, Ph.D.)

Task 3. Nutrition, Stress and Body Weight Regulation (Roy Martin, Ph.D., Task Leader)

Task 4. Laboratory for Human and Food Samples (Richard Tulley, Ph.D., Task Leader)

Task 5. Stable Isotope Laboratory (James DeLany, Ph.D., Task Leader)

Task 6. Nutrient Database Laboratory (Catherine Champagne, Ph.D., Task Leader)

Task 7. Metabolic Unit Project (Steven Smith, M.D., Task Leader)

TASK 1: SUSTAINING PERFORMANCE AND HEALTHY WEIGHT IN CAREER PERSONNEL

A. Problem to Be Studied

Weight and performance standards are in place across all services of the military and are applied to all military personnel. Failure to meet minimum standards currently results in dismissal of approximately 5,000 personnel each year across the military forces (including 2,000 Army personnel).

The current military practice requires evaluation of all personnel for weight, body composition, and performance at 6-12 month intervals. If personnel fail to meet performance standards (generally a 1½-3 mile run or submaximal bicycle ergometer test to assess aerobic capacity and push up and sit-up requirements) and/or weight standards (based upon BMI and body composition derived from circumference-based formulae; BMI $\leq$ 25 O+, BMI $\leq$ 27.5 O→), they are "tagged" for remediation. Approximately 6% of military personnel fail to meet standards on the first attempt. These personnel are allowed a one-hour session with a dietitian, and in some instances, a one-hour session with an exercise trainer. If overweight, they are required to lose 3-8 pounds/month until standards are met. If they fail to meet this target on two consecutive months, they are subject to discharge.

In our recent study of soldiers entering the Army in Basic Combat Training (BCT) at Fort Jackson, we found that approximately one-quarter of the recruits were overweight, as defined by military standards. During BCT, overweight soldiers generally lost weight and most were no longer overweight. They lost weight primarily due to the extreme physical demands of training during BCT, since they averaged consuming approximately 4,000 kcal/day. It is well known that people who have been overweight as adolescents and young adults, and lose weight as adults, are at greater risk for regaining weight. Thus, it is possible that some proportion of the career soldiers who fail to meet the weight standards of the military may be people who had been overweight upon entering the military. If this hypothesis is correct, then the problem of overweight career military personnel is likely to worsen given the current epidemic of obesity in the U.S. No age group, including those likely to be recruited by the military has escaped this epidemic.

Over the past 30 years, effective strategies for lifestyle behavior modification for weight loss have been developed. This research has found that intense, clinic-based interventions that target changes in eating

habits, nutrition, and exercise can lead to substantial reductions in body weight. However, weight loss is generally maintained only if regular contact with a lifestyle counselor is continued over an extended time period. There are many obstacles to maintaining extended therapeutic contact, including work, family, and recreational responsibilities. Therefore, it is desirable to develop means of maintaining therapeutic contact in ways that are not disruptive for the individual. Use of the internet to provide a "home study course" is one approach that could be used to overcome some of these obstacles. It is our understanding that most career military personnel have internet access at work and/or at home, which makes this approach very viable for application with military staff who are overweight. A primary aim of this project will be to develop and test the efficacy of an internet-based approach for weight management that will be directly compared to a clinic-based lifestyle behavior modification program.

B. Outline of Proposed Research

Since 6% of personnel are eligible for remedial efforts each year, remediation programs must be designed, mindful of the following factors:

- Safety: preservation of bone and muscle mass and function, as well as cardiovascular function.
- Efficacy: sustainable weight loss must be accomplished primarily by fat loss with maintenance/improvement of performance.
- Cost: Must be cost-effective, not just inexpensive to implement.
- Suitability for active duty military personnel
- Program portability: since the military forces are based at various sites around the world, ease of implementation and broad applicability are paramount.
- Consumer satisfaction: since behavioral programs are effective only when the person uses the program and expresses satisfaction with the way that it is structured, the establishment of a higher degree of consumer satisfaction will be important if the program is to be widely disseminated.

The initial phase of this project will span the first two years and it will have two components: 1) program evaluation of a clinic-based weight management program that is grounded in behavior theory and results of current lifestyle behavior modification research, and 2) development of an internet weight management program application that is based upon the same behavioral research, but is designed to be similar to a "home study course." Both interventions will be specifically designed for career soldiers in the military setting. In the second phase of the project, the relative efficacy of the clinic-based and internet-based interventions will be directly compared in a randomized controlled trial that will span two years. Recruitment of participants for this study will require one year, so the second phase will require three years. There is considerable research evidence that an intensive, clinic-based intervention should be an effective means for assisting the soldier with weight loss, to meet military weight regulations. Unknown factors relevant to military staff are the profiles of successful versus unsuccessful participants in a clinic-based intervention and perceived obstacles to success. These factors will be studied in Phase 1. The most important questions will be addressed in Phase 2. We anticipate that the demands of military life, family responsibilities, etc, will make it quite difficult for military personnel to attend a weight loss clinic on a long-term and regular basis. As noted earlier, the use of a website/interactive counseling approach could overcome many of these obstacles. Whether this approach can be as effective as a clinic-based intervention in the short-term or over a longer period of time is not known since no studies have compared these two approaches. If the internet-based approach can be as effective or more effective, then it may be the preferred method for addressing the problem of overweight career military personnel, since it should be more convenient, less intrusive, and less costly to implement (after the initial costs associated with development).

These interventions will be tested at Fort Bragg, North Carolina, which is home to ~50,000 military personnel and is composed of the 18th Airborne Division, as well as U.S. Army Special Operations Forces. The base population can be very stable, with personnel staying at this base for 10 or more years of their career. There is, thus, a spectrum of personnel who might benefit from a formalized program directed at those who

are "tagged" as failing or receiving cautionary appraisals on the weight and performance standards. Fort Bragg is also the site of a modern hospital facility with capacity to provide space and resources for housing the study.

Project Design Overview

Phase 1. One aim of Phase 1 is development and evaluation of a clinic-based weight management program for military personnel. The program will incorporate state-of-the-field weight management approaches with focus on safety, effectiveness, cost effectiveness, and portability. The first step in the development of the clinic-based and the internet-based interventions will be a study of the needs of overweight soldiers, perceived obstacles for successful weight management and assessment of the clinic facilities and staff. To accomplish the objective, PBRC staff will interview soldiers and clinic staff and conduct focus groups and surveys. The clinic-based Program will evaluate changes in weight and performance measures over a one to two year period. **This program evaluation will not include a control group as a part of the research design since the primary aim of the first phase is the development of two alternative interventions that will be compared in a randomized controlled trial in Phase 2.** The outcome measures for this program evaluation will include fat loss and performance evaluation, as well as quality of life and cost-effectiveness measures. Also, the research program will assess the association between personal, occupational, and family variables with adherence to the program components and with weight loss. These variables will be evaluated in an effort to develop a profile of successful versus unsuccessful participants in the Clinic-based Program. This aspect of the program evaluation will facilitate refinement of the program to address the needs of individual soldiers. The following variables will be tested as correlates of relative success with the program:

1) Personal factors: obesity status of parents, gender, ethnic group, age, history of childhood/adolescent obesity, history of weight cycling, eating habits, and exercise habits.
2) Occupational factors: length of service, type of job (e.g., sedentary vs. active), perceived job stress, perceived time to devote to weight management, and rank.
3) Family: obesity status of spouse, number of overweight children, family eating habits, perceived family stress, and perceived family support for weight management.

The outcome measures for this program evaluation program will be:

1) Changes in body fat and body mass index
2) Changes in fitness
3) Cost of treatment
4) Adherence to the clinic-based program, including attendance to sessions, completion of homework assignments, adherence to the prescribed diet, and adherence to the prescribed exercise program.

As a part of this program evaluation program, treatment manuals specifically tailored to the demands of different jobs, ranks, and other responsibilities will be developed. Also, therapist manuals will be developed and lifestyle counselors will be trained to deliver the clinic-based Program. These functions will be directed and conducted by PBRC research staff. The program materials will be modeled after the manuals and training procedures developed for the LookAHEAD and DPP project sponsored by NIH. Dr. Williamson has been highly involved in the development of these materials.

We anticipate that the initial six months of Phase 1 will be devoted to surveys, focus groups, and development of treatment materials. Soldiers will be enrolled in the Clinic-based program over the next six to nine months. The program evaluation will focus upon data from the first six months of the clinic-based Program. We will enroll 60 male and female soldiers in this program evaluation.

The second aim of Phase 1 will be the development of an internet-based alternative for the clinic-based Program. This program will require the development of an interactive website for the delivery of internet counseling for lifestyle behavior modification. The internet-based program will include an online treatment

manual that will be similar to the (hard-copy) treatment manual that will be developed for the clinic-based program. We believe that some face-to-face counseling and computer training will be required for the internet-based intervention. The extent of this face-to-face counseling will be determined by the findings of a pilot study. The website will be designed with the following components:

1. Asynchronous and synchronous internet counseling for lifestyle behavior change
2. Self-monitoring of food intake and physical activity using a drop-down menu format
3. Automatic feedback about weekly weight changes
4. Automatic feedback about the nutritional quality of food selections using feedback about calories and Food Guide Pyramid categories
5. Personalized reminders for behavioral tasks, e.g., behavioral contracts and self-monitoring
6. Threaded discussions to promote group problem solving for common problems
7. Chat room discussions to simulate group discussions
8. Remote data collection
9. Treatment planning based upon these data

The development of the internet-based program will take place in the first 12 to 18 months of Phase 1. A pilot trial of the program will take place in months 18-24 of Phase 1. We will recruit 30 overweight nonmilitary adult volunteers (males and females) with a BMI between 25 and 30 to participate in the pilot study. The primary purpose of the pilot study will be to test the feasibility of the program design, computer training procedures, and the need for face-to-face sessions.

Phase 2. The primary aim of Phase 2 will be to evaluate the relative efficacy of the clinic-based and internet-based Clinic-based Programs using a randomized controlled clinical trial. Overweight soldiers who volunteer for the research project will be randomly assigned to either the clinic-based or internet-based interventions. We propose to recruit 75 participants for assignment to each of the treatment arms (total of 150 volunteers). There are approximately 50,000 military personnel at Ft. Bragg. Therefore, if 6% of the soldiers are overweight and eligible for participation, there should be approximately 3,000 soldiers who should meet the weight requirement for participation in the study. given these considerations, recruitment of 150 volunteers in the first year of Phase 2 would appear to be reasonable. The treatment period will last two years. Assessment measures of outcome will be administered at Baseline and at six month intervals, i.e., 6, 12, 18, and 24 months.

The timeline for the components of Phases 1 and 2 is projected below.

Phase 1:Protocol/treatment manual development for clinic-based intervention	11/1/01 – 4/1/02
Phase 1: Website development for internet-based intervention	11/1/01 – 3/1/03
Phase 1: Program evaluation for clinic-based intervention	4/1/02 – 11/1/03
Phase 1: Pilot testing of internet-based intervention using nonmilitary volunteers	3/1/03 – 11/1/03
Phase 2: Enrollment of participants into the randomized controlled study	11/1/03 – 11/1/04
Phase 2: Intervention phase	1/1/04 – 9/1/06
Data validation, analysis and publication	11/1/03 – 11/1/06
Protocol development begins for dissemination studies	8/1/06

PBRC: Role in the Project

PBRC has well-established research programs in basic and clinical science research in obesity, comprising the most eminent obesity research programs in the world. Of relevance to this project is the

Center's role in multicenter obesity trials such as SHOW (Study of Health Outcomes of Weight Loss; now called LookAHEAD), which will enroll 6,000 participants and DPP (Diabetes Prevention Program), which enrolled 4,000 participants. The Center has established expertise in behavioral approaches to weight loss through dietary and physical activity measures, as well as assessment of a variety of relevant endpoints (i.e., body composition, biochemistry, physical activity, and food intake assessment, et al.). Additionally, PBRC has served as a data coordinating center for a multicenter weight loss trial and has experience in database creation, data collection and management and statistical data analysis.

Donald A. Williamson, Ph.D., the project leader, has extensive experience in the development of clinic-based and internet-based interventions for lifestyle behavior modification. Dr. Williamson has supervised the Diabetes Prevention Program at PBRC and has been a member of the committee that developed the treatment and training materials for the new LookAHEAD trial; both are multi-center NIH prevention trials. Dr. Williamson is Principal Investigator of an NIH sponsored study of internet-based intervention for overweight adolescents and is co-investigator on a second NIH sponsored study of an internet-based intervention for overweight adult women.

The PBRC will use this experience and expertise to guide the development of the clinic-based and internet-based interventions in Phase 1. During Phase 2, PBRC will conduct the controlled trial comparing the two interventions and will provide the internet-based counseling for one of the treatment arms.

Role of USAMRMC (U.S. Army Medical Research and Materiel Command) and USARIEM (U.S. Army Research Institute of Environmental Medicine)

Oversight of the project, guidance with regard to military relevance, and coordination of this project with similar ones undertaken by the military will be key roles for USARIEM and USAMRMC. There is a comprehensive internet application for physical training, physical fitness, and health advise being developed at USARIEM by Dr. Lieberman and this and other projects can inform the Fort Bragg intervention. For this reason we will have representatives from USARIEM and USAMRMC on the Steering Committee and the Executive Committee.

Suggestions for the Project

1. Administration

A formal organizational structure for the Task will be developed with clear roles, responsibilities and chain of command. We propose an Advisory Committee to meet annually to review and direct the project. Members include: Ryan and Williamson (PBRC), Obusek and Young (USARIEM), Friedl (USAMRMC), Fort Bragg Commander and Hubbard (NIH).

We propose a Steering Committee chaired by Don Williamson. The Steering Committee is responsible for Protocol and Manual of Operations development. The Steering Committee should consist of the following personnel:

- Task Leader - Williamson
- Fort Bragg Coordinator – To Be Named by Post Commander
- Representative from the clinic/hospital site at Ft. Bragg--To be named
- USARIEM Coordinator – LTC Bathalon
- Dietary Intervention and Assessment -- Champagne
- Physical Activity Assessment and Intervention – Larson
- Clinical Lab Assessment – Rood
- Body Composition Assessment Consultant – DeLany
- Data Coordination and Statistical Analysis – Volaufova
- Computer Services – Calderon

- Additional Consultants: LTC Freund (USARIEM), COL Obusek (USARIEM), Young (USARIEM), Lieberman (USARIEM), COL Friedl (USAMRMC)

In order to promote management efficiency, conference calls can be used to assemble committees. An e-mail list server should be developed to aid efficiency.

Finally, for the project to be successful, it will be important to have the full endorsement support of the Commander of Fort Bragg and of the operational units headquartered and stationed at Fort Bragg.

2. General Design Recommendations

Early in the course of protocol development, we must develop a system to assure confidentiality of data reported on soldiers and other military personnel. Because of the sensitive issues of standards with regard to potential discharge, we recommend that PBRC oversee data collection instruments and that the instruments be designed so as to preserve anonymity. The study data should not be identifiable for an individual to preserve anonymity. In our last grant period, Dr. Williamson and his research team developed a method to accomplish this objective.

Early in the planning phase, potential participants in the program should be evaluated as to their perceived needs, perceived obstacles to utilization of the program, and motivation for participation in the program, e.g., removal of stigma by military, personal health, appearance, social pressure by family, etc. The content and structure of the program can be in response to the findings. This can be done by focus group or survey technique.

The primary study of Phase 2 will be a randomized controlled experiment. This randomization will be at the level of the individual. We considered using a control group that would receive delayed intervention and/or receive a self-help program that is more structured than the procedures that are currently in use and/or the passive health education program that will be developed as one component of the internet-based treatment. We elected to not include this type of control group for a number of reasons: 1) there have been many controlled studies showing that clinic-based behavioral interventions are more effective than various control conditions, 2) overweight soldiers can benefit immediately from behavioral treatment and withholding treatment for a period of time could cause recruitment problems, and 3) the most important questions for the military relate to usage of the program and cost-effectiveness of the interventions, which will be addressed in the controlled study of Phase 2. Use of another military base as a unit of randomization is less desirable for several reasons, including, potential for selection bias, difficulty in collecting outcome measures on the control sample, potential confounding of a site effect with treatment, and logistical problems stemming from having two sites.

3. Intervention

A strong lifestyle behavior modification program should be developed as the backbone of the intervention. To accomplish this aim, the Clinic-based Program will be developed and evaluated in Phase 1. As noted earlier, long-term adherence to clinic-based interventions is often problematic and can be quite costly. Given these considerations, an internet-based intervention (with some clinic visits), will be developed during Phase 1.

A multidisciplinary staff is required to deliver the clinic-based intervention and conduct the outcome measures. This staff would ideally include the following: study coordinator, physician, nurse, behavioral psychologist, dietitian, and exercise physiologist. The delivery of the intervention will be supervised by a clinician with expertise and experience in the delivery of lifestyle behavior modification strategies of obesity/overweight. This clinician will be provided by PBRC by the hire of an Assistant Professor of Health Psychology during year 1. Design of the internet-based intervention will require a multidisciplinary team of

psychologists, dietitians, telemedicine specialists, and computer programmers and web designers. In year 2, an expert in telemedicine will be recruited to guide this effort.

4. Assessments

The primary endpoint for the study is body weight (BMI). Body composition measures, performance/ fitness, and meeting military weight regulation standards will be secondary endpoints. Other secondary endpoints are: utilization of various components of the lifestyle behavior modification program, compliance/adherence with various aspects of the program, various biological/health outcomes, quality of life, costs, and motivation for behavior change and weight management.

Cost-effectiveness of the intervention must be studied. Direct costs associated with the delivery of treatment are relatively easily calculated. Also, benefits such as weight loss, improved fitness, and other health benefits are easily measured. Indirect costs and benefits are more difficult to calculate. In year 3, we plan to hire a statistician with expertise in cost-effectiveness analysis

Data will be gathered to study personal characteristics of soldiers that are associated with success versus failure with weight loss and long-term weight maintenance. These characteristics will include personal, occupational, and family variables that were described earlier.

What Will Be Delivered at the Completion of Task 1?

1. A clinic based weight management program and an internet-based weight management program will be developed specifically tailored to the military and implemented at a military base.
2. Comparison of the clinic-based vs. internet-based programs will be analyzed and reported to the Army.
3. Guidelines for staff selection, protocols for staff training, and program management guidelines for the clinic-based program will be delivered to the Army.
4. Participant and provider manuals for the clinic-based programs will be delivered to the Army.
5. Data collection procedures, e.g., the use of barcodes to maintain the anonymity of participants, biological assays, etc., will be available for use by the military in other research projects.
6. Following interpretation of the two phases, PBRC scientists will participate with USARIEM and USAMRMC in planning the next stages of ongoing program development and evaluation to address military weight loss needs.

C. Significance and Uniqueness of the Proposed Project

While the military setting is a unique paradigm, there are some parallels to worksite health promotion programs. Programs that are successful in the Fort Bragg military setting could be translated to other military settings at home and abroad and could then be translated to non-military worksites. Thus, a program of proven efficacy could have a public health impact if implemented on a broader scale. The military setting provides a unique opportunity to develop behavioral weight and fitness improvement programs. Because the program will target career personnel, the ability to follow progress over years is a unique opportunity, unavailable in most worksites where out-migration is more common than in the military.

D. Potential Military Relevance

Maintenance of military readiness is essential to the defense mission. An optimally physically fit force requires attention to maintenance of healthy weight. Military personnel, along with the general American population, are subject to environmental factors that have resulted in prevalence of 55% overweight (BMI $\geq$ 25) and 22% obesity (BMI $\geq$ 30) in a representative sample of the American population (NHANES, 1992-1996). Aside from the association of overweight and obesity with a number of disease states (diabetes, hypertension, cardiovascular disease, hyperlipidemia, some cancers, et al), overweight and obesity can lead to increased risk for musculoskeletal injury, potentially impacting performance in military tasks. Obesity may

impact ability to recruit. Indeed, the rising prevalence of obesity in children (NHANES data) impacts the military acceptability of the pool of recruit-age Americans.

One factor to consider in enforcing weight standards is the potential negative impact of "crash dieting" to meet those standards. If a quick-fix approach is applied to overweight, extreme diets and fasting can have deleterious metabolic consequences. When very low calorie (< 800 kcal/d) diets are employed without medical supervision, electrolyte imbalance, loss of lean muscle mass and even death can result. Even less stringent caloric restriction is not without deleterious effects; calorie restriction without a physical activity component produces greater loss of lean tissue than fat. The goal of any weight loss program must be to preserve and improve physical performance while achieving loss of excess fat. Thus, a program of proven efficacy is preferable to the do-it-yourself approach.

It is, therefore, of critical importance that the Department of Defense addresses the issue of sustaining healthy weight and performance in military personnel through the development of programs targeting those who fail or are at risk of failure of weight and performance standards.

E. Proposed Duration of the Study

Phase 1 will require two years to develop and evaluate the clinic-based and internet-based interventions. Phase 2 will require three years to complete a randomized controlled trial that compares the efficacy of the clinic-based and internet-based interventions for weight loss and long-term weight maintenance.

F. Names, Titles, Roles and Percent Effort of Participating Personnel

Donald A. Williamson, Ph.D. the PBRC project leader is budgeted at 30 percent effort. During the first phase, Dr. Williamson will divide his time (15 % effort for both projects) between development of the clinic-based intervention and the internet-based intervention. During the second phase, Dr. Williamson will supervise the randomized controlled trial. Dr. Williamson will have responsibility for all aspects of the project where PBRC contributes in developing the research protocol, collection of data, developing and delivering the interventions, oversight of data analysis, and dissemination of the findings. Dr. Williamson has served as the Project Leader for Task VII, Enhancing Military Diets. He has visited Ft. Jackson, SC eight times over the past 18 months and is quite familiar with Army military structure and environment. Dr. Williamson has supervised the "Lifestyle Intervention" arm of the DPP at PBRC, since 1996. Also, as a member of the Lifestyle Intervention Committee of the LookAHEAD trial, he has gained extensive experience in designing lifestyle interventions. He has written portions of the participant and therapist manuals for the Look AHEAD trial and will play an important role in training staff from the 15 sites around the U.S. Dr. Williamson also has experience in the development of interactive websites for weight management. He is PI for one study (sponsored by NIH) and is co-investigator for a second study (sponsored by NIH) that uses this new technology.

Donna Ryan, M.D. is the PBRC's Special Advisor to the project. She serves on the Executive Committee of the Task, bringing expertise in clinical obesity research (Co-PI LookAHEAD Trial, Medical Investigator of numerous pharmaceutical and behavioral weight loss trials). As PI of this cooperative agreement, she assures the integration and support of Tasks 4, 5, and 6 laboratories to execute this Task.

An Assistant Professor of Psychology, To be named (TBN), will be budgeted for 100 percent effort. The primary responsibilities of the Assistant Professor during Phase 1 will be development of the clinic-based intervention and evaluation of this program. During Phase2, this person will supervise the delivery of both the clinic-based and internet-based interventions. The Assistant Professor will be an expert in lifestyle behavior modification for obesity. This person will be responsible for developing the clinic-based protocol, participant manual, therapist manual, and training of staff. This person will work very closely with the staff at Ft. Bragg and will travel to the site on a regular basis.

Michelle Burton, M.A., R.N. will be the primary dietitian for the project. She will devote 95 percent effort to the project. During Phase 1, she will divide her time developing the clinic-based and internet-based interventions. She has worked closely with Dr. Williamson on Task VII and she developed the dietary interventions for one internet-based study at PBRC and she is an online dietary counselor in this project. During Phase 2, she will serve as the dietary internet counselor.

Heather Walden, M.A., Research Associate, will be budgeted at 100 percent effort. Ms. Walden has assisted Dr. Williamson develop the treatment manuals and therapist manuals for two internet-based interventions and is currently an internet behavioral counselor. During Phase 1, she will assist the Assistant Professor in the preparation of the participant manual by writing and preparing these materials. During Phase 2, she will serve as an internet behavioral counselor.

Ray Allen, Ph.D. will be budgeted for 20% of his time for Years 1, 2, 4, and 5. During year 3, he will be budgeted at 40% effort. Dr. Allen is an expert in information systems and designing databases. He worked closely with Dr. Williamson in Task VII and he was instrumental in developing the system for gathering data with anonymity. In Phases 1 and 2, the primary responsibility of Dr. Allen will be supervision of the website design, development of the computer databases, and gathering data via scanned forms and via the internet.

Research Associate—Post-doctoral fellow in Psychology (TBN) will be budgeted at 100% effort during the entire project. This person may change from Phase 1 to Phase 2. During Phase 1, the fellow will divide time between development of the clinic-based and internet-based interventions. The fellow will assist in the development of materials and will be responsible for the collection of program evaluation data and profiles of successful weight loss. During Phase 2, the person in this position will serve as an internet counselor and will assist in the conduct of the clinical trial.

Pam Martin, Ph.D., the Assistant Project Leader, is budgeted for 30% effort. Dr. Martin will serve in place of Dr. Williamson when necessary and she will be aware of all aspects of the program. She has worked in this capacity in Task VII and has worked closely with Dr. Williamson in developing the content of one internet-based intervention. Her primary responsibility during Phase 1 will be supervising the development of the internet-based intervention and conducting the pilot study of this approach. During Phase 2, she will supervise the internet counselors. She will also be involved in the dissemination of findings.

Andres Calderon, M.A. is Director of Computer Services at PBRC. He will be budgeted at 15%. He will provide oversight of the development of the internet-based intervention and maintenance of the website after it is developed. He will be responsible for supervising the computer programmers and website developers.

Eric LeBlanc, Research Associate, will be one of the key computer programmers for the internet-based intervention. He will be responsible for design of the website and programming of automatic feedback functions and remote data collection. He is budgeted at 40% in Year 1, 60% in Year 2, 100% in Years 3, 4 and 5. During Phase 2, he will be responsible for troubleshooting programming issues and maintenance of the website.

Webmaster, Research Associate (TBN), will have the responsibility of designing the website during Phase 1 and servicing the website during Phase 2. This person will be budgeted at 100% throughout the project. This position is essential so that modifications of the website can be made quickly, in response to the demands of the study.

Stephen Mayville, M.A., Research Assistant, will be budgeted for a 20 hour per week research assistantship. Mr. Mayville is a graduate student under the supervision of Dr. Williamson. He has worked on Task VII for two years and his primary responsibility in Phases 1 and 2 will be the development of automated data collection and working with Dr. Allen for the collection of data using computer and internet technology.

Emily York, B.A., Research Assistant, will be budgeted for a 20 hour per week research assistantship. Ms. York is a graduate student under the supervision of Dr. Williamson. She has worked on Task VII for one year and she developed the system for scanned scoring of survey instruments. She has also assisted in the development of an internet-based intervention and is now an internet counselor. Her primary responsibilities will be assisting Dr. Martin in the development of the internet-based intervention and managing the remote data collection process during Phases 1 and 2.

During Year 2, an Associate Professor (TBN) with specialty in telemedicine will be recruited. This faculty member will have the primary responsibility of managing the technical aspects of the internet-based intervention and ensuring that the intervention is "user-friendly". This faculty member will have the primary responsibility of supervising the technical and clinical aspects of the internet-based intervention.

During Year 3, an Associate Professor (TBN) in statistics, cost-effectiveness analysis, and clinical trials will be recruited. This person will be essential for the conduct of the clinical trial and for analysis of the data from this study.

During Year 3 a post-doctoral fellow in psychology will be recruited. This person will assist the faculty member in telemedicine in the refinement of the website so that it meets high standards of consumer satisfaction. Also, this fellow will serve as an internet counselor.

Student workers are budgeted for $8,000 per year to provide personnel for data entry, including dietary intake data.

The following key personnel will assist in the design and implementation of the program and are funded in other tasks of this grant:

- Coordinator of Dietary Intervention and Assessment – Catherine Champagne, Ph.D. Dr. Champagne has extensive experience in dietary counseling (Co PI, Premier) and food intake assessment. She will oversee the PBRC's contribution in these areas for the Task.
- Coordinator of Physical Activity Assessment and Intervention – Ennette Larson-Meyer, Ph.D.
- Director of Clinical Lab Assessment – Jennifer Rood, Ph.D. Dr. Rood will oversee the activities of the PBRC's clinical laboratory in serving as a reference lab for the Task.
- Body Composition Studies – James DeLany, Ph.D. Dr. DeLany oversees the Stable Isotope Lab at the PBRC. He is available for consultation on body composition issues.
- Data Coordination and Statistical Analysis – Julia Volaufova, Ph.D. Dr. Volaufova will oversee the data collection, coordination and statistical analyses.

During Year 2, an Associate Professor will be added at 100 percent effort to provide expertise in data management and data analysis for large-scale clinical trials.

In Year 3, an Associate Professor with expertise in telemedicine will be added at 100 percent effort to manage the development of web sites and telemedicine initiatives. Also, a computer programmer with expertise in the development of interactive websites will be added at 100 percent effort. To support this telemedicine effort $30,000 in computer equipment and $10,000 in software acquisition will be added to the budget.

G. Major Capital Equipment

During each budget year, computer equipment is purchased. This equipment includes five servers for the internet-based intervention, computers for new faculty and staff, and computers that will be used for the internet laboratory. During year 1, $20,000 is requested. In Year 2, $56,000 is requested and in years 3,4 and 5, $50,000 is requested. These funds will be used to purchase computer equipment for: servers, internet

access, computer training at PBRC and Fort Bragg, internet counseling, and computers for new faculty and staff.

H. Subcontracts

None.

I. Brief Description of Human Use

In this study, human subjects will be enrolled in a controlled outcome study of lifestyle behavior modification. Overweight soldiers at Fort. Bragg will volunteer for the study and will sign consent forms to participate in the study. The research protocol and consent process will be evaluated and approved by the IRBs of the Army and, where relevant, the PBRC.

J. Conclusion

In this project, a series of studies will be designed to test the efficacy of weight management programs for overweight soldiers who volunteer to participate. The primary aims of the project are to develop and test cost-effective weight management programs that could be implemented on U.S. military bases around the world. This Task forms the centerpiece of this Cooperative Agreement. It builds on existing strengths at the PBRC and the collaboration provides a unique opportunity for the PBRC to play a role in work site health promotion. Tasks 4, 5 and 6 support the project. Findings from Task 2, and even Task 3, could be translated into interventions for this Task. This Task provides the military with the unique opportunity to collaborate with leading scientists in weight management approaches to develop programs that are specifically tailored to military needs.

TASK 2: CLINICAL STUDIES IN HEALTH AND PERFORMANCE ENHANCEMENT

A. Problem to Be Studied

Achieving an ideal body weight and body composition is essential for optimal performance, especially for Army personnel. It is therefore imperative to better understand the interactive process between physiological functions, genetic makeup, and environmental conditions that favor weight gain. Similarly, studies designed to identify the most appropriate dietary regimen for achieving peak performance are necessary. Such studies will be essential to identify strategies that will allow Army personnel to maintain ideal body weight, while also retaining optimal physical and cognitive performance. Because of the alarming increase in the prevalence of childhood obesity, it is now clear that the DOD will have increasing difficulty recruiting as the pool of young, fit individuals shrinks. Since the seeds of adult obesity are sown in childhood when behavioral patterns are set and since childhood overweight strongly correlates with adult overweight, this Task will have a component that examines childhood obesity.

The mission of Task 2 is, therefore, to conduct innovative clinical research designed at improving health and performance in people with relevance to military personnel. The task leader is Eric Ravussin, Ph.D. a renowned investigator in the field of energy balance, diabetes and physical activity. Task 2 is structured around the following four sections: 1) Metabolic Understanding of Energy Balance, 2) Influence of Dietary Fat on Training and Performance, 3) Obesity Prevention, and 4) Functional Genomics of Energy Balance and Training.

The overall aims of the four sections are:

1. Metabolic Understanding of Energy Balance. This section is led by Eric Ravussin, Ph.D. with the assistance

of Lilian de Jonge, Ph.D. Studies of energy metabolism in relation to diet and exercise will be conducted. There is growing evidence that obesity is caused not only by chronic positive energy balance, but also by a lack of quick adaptation to acute perturbations in energy balance or in dietary fat content. We will use state-of-the-art methods including respiratory chambers, metabolic carts, and doubly labeled water to assess energy metabolism, heart spectral analysis and microneurography to measure autonomic nervous system balance and microdialysis to determine local substrates and hormone release. To elucidate some of the underlying variability in energy metabolism caused by energy balance perturbations or training, tissue samples will be collected for gene expression determinations analyzed by the functional genomics laboratory (see Section 4, Task 2).

2. Influence of Dietary Fat on Training and Performance. This section is headed by Enette Larson-Meyer, Ph.D. to investigate the effect of diet composition (especially dietary fat) on performance, including endurance and strength. Studies will be designed to assess the interaction between diet and training on performance. Later, we plan to hire a muscle biochemist (post-doctoral level) to expand investigation of muscle adaptation to training and diet at the biochemical and molecular level.

3. Prevention of Obesity. Melinda Sothern, Ph.D. leads this section. We will conduct a three-year controlled intervention study in 14-16 year old obese adolescents (pre-recruit age population) targeting diet and physical activity in a school- and family-based setting.

4. Functional Genomics of Energy Balance and Training. George Argyropoulos, Ph.D. leads this section. To complement the above studies, tissue and DNA samples will be collected from subjects to investigate the molecular mechanisms underlying the variability in energy metabolism adaptation in response to diet and training. We will implement state-of-the-art methods to measure gene expression in adipose tissue and skeletal muscle and screen for variants in genes involved in the etiology of obesity and the response to training. Functional consequences of the identified genetic variants will be investigated.

Project 2A. Metabolic Understanding of Energy Balance: Mechanisms of Energy Metabolism Variability in Response to Perturbation of Energy Balance

A. Problem to be Studied

There are large differences among individuals in degree of body weight gain or body weight loss in response to overfeeding or negative energy balance. We know that those subjects who can increase their metabolic rate and fat oxidation in response to overfeeding are less likely to become obese. However, most of the mechanisms underlying this variability are unknown. In the first study, we aim to investigate some of the potential mechanisms underlying the variability in metabolic adaptation to perturbations of energy balance. The time line for this first project requires three and a half years for completion. We would pursue hypotheses developed from observations derived from this experiment in subsequent studies in later years of the grant.

B. Outline of Proposed Initial Research

We wish to study the metabolic response to acute overfeeding (three days duration) and complete starvation (three days) on 24-hour energy expenditure and macronutrient oxidation measured in a respiratory chamber in obesity-prone (non-obese individuals with a family history of obesity and post-obese individuals) and obesity-resistant (never obese individuals without a family history of obesity) individuals. We also wish to determine the relationship between metabolic responses to short-term overfeeding and fasting with the activity of the autonomic nervous system. Finally, since these metabolic adaptations are expressed primarily in skeletal muscle and adipose tissue, we wish to measure gene expression in these tissues in response to overfeeding and fasting.

Each volunteer will be admitted to the inpatient unit of the PBRC on three separate occasions. On each visit, they will spend three days in the metabolic chamber while either fasting or receiving 100 percent or 200 percent of their initially estimated 24-hour energy expenditure. All metabolic chamber periods will be preceded by a three-day run-in period during which a weight-maintaining diet containing 37 percent fat will be eaten. All individuals will participate in all three conditions starting with the isocaloric condition. The order of the two other test periods will be at random.

Hypothesis and Specific Aims

Obesity is the result of a positive energy balance due to energy intake exceeding energy expenditure over a prolonged period of time. Studies in pre-obese and post-obese have shown that people prone to obesity have a low metabolic rate and impaired fat oxidation. However, obesity may be favored not only by a low metabolic rate and/or fat oxidation at a given time, but also by an impaired response to acute changes in energy balance and/or weight change. This response is mostly regulated by central mechanisms, but also depends on peripheral tissue responses in terms of gene expression and biochemical adaptation.

The specific aims of this study are therefore:

1. To determine the effect of acute (three-day) overfeeding and fasting on 24-hour energy expenditure and 24-hour (RQ) in obesity-prone and obesity-resistant individuals.
2. To determine the relationship between the metabolic responses to short-term overfeeding and fasting and the activity of the autonomic nervous system.
3. To identify differences between groups in gene expression in adipose tissue and skeletal muscle at baseline (isocaloric feeding) and in response to overfeeding and fasting.

We hypothesize that:

1. Obesity-prone subjects (never-obese individuals with a family history of obesity and post-obese individuals) have a lower increase in 24-hour energy expenditure, and a higher increase in 24-hour RQ (lower fat oxidation) in response to overfeeding when compared to never-obese individuals without a family history of obesity (obesity resistant). Also, they will have more of a decrease in energy expenditure and less of a decrease in 24-hour RQ in response to fasting.
2. In obesity-prone subjects, the impaired response in energy metabolism (24-hour energy expenditure and 24 hour-RQ) is associated with an impaired sympathetic nervous system (SNS) response to acute overfeeding and fasting.
3. The third aim is more of an exploratory nature. We will identify genes being overexpressed or suppressed in response to the dietary manipulation and look for differences between groups. These preliminary data will be used for the development of a protocol of long-term overfeeding.

Primary Endpoints

To determine how an acute change in energy intake will affect energy metabolism in obesity prone and obesity resistant individuals we will measure **24-hour energy expenditure** and **24-hour RQ** in a metabolic chamber over the three days of overfeeding and fasting.

Secondary Endpoints

The main secondary endpoints will be the effects of fasting and overfeeding on **autonomic functioning**. We will measure **24-hour urinary catecholamine excretion** during every chamber day, the **response in energy expenditure to i.v. infusion of the sympathomimetic isoproterenol, and heart rate variability** during fasting and after a standard meal. In addition, we will measure **gene expression** in fat and muscle tissue.

The following parameters will be measured:

1. Body composition by DEXA using a Hologic QDR-2000 whole body scanner.
2. Maximal aerobic capacity will be determined by submaximal cycle ergometer testing using the YMCA protocol. This protocol uses three to four, four-minute stages of continuous exercise, and is designed to raise the steady state heart rate of the individual to 110-150 beats/min for two consecutive stages in order to predict VO_{2max}.
3. Twenty-four hour energy expenditure and substrate oxidation (RQ) will be measured for three consecutive days in a respiratory chamber under each experimental condition.
4. Sympathetic nervous sensitivity will be assessed by the increase in resting energy expenditure during the infusion of increasing doses of isoproterenol with each dose being administered for 45 minutes. During the entire experiment heart rate will continuously be recorded. Using linear regression analysis the dose of isoproterenol required to obtain a 25 percent increase in energy expenditure will be calculated.
5. Autonomic nervous balance will be assessed in the fasting state and 30 minutes after the ingestion of a meal by means of spectral analysis of heart rate variability. Subjects will then receive a standard meal (Ensure, Abbott Laboratories) containing 30 percent of total daily energy expenditure.
6. Gene expression will be measured by micro-arrays from fat and muscle biopsies.

Power Calculations

The power analysis was based on the assumption of an average energy expenditure of 2200 kcal/d and a within subject standard deviation of 124 kcal/d that corresponds to a four percent coefficient of variation. The minimal detectable difference in change in 24-hour energy expenditure between groups was allowed to vary between 100 and 200 kcal/d.

The following table shows the results of the power analysis. If we expect a difference in response between groups of 100-150 kcal/d, then 15-31 subjects per group would provide good power to detect treatment differences within a person. We have elected to enroll 20 individuals in each group for this study.

Difference to be detected (kcal/d)	Number of subjects/per group	Power (percent)
100	31	80.49
150	15	82.38
200	9	82.74

C. Significance and Uniqueness of the Proposed Effort

Obesity is a result of a prolonged period of excess energy intake over energy expenditure. In addition to energy balance, the individual macronutrient balances (protein, carbohydrate, and fat) have become of increasing interest since there is growing evidence illustrating that a reduced ability to increase fat oxidation in response to an increased fat intake is an important factor in the development of the disease. Although a low resting metabolic rate and an impaired fat oxidation are predisposing factors for obesity, an impaired short- to mid-term capacity to regulate energy and fat balances in response to acute imbalances may also contribute to the propensity to weight gain. This study will represent the first attempt to identify the variability in energy metabolism responses to acute manipulation of energy balance in obesity-prone and obesity-resistant subjects. Furthermore, assessment of autonomic nervous system activity and gene expression analysis will provide information on the potential mechanisms underlying this variability.

D. The Potential Military Relevance

Overweight and obesity not only impact the health status of the general population, but also have a negative effect on the health and performance of military personnel. Of particular concern are potential effects on career military personnel. Due to the variability of physical activity of young recruits, including

periods of vigorous basic training and periods of more sedentary behaviors, it is important to identify the potential mechanism of weight gain caused by cycles of positive and negative energy balance. Research in the PBRC metabolic chambers has demonstrated not only variability of response in fat oxidation to high fat diet challenges, but also impaired adaptation in sedentary individuals compared to fit individuals. Our proposed study will not only identify potential physiological mechanisms underlying the variability in energy metabolism responses, but may also identify predictors of health outcomes. The proposed studies may yield information to be incorporated into targeted interventions developed in Task 1.

E. Proposed Duration of the Initial Experiment

We anticipate enrolling approximately 20 subjects per year over a three-year period in this protocol. The proposed time line is as follows:

Year 1, first six months	Years 1, 2, 3 and 4	Year 4, last six months
Study design, staff recruitment, organization and implementation of the study.	Subject enrollment in the study.	Data analysis, manuscript writing and publication

Following completion of the study, we will develop additional studies based upon hypotheses derived from this experiment.

F. Names, Titles, Roles and Percent Effort of Participating Personnel

Eric Ravussin, Ph.D., Head, Division of Health and Performance Enhancement and project leader of Task 2, will devote 40 percent effort to this section (administration is 20 percent and Projects 2, 3 and 4 are 10 percent each). He is key to development of the study design and its implementation, supervising its progress, as well as assuring quality control of all procedures. Dr. Ravussin is also responsible for supervision of data analysis and reporting of its results.

Lilian de Jonge, Ph.D. devotes 50 percent effort to the project contributing to the study design, as well as its implementation in the metabolic chamber. She is responsible for accuracy of chamber data collection and collaborates on data analysis and reporting. She is a graduate of the University of Montreal in Quebec, Canada, and received her doctoral degree there in 1996. During her Ph.D. course work she worked on the role of the ANS in resting energy expenditure in lean and obese individuals. She trained as a post-doctoral fellow with George Bray, M.D. at the PBRC, and has directed the indirect calorimetry laboratory at the PBRC since 1997. She has conducted three studies investigating the metabolic adaptation to changes in dietary fat intake using the metabolic chambers.

We have recruited a Physician (clinician scientist) to support Task 2. Michael Hamilton, M.D. was Director of the Duke University and Fitness Center in Durham, North Carolina from 1985-1999 before becoming the Director of the Weight Management Program at the Pritikin Longevity Center in Florida. He will join the PBRC in May 2001. For this section the physician provides medical supervision, performs tissue biopsies, and supervises isoproterenol infusion testing.

Damian Blanchard, M.S., Research Associate (100 percent effort), is responsible for study coordination and data collection, entry and processing.

A Post-Doctoral Student will be recruited at 100 percent effort to assist with the execution of the project under Dr. de Jonge and Dr. Ravussin's supervision.

A Research Associate will oversee the muscle library for all projects at 100 percent effort.

Tuong Nguyen, Metabolic Chamber Engineer (10 percent effort), is responsible for maintenance and calibration of the metabolic chamber and routine quality control of data generated. He obtained a B.E. in biomedical engineering from Tulane University in New Orleans, Louisiana in 1999 and has been working in the metabolic chambers laboratory at the PBRC since that time.

Jason Fuqua, Research Associate (25 percent effort), is responsible for maintenance and calibration of the metabolic cart and respiratory chambers. He contributes data acquisition and entry, as well as scheduling of patients. He holds a B.Sc. in biology from McNeese University in Lake Charles, Louisiana.

Dietary support (planning of experimental diet, education of research volunteers, and collection of food intake data) will come from Tasks 6 and 7. Personnel in Task 7 will provide preparation of food for the experimental diets. Clinical chemistry determinations will be provided by Task 4. Stable isotope determinations are provided by Task 5.

G. Itemized List of Major Capital Equipment

Equipment in Place:

- Two 27,000 L respiration chambers – each chamber is provided with furnishings and equipment necessary to perform metabolic studies over extended periods of time in a precisely controlled environment.
- A Deltratrac II metabolic monitor (Datex-Ohmeda, Helsinki, Finland) with ventilated hood – room air is drawn through the hood with a known fixed flow, and the concentration of oxygen and CO_2 in inspired and expired air are measured to determine metabolic rate.
- A Hologic QDR-2000 dual energy x-ray absorptiometer (DEXA) – for the measurement of body composition.

There are no anticipated major purchases.

H. Subcontracts

None.

I. Brief Description of Human Use

1. The human subjects involved in this research will be 60 women from the Baton Rouge area, who are at least 18 years of age. There will be 20 never-obese, dietary restrained women with a family history of obesity, 20 post-obese women who have lost at least 20 percent of their body weight and have maintained this weight for at least six months within 2 kg, and 20 never-obese women without a family history of obesity. For power purposes, especially due to the gene expression work, we will study only women. We hope to be able to expand the study to men in future studies, depending on the results of this study.
2. We will collect data on energy expenditure, substrate oxidation, body composition, physical fitness, autonomic nervous system activity, clinic blood chemistry measures, body image, food intake, and gene expression from muscle and fat biopsies.
3. Consent forms will be discussed with each participant, and all questions will be answered. The consent forms will then be signed by the participant, the PI, and a witness.
4. The anticipated risks associated with this study are the potential for bruising and infection related to the blood draws and the biopsies, weakness and fainting related to the three day fast, bloating and nausea related to the overfeeding, and exposure to a small dose of radiation related to the DEXA. All data will be stored in a private manner to avoid identification of the subjects.
5. The protocol will be approved by the HSRRB and the PBRC's IRB prior to initiation of the study.

J. Conclusions

The increased incidence of overweight and obesity among military personnel parallels national patterns. The present study will identify potential mechanisms underlying the susceptibility to weight changes in response to acute changes in energy balance, a factor common during training and subsequent careers of our military forces. More importantly, the proposed studies may provide some predictors of weight gain in populations at risk for obesity. These predictors, if measurable, may then be used to target those at risk with prevention programs very early in their military career obviating remedial measures. The PBRC is uniquely positioned to conduct these studies because of the availability of respiratory chambers (two of approximately 10-15 in the world) and the expertise in energy metabolism and molecular biology.

Project 2B. Influence of Dietary Fat on Training and Performance

A. Problem to Be Studied

The quantity of lipid droplets stored within skeletal muscle fibers (intramuscular lipids – IML) may be important in controlling lipid utilization and performance during exercise. Under normal dietary conditions, skeletal muscle contains significant stores of IML that serve as an important fuel during exercise, particularly prolonged moderate-intensity exercise. Several previous studies have demonstrated that IML are significantly depleted after moderate to strenuous endurance exercise, i.e., endurance running, endurance cycling and possibly prolonged vigorous marching. Studies have also alluded to the possibility that extremely low-fat diets (i.e., 10-15 percent of total energy from fat) may be detrimental to performance in endurance-trained individuals, possibly by compromising IML stores.

While it is well established from studies in male athletes that a certain quantity of dietary carbohydrate (6 to 10 g CHO per kg of body weight) is necessary to maintain adequate muscle glycogen stores and optimize performance during both endurance exercise and repeated bouts of high-intensity exercise, little is known concerning the quantity of dietary fat that will both optimize performance and promote good health in active individuals (such as military troops) and highly trained individuals (endurance athletes and Military Special Forces).

B. Outline of the Proposed Research

We wish to initiate two studies that form the basis for subsequent experiments. These two experiment evaluate:

1) the influence of two diets, one low in fat and high in carbohydrate (20 percent fat, 65 percent carbohydrate, 15 percent protein) and one moderate in both fat and carbohydrate (35 percent fat, 50 percent carbohydrate, 15 percent protein) on the adaptation to an intense exercise training program in healthy, but previously inactive individuals (study 1) and;
2) the influence of a controlled very low-fat (10 percent fat) and moderate-fat (35 percent fat) recovery diet on replenishment of IML and glycogen stores following a bout of prolonged moderate exercise, and subsequent performance in trained endurance athletes (study 2).

In both studies we will measure the influence of the diet-training interaction (study 1) and the composition of the recovery diet (study 2) on gene expression. The combination of these two studies will help determine the optimal dietary fat composition in two groups of subjects, a previously inactive group entering an intense training program (similar to military basic training), and a highly active endurance-trained group (similar to Military Special Forces, Rangers, Seals, etc., during periods of intense training).

Study 1.

In study 1, healthy college-age volunteers will be randomly assigned to either the low- or the moderate-fat diet and provided instruction for following and maintaining the assigned diet composition for the duration of a 16-week training program. Fitness and performance tests (primary outcomes), as well as specific health outcomes (secondary outcomes), will be completed at baseline, midpoint, and at the end of the training program. Biopsies of muscle and adipose tissue will also be obtained at these same time points.

Hypothesis:

We hypothesize that: (a) in response to training, performance improvements will be greater when consuming a moderate-fat diet compared to a low-fat diet, and that (b) health outcomes will not be impaired by a moderate fat diet that contains mostly mono- and poly-unsaturated fat sources. Adaptation to the moderate fat diet in combination with training would enhance the ability to store and utilize IML during moderate prolonged exercise, thereby sparing muscle glycogen for use during more strenuous activities.

Specific Aims:

- To determine the influence of two levels of dietary fat in combination with exercise training on aerobic power, endurance, strength and body composition outcomes (VO_{2max} test, preloaded endurance test to exhaustion, 1.5 mile run, 1 repetition max (RM) lifts on specific exercises, DEXA).
- To determine the influence of two levels of dietary fat in combination with exercise training on the quantity of IML by both invasive (biochemical analysis of muscle biopsy sample) and noninvasive techniques (CT scan of mid-thigh); and the intracellular location of IML by electron microscopy morphometric analysis.
- To evaluate the influence of two levels of dietary fat (with similar levels of saturated fat, e.g., <10 percent) in combination with exercise training on fasting insulin and lipoprotein profile.
- To measure the influence of two levels of dietary fat in combination with exercise training on activity of key metabolic enzymes, i.e., creatine kinase, PFK, citrate synthase, HADH, cytochrome C oxidase and muscle gene expression by real-time PCR or micro-arrays.
- To evaluate the influence of two levels of dietary fat in combination with exercise training on energy expenditure and fat oxidation by respiratory chamber.

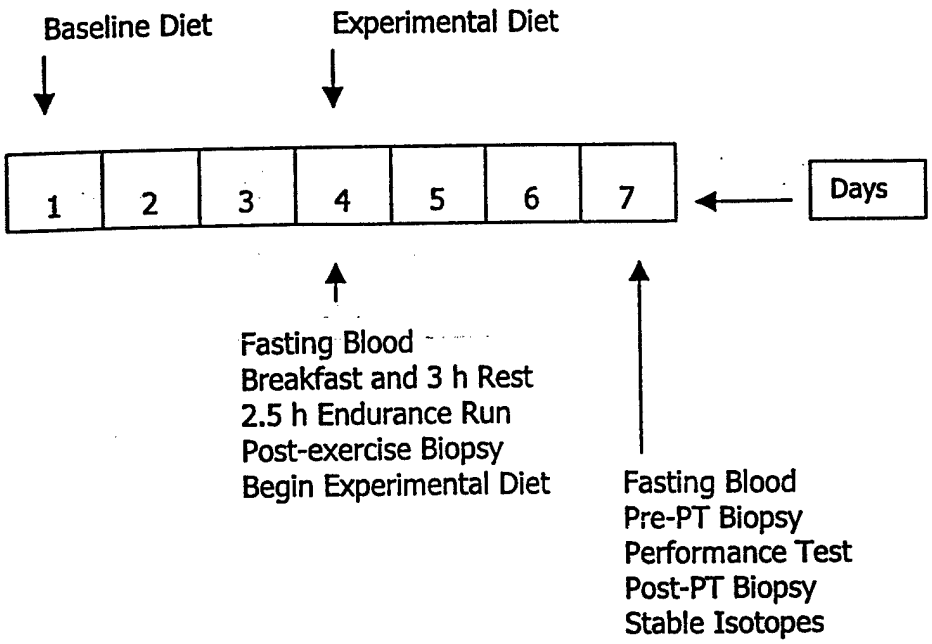
Study 2.

As outlined in Figure 1 (see on the following page), endurance athletes will perform a controlled bout of endurance exercise (approximately 150 minutes of running or cycling at a moderate pace, 65 percent VO_{2max}, i.e., a IML-depleting exercise) and subsequently be randomly assigned (in a cross-over design) to either three days of a very low-fat or a moderate-fat recovery diet. An endurance test designed to evaluate whether differences in IML (and possibly glycogen stores) (primary outcomes) will result in differences in endurance performance will then be performed after 72 hours of consuming the recovery diet. Biopsies for measurement of IML, glycogen, lipoprotein lipase and candidate gene expression will be obtained at baseline, immediately following the IML depleting exercise, after three days (72 hours) of consuming the experimental recovery diet and immediately following the endurance performance test. The crossover diet treatment will be performed approximately one month apart in men and one menstrual cycle apart in women (testing will be done in the follicular phase of the menstrual cycle).

Hypothesis:

We hypothesize that compromised IML stores, as a result of following a very low-fat diet, will result in impaired performance, and that the improvement in performance will correlate with a sparing of muscle glycogen by higher initial stores of IML (at the start of the performance test).

Figure 1
Experimental Protocol



Specific Aims:

- To determine the influence of "compromised" IML content on endurance performance, three days after a bout of IML-depleting endurance exercise.
- To correlate the changes in performance with differences in intramuscular triglyceride and glycogen stores (via muscle biopsy biochemical analysis), IML location via electron microscopy morphometric analysis, and substrate utilization using stable isotopes (in collaboration with Task 5).
- To measure the influence of fat content in the recovery diet on fasting insulin and lipoprotein profile.
- To measure the influence of fat content in the recovery diet on activity of key metabolic proteins and enzymes, i.e., LPL, fatty acid binding protein, carnitine palmitly transferase, HADH, etc., and muscle gene expression by real-time PCR or micro-arrays.

C. Significance and Uniqueness of the Proposed Effort

Several recent studies have suggested that endurance-trained individuals participating in vigorous endurance training programs may perform better on diets that are moderate compared to those very low in fat. This is probably related to the fact that endurance training increases the capacity of skeletal muscle to utilize free-fatty acids (FFA), particularly FFA derived from fat depots within skeletal muscle. Increasing metabolic utilization of IML as a fuel source is further thought to spare muscle glycogen, which could improve endurance performance. A recent study by Dr. Larson-Meyer has found that, following two hours of endurance running, IML stores were not replenished by 22 hours post-exercise with a very-low fat recovery diet (10 percent fat) but were replenished with a moderate fat diet (35 percent fat). Interestingly, IML stores were not replenished even three days post-exercise with the low-fat diet. Other recent investigations have further suggested that low-fat diets may contribute to exercise-induced amenorrhea, compromise immune function and elevate serum triglycerides.

An optimal diet for active individuals would be one that allows for replacement of both muscle glycoger and muscle triglycerides (in sufficient time to continue training or hard work) and also promotes good health. Few studies, however, have compared whether diets more moderate in fat (with the increased fat coming

from mono- and polyunsaturated fats) compared to those low or very low in fat would:

1) influence adaptation to an exercise training program;
2) promote a more balanced recovery of muscle glycogen and IML following prolonged exercise (known to compromise both muscle glycogen and IML stores) and;
3) influence health parameters important for preventing acute illness/injury and chronic disease in active populations.

In this regard, the few published studies have focused mostly on men. Little information is known concerning the sport nutrition requirements of active and highly trained women. Our proposed studies are timely and very important because an increasing number of active individuals, particularly women, have been adopting extremely low-fat diets (i.e., <10-20 percent of energy from fat) with the belief that dietary fat consumption will increase adiposity and impair health and/or performance.

D. The Potential Military Relevance

It is imperative that the military know the dietary composition that will optimize both the performance and health of their male and female personnel. Since women make-up about 16 percent of those entering basic training in the Army, Air Force and Navy, it is even more important to understand whether the nutritional needs of active women are different than those of active men. Results from our proposed studies will help in providing adequate nutrition and nutrition education for new recruits during basic training (study 1) and for elite troops during excessively demanding training (study 2). In both cases, the target study groups serve as analogue populations, since it is not feasible to perform these experiments in military personnel. In the latter case we feel that trained endurance athletes serve as the ideal group for Special Military Forces/Elite Troops due to the probability that both groups utilize significant IML during prolonged bouts of training and active duty/combat. Certain body fat and energy expenditure parameters obtained in study 1 (e.g., 24-hour energy expenditure and fat oxidation, composition of weight-loss, fasting insulin concentration) will also ensure that consumption of a moderate-fat versus a low-fat diet would not likely lead to weight gain (and obesity) following basic training. Finally, since the fat composition of the diet may also be related to menstrual cycle function, future studies could focus on how fat composition would influence these parameters and perhaps prevent loss of duty time due to acute illness or skeletal injury.

E. Proposed Duration of the Study

Study 1. We anticipate enrolling a total 60 subjects to have an end retention sample size of 48 subjects (12 males and 12 females in each diet group). Assuming an 80 percent power, this sample size would give the power to detect a performance difference of about 12 minutes in a preloaded time trial endurance test. We will enroll approximately 10 individuals at a time in five to six training groups spaced approximately six months apart.

Year 1, first six months	Years 1,2,3,4	Year 5
Study design, staff recruitment, organization and implementation of the study	Subjects enrolled in training study	Data analysis, manuscript writing and publication, development of subsequent experiments

Study 2. We anticipate enrolling 10 well-trained female athletes during the first year and a half, and then enrolling 10 well-trained male athletes during the second year. Assuming an 80 percent power, this sample size would give the power to detect a performance difference of about 12.5 minutes in a preloaded time trial endurance test. Studying the female athletes first will allow us to group match the male subjects to the female subjects based training and aerobic fitness.

Year 1, first six months	Years 1 and 2	Years 2,3	Years 4,5
Study design, staff recruitment, organization and implementation of the study	Female subjects enrolled in crossover study; Data analysis, manuscript writing and publication of data in women	Male subjects enrolled in cross-over study; Data analysis, manuscript writing and publication of data in men and final analysis of project in both genders; Development of subsequent experiments	Design of future investigations based on information gained in this study (e.g., role of dietary fat in menstrual function in highly trained women)

F. Names, Titles, Roles and Percent Effort of Participating Personnel

Enette Larson-Meyer, Ph.D., R.D., Head, Nutrition and Exercise Laboratory is responsible for the day-to-day leadership in directing this project at 100 percent effort, with advice and oversight by the Task Leader, Dr. Ravussin, at 15 percent effort. Dr. Larson-Meyer has conducted previous clinical studies evaluating the effect of diet on performance and has extensive experience conducting studies in female populations. Dr. Larson-Meyer is knowledgeable in the area of nutrition and performance; she served as the sports nutritionist for the University of Alabama at Birmingham (UAB) athletic department for five years and taught undergraduate and graduate level sports nutrition classes. In addition, she has a successful track record of collaborating with Dr. Ravussin during her pre-doctoral work as the research dietitian at the NIH, NIDDK in Phoenix.

A postdoctoral level position will be recruited (under Dr. Larson-Meyer and Dr. Ravussin's supervision) to perform muscle biochemistry and electron microscopy in association with the experiments described in this project. It is anticipated that the successful candidate will be experienced performing biochemical, histochemical and molecular analysis in human skeletal muscle.

The physician scientist described in Task 2A, Michael Hamilton, M.D., will perform all the medical procedures required in the project, including physical examination and medical history, muscle and adipose tissue biopsies, infusion tests, such as stable isotope infusion during exercise, etc.

Damian Blanchard, M.D., Research Associate (100 percent effort), is responsible for study coordination and data collection, entry and processing.

Dietary support (planning of experimental diet, education of research volunteers, collection of food intake data) will come from Tasks 6 and 7. Personnel in Task 7 will provide preparation of food for the experimental diets. Clinical chemistry determinations will be provided by Task 4. Stable isotope analysis will be provided by Task 5.

An Exercise Biochemist (TBN) at 100 percent effort will be recruited in Year 2 of the grant and will be responsible for histochemical and biochemical analysis of muscle biopsies, preparation and analysis of muscle samples for electron microscopy (EM), and gene expression work.

G. Itemized list of Major Capital Equipment

None.

H. Subcontracts

None anticipated.

I. Brief Description of Human Use

Study 1. Healthy college age men and women will be recruited to participate in the study. To be eligible for the study, volunteers must not be participating in a regularly scheduled exercise program, be free from chronic disease, not be taking any medications/supplements known to influence body composition and/or energy expenditure, and be either of normal body weight or slightly overweight (BMI<27). Women must be having regular menstrual cycles.

Study 2. Well-trained male and female endurance athletes will be recruited to participate in the study. To be eligible for the study, volunteers must be currently performing regularly scheduled endurance training (> four days per week), which includes regularly scheduled bouts of exercise of two hours or more, have been performing this type of training for at least a year, have a VO_{2max} of >49 ml/kg/min for women and 59 ml/kg/min for men, and have a negative history of eating disorders or a recurrent pattern of overuse injuries. Volunteers must also be free from chronic disease and not be taking any medications/supplements known to influence body composition and/or energy expenditure. Women must be premenopausal and be having regular menstrual cycles and/or taking cyclic estrogen/progesterone.

The protocols for Studies 1, 2 and any subsequent studies will be approved by HSRRB's and PBRC's IRBs before the study is initiated.

J. Conclusions

The optimal dietary composition of fat for individuals that are highly active is not known. Diets that are either too high or too low in fat may impair both health and performance. While diets too high in total fat and saturated fat may compromise muscle glycogen stores and increase the risk for developing cardiovascular disease and diabetes, diets that are too low in fat may be linked to amenorrhea, compromised immune function, elevated serum triglycerides and poor performance via compromised IML levels. These studies propose to determine whether diets that are more moderate in fat (with the additional fat coming from mono- and poly-unsaturated sources) will influence positively the adaptation to an intense exercise training program in untrained individuals, and influence the recovery of highly-trained athletes after a bout of endurance exercise. The results of these studies will provide important information concerning the optimal nutrient composition to enhance physical performance and maintain health in both male and female military personnel. The PBRC is positioned to provide the scientific expertise and infrastructure to successfully address these important questions.

Project 2C. A School/Community Based Intervention to Prevent Adult Obesity in Overweight Adolescents: A Three-year Randomized and Controlled Trial

A. Problem to Be Studied

Americans are becoming obese earlier and the long term physiologic and metabolic consequences, even if not fully understood, are deleterious. Therefore, research efforts should focus on methods and techniques that primarily halt the onset of obesity early in life, but moreover, prevent overweight teenagers, who currently number 30 percent of the adolescent population, from becoming obese young adults. The PBRC has conducted studies in 140 Baton Rouge school children, 12-14 years of age, documenting that more than 50 percent of these children have a BMI in the top 15th percentile, according to U.S. normative data (DeLany et al., in review), and demonstrating the availability of a local target population for study.

B. Outline of Proposed Research

We propose to examine the feasibility and efficacy of a three-year randomized and controlled trial of a community/school-based intervention program to prevent adult obesity in overweight adolescents, 13-15 years of age.

The goal of this study is to provide an intervention that will prevent the onset of a clinically significant obese condition (>95th percentile for age-adjusted BMI USCDC) in adolescents who are classified as overweight (>85th percentile age-adjusted BMI) but not yet obese. **It is well documented that over 80 percent of these overweight adolescents will become obese adults.**

The intervention program will be implemented in adolescents in a family-based, school/community setting. We think this type of setting provides a practical and effective venue for wider implementation.

If our interventions prove to be feasible and effective, they could be implemented on a larger scale. We propose to adopt an enhanced version of the Committed to Kids program that we have used at the PBRC and at the LSU Health Sciences Center in New Orleans for over 8 years.

We will use standard field techniques to annually measure treatment and control school-aged subjects during a critical period of development (as defined in the research literature): Adolescence, approximately 13-15 years of age (9th grade). Body mass index will be the primary outcome variable.

The annual field measurements include weight, height, body mass index, waist and hip circumference, body composition using bioimpedence and skinfolds, blood pressure by Dynamap, physical fitness by standard field testing, family and medical history by questionnaire, self-report Tanner stage, and physical activity by questionnaire.

Experimental Research Design (two group, repeated measures):

Risk Groups	Age	Treatment (N=60)	Control (N=60)
Overweight (>85 <95 percent age-adjusted BMI)	13-15 years	Overweight adolescents and families participate in an enhanced three-year Committed to Kids Weight Management Program, implemented in school/ community sites.	Overweight adolescents receiving standardized health information.

C. Significance and Uniqueness of the Proposed Effort

The escalating rates of adolescent obesity will impact the future health of Americans. Eighty percent of overweight 10-15 year olds will become obese adults if successful interventions are not developed. The increase in the prevalence of obesity is well documented in male and female adolescents and in those from all ethnic backgrounds.

Reducing the body mass index of overweight children of all ages has been shown to result in an improvement of chronic disease risk factors. The long-term health benefits of family-based weight management programs in a clinical environment are well-established in 9-12 year old overweight children. Benefits in lipid profiles and body composition have been documented in these children 10 years later during adulthood. In addition, the PBRC and the Louisiana State University Health Sciences Center (Committed to Kids Pediatric Weight Management) have published numerous reports demonstrating significant improvements in obesity, lipid profiles, and aerobic endurance over three-month and one-year periods in overweight and

obese children, 5-18 years of age in a clinical setting. The PBRC program combines nutrition education, behavior modification, but more importantly, specialized exercise techniques and targeted methods to reduce sedentary behaviors based on scientifically proven theoretical models (see example below).

Sample PBRC Committed to Kids Sample Weekly Clinic Intervention Schedule:

WEEK	TOPIC	ACTIVITY DESCRIPTION	TIME
1	Nutrition Behavior Exercise	Healthy eating and portion control Goal-setting and Self-Monitoring Safety and Exercise (Moderate-intensity Progressive Exercise Program [MPEP] /The MPEP Step to increase daily physical activity.	5:00-6:00 6:00-6:20 6:20-6:30
2	Nutrition Behavior Exercise	Menu planning, recipe substitution Commitment Rating The Metabolic Systems of the Body: Aerobic versus Anaerobic Metabolism – Low Impact Aerobics to Music	5:00-5:15 5:15-5:30 5:30-6:30
3	Nutrition Behavior Exercise	Fun in the Kitchen – Oven Fried Chicken Benefits and Sacrifices: Limit Setting & Rules for eating Modified Aerobic Field Sports	5:00-5:30 5:30-5:50 5:50-6:30
4	Nutrition Behavior Exercise	Fun in the Kitchen – Low Calorie Pizza Habit Formation: ABC's of Behavior; Behavior Chain The MPEP-pump – strength training	5:00-5:30 5:30-6:00 6:00-6:30
5	Nutrition Behavior Exercise	Dining out: restaurant choices Eating Patterns; Goal Check; Practical Solutions The Flex Test to test for imbalances in posture and flexibility – Stretch 'n Flex Series	5:00-5:30 5:30-6:30 6:00-6:30

To date the PBRC Committed to Kids program has not been examined under randomized and controlled experimental conditions in a non-medical setting. Also, it is not known whether similar approaches applied to overweight adolescents in a community/school based setting will prevent the onset of adult obesity. Previous attempts to reduce the BMI of overweight children and adolescents in a school based environment have been unsuccessful. It is proposed that subjects compensate at home by increasing sedentary behaviors, such as TV-watching, and also through increased consumption of high-calorie snacks. Targeted, family-based programs conducted in school/community based settings may provide a viable means for the prevention of adult obesity in adolescents. By focusing on both the home and community environment, in addition to the school setting, this tendency toward compensation outside of school may be reduced. Furthermore, by targeting adolescents who are at risk for obesity (due to an elevated BMI) during a critical period of development we will increase the opportunity for achieving significant results. Our experienced faculty and staff are perfectly suited to accomplishing this task.

D. The Potential Military Relevance

Why should this project be a part of military nutrition research? First, there an increasing prevalence of obesity and Type 2 Diabetes in young adults. The pool from which the armed forces recruits has a higher proportion of unsuitable individuals and even those who meet entrance standards may be marginally effected. New recruits are required to participate in a vigorous basic training program and overweight or obesity significantly impairs their physical ability. Overweight decreases exercise tolerance and increases the risk of injury to the joints of the lower extremities during training and in times of physical stress. The declining fitness level of new recruits may be related to the increase in the prevalence of overweight and decrease in physical activity nationwide. If the national epidemic of adolescent obesity is not sufficiently addressed, it will become

increasingly more difficult to train and maintain healthy and physically fit Army personnel. Lastly, and most importantly, this study must be viewed in the larger context of the overall theme of the grant. If obesity is of military relevance, then adolescent obesity cannot be ignored.

E. Proposed Duration of Study

We anticipate that the study participants will be drawn from three high schools (based on estimates from the Ascension Parish School Board) and will participate in the study over a five-year period.
1) Year 1: We will refine our intervention techniques in preparation for the adolescent study intervention trial, train the testing and intervention staff and conduct a 3-month pilot study. This will be followed by a program evaluation including adolescent, family and school personnel focus groups.
2) Year 2: We will target overweight (defined as >85th <95th percentile age-adjusted BMI) 9th grade students with a family-based weight management intervention utilizing school, health and community centers for a three-year period. We will conduct baseline measures in a cohort of 120 ninth grade students. Schools will be randomly assigned as treatment or control. Volunteer overweight subjects and family members from treatment schools will attend 90-minute dynamic, educational classes based on an enhanced version of the Committed to Kids approach (adding community links and web-based technology) weekly, after school for a one year period. The subjects will be measured annually thereafter with a final follow-up measure three years later in 12th grade. Control subjects will receive standardized health information classes once per month after school on topics such as smoking prevention, substance abuse prevention, firearm safety, fire safety, and prevention of sexually transmitted diseases.
3) Year 3: Annual measures will be conducted. Treatment subjects and families will attend monthly 90-minute maintenance sessions after school. Control subjects will continue to receive standardized health information classes once per month after school.
4) Year 4: Annual measures will be conducted. Treatment subjects and families will attend monthly 90-minute maintenance sessions after school. Control subjects will continue to receive standardized health information classes once per month after school.
5) Year 5: In the final year of the study we will conduct three-year follow-up testing in 120 ninth grade student treatment and control subjects. These students will be in 12th grade and will be between 16-18 years of age.

The proposed time line is as follows:

Year 1 9/2001-9/2002	Year 2 9/2002-9/2003	
Refine intervention program; Staff training; 3-month pilot study followed by a program evaluation and revision.	Baseline testing: Ninth grade treatment and control subjects. Implement intervention trial in 9th grade treatment subjects (N = 60 [refer to justification below]). Treatment subjects receive weekly intervention meetings. Control subjects receive standardized health information.	
Year 3 9/2003-9/2004	Year 4 9/2004-9/2005	Year 5 9/2006-9/2007
One year follow-up testing in 9th grade treatment and control students (N=120) who are now in the 10th grade. Monthly intervention meetings for treatment subjects. Control subjects receive standardized health information.	Two year follow-up testing in 9th grade treatment and control students (N=120) who are now in the 11th grade. Monthly intervention meetings for treatment subjects. Control subjects receive standardized health information.	Three-year follow-up testing in 9th grade treatment and control students (N=120) who are now in the 12th grade. Data analysis and reports.

Schools will be randomly assigned to treatment or control conditions. We estimate that we will recruit approximately 60 treatment and 60 control subjects from either one or two of three high schools existing in the parish. Both treatment and control ninth grade student subjects will have measures at the beginning of 10^{th}, 11^{th} and 12^{th} grade.

F. Names, Titles, Roles and Percent Effort of Participating Personnel

Melinda S. Sothern, Ph.D., C.E.P., Laboratory Director (75 percent effort), project leader, is responsible for study design, implementation and follow-up. She oversees staff recruitment, training and supervision. She supervises data acquisition, entry, analysis and reporting and assists with the delivery of the study intervention.

Eric Ravussin, Ph.D., Head, Division of Health and Performance Enhancement (15 percent effort), Co-Investigator, assists Dr. Sothern in study design, data analysis and reporting.

Michael Hamilton, M.D., Clinical Investigator, will provide medical supervision for the project (see Project 2A for more details).

James Delany, Ph.D., Director, Stable Isotope Laboratory Head-Task 5, will collaborate on and advise the project. Dr. DeLany has conducted a descriptive study of risk for obesity by longitudinal follow up of 116 Baton Rouge school children followed over six years.

Timo Lakka, M.D. is a visiting epidemiologist at the PBRC. He will advise the project on a gratis basis.

Enette Larson-Meyer, Ph.D., R.D. will advise on physical activity testing (see Task 2B).

David Harsha, Ph.D. has extensive experience in observational and family-based interventions in children. He was active in the Bogalusa Heart Study.

Donald Williamson, Ph.D. (see Task 1) is available for behavior intervention advice.

Melissa Terry is a Research Associate at 50 percent charged with overseeing the field testing (scheduling, assessments, data acquisition, as well as participating in the delivery of intervention classes).

Robin Ours, a Research Associate at 50 percent effort, will assist with data acquisition and assist in conducting intervention classes.

To be announced: Research Associate at 15 percent effort, will assist with data acquisition and assist in conducting intervention classes.

G. Itemized list of Major Capital Equipment

None.

H. Subcontracts

None.

I. Brief Description of Animal and Human Use

Children ranging from 13-18 years of age will participate in the study. Recruitment will target local high schools. Participation will be voluntary and parental consent will be obtained prior to any screening or testing. Patient confidentiality will be upheld at all times.

Subject Inclusion Criteria: To be included subjects must be:
1. At least 13 years old, but not yet 15 years old. Body mass index >85th <95th % age-adjusted BMI.

Subject Exclusion Criteria: Subjects will be excluded if they have any of the following conditions:
1.Age adjusted BMI <85th% or >95th% based on national normative values.
2. Heart Disease (such as atherosclerotic cardiovascular disease, cardiac arrhythmia)
3. Liver Disease
4. Viral Disease (such as hepatitis, etc.)
5. Currently taking medications including diuretics, steroids, or other drugs that stimulate the adrenal gland.
6. Lung disease, nervous system disease.

Potential Risks and Benefits:

The field tests are non-invasive and not physically demanding. The intervention includes nutrition and fitness education and family counseling. There are no known risks to participation in the study other than those associated with a typical fitness regime. The benefits to participation include health status information for all subjects and positive changes in obesity and related disease risk in the treatment subjects.

Human Subjects Review:

The protocols will be reviewed and approved by the HSRRB and the PBRC IRB prior to initiation of the study.

J. Conclusions

As part of the overall theme of the grant, it is appropriate to include a component that addresses the childhood origins of obesity. Programs that positively alter the obesity and health status of U.S. youth may indirectly improve the health and performance of Army recruits. The health and performance of future Army recruits is dependent upon slimming the nationwide epidemic of childhood and adolescent obesity. More importantly, our country's future health requires preventing the onset of obesity. The PBRC is uniquely positioned to provide the scientific expertise and infrastructure to successfully address the important issue of obesity prevention.

Project 2D. Functional Genomics

A. Problem to Be Studied

A plethora of evidence from human and animal studies and *in vitro* data indicate that genetic factors are involved in the regulation of food intake and energy expenditure. We classify the genetic factors as central or peripheral, depending on the tissue and location of gene expression. Our studies are designed to address both types of genetic factors by looking at genes that express in the central nervous system (i.e., central genetic factors) and genes that express in fat and muscle (i.e., peripheral genetic factors). The main goal of the proposed study is to identify and functionally characterize the genetic pathways that regulate food intake and energy homeostasis in humans and more particularly in military personal undergoing variable exercise programs and diets.

B. Outline of the Proposed Research

Body-weight is the result of the balance between energy intake and energy expenditure (i.e.

energy homeostasis). Body weight can significantly vary according to the amount of caloric intake and physical activity, while the balance of energy homeostasis is ultimately determined by cellular functions that are responsible for energy utilization to maintain a constant body temperature, muscle tone, and CNS activities. Any one of these functions (i.e., caloric intake, physical activity, body temperature, muscle tone, and CNS activity) are manifestations of gene-gene and gene-environment interactions. However, certain genetic variations like random mutations, population-specific polymorphisms, or gene expression levels due to polymorphisms in the promoters of genes, can perturb the fidelity of this highly tuned genetic and biochemical mechanism. To study this mechanism, we will employ two genetic strategies: (1) identification of new genetic pathways during high and low physical activities and variable diets, and (2) functional characterization of candidate genes involved in food intake (appetite) and energy expenditure.

Two hypotheses have been developed to address our research objectives.

Hypothesis 1: *We hypothesize that there are complex genetic pathways that are activated by perturbations of energy homeostasis in response to hyper- or hypo-caloric diets and in response to prolonged exercise training.*

Hypothesis 2: *We hypothesize that polymorphic variants in certain genes that express centrally and/or peripherally could predispose individuals to increased appetite and diminished energy expenditure.*

B.1 The Impact of Diet and Exercise on Gene Expression

Genome-wide approaches will be employed to identify genes that are involved in metabolic and energy balance pathways. ***Hypothesis 1:*** *We hypothesize that there are complex genetic pathways that are activated by perturbations of energy homeostasis in response to hyper- or hypo-caloric diets and in response to prolonged exercise training.* To address this issue, two major experimental approaches have been developed. The first approach will concentrate on a selected number of candidate genes that could express differentially during variable diet and exercise programs in the two major tissues for energy balance, fat and muscle. The second approach will employ the microarray technology to identify genome-wide expression pathways involved in the regulation of energy homeostasis, again, in fat and muscle.

It is expected that certain genes might be up- or down-regulated during disturbance of energy balance (Project 2A) or in response to prolonged exercise training (Project 2B). Our procedures involve the isolation of mRNA from fat and muscle from individuals during complete rest or individual that participate in vigorous exercise. There will be no additional recruitment of volunteers for these studies. The mRNA will be extracted from muscle and fat biopsies from individuals participating in the studies described under Task 2, Projects 2A and 2B. In Project 2A, muscle and adipose tissue biopsies will be obtained from obesity-prone and obesity-resistant female volunteers. Gene expression in these tissues will be compared with the energy metabolism responses to acute disturbances of energy balance by a three-day fast and a three-day overfeeding. In Project 2B, volunteers will be college age men and women that either do not exercise regularly or participate in regularly scheduled endurance training. The mRNAs from each category of individuals, and each gender represented separately, will be pooled and used to synthesize the first cDNA strand of all the genes that are expressed during the two different states of physical activity. Following standardization and normalization, these pooled cDNAs will be labeled and used to screen the

Genechips and the Genefilters to identify genes that are over- or under-expressing or genes that express exclusively depending on the activity levels, in the two genders. Microarray technology promises to provide a new fundamental tool to examine gene expression in specific tissues or during particular physiological phases. Modification of gene expression is a common feature in all mammals subjected to environmental stresses. Evidence suggests that a high expression of stress-induced genes is correlated with high stress resistance. An advantage of the genome-wide approach is that not only it offers the entire spectrum of the human genome to be screened but it also provides functional information at the same time. Indeed, the entire concept is based on the fact that genes may be up- or down-regulated or not regulated (expressed) at all, or uniquely expressed under different dietary and physical activity conditions. As mRNA will be collected from fat and muscle biopsies from individuals undergoing the aforementioned diet and physical activity regimes, we will be able to identify not only the genes that express differently, but also by how much and under which conditions.

B.1.a Expression studies of candidate genes

The main advantage of this approach is that it allows the detailed examination of the expression levels of selected (candidate) genes. This will be achieved by employing the technology of Real-time RT-PCR. As described in projects 2A and 2B of Task 2, fat and muscle biopsies will be performed on subjects during variable diet and exercise programs. mRNA will be isolated and examined separately for each individual and for each stage of the exercise and diet programs. This will allow us to examine the expression levels of candidate genes as they may be altered in response to exercise and diet. A battery of candidate genes will be studied and they will be selected on the basis of their putative functions. The list includes the UCP family of genes (2, 3, and 4), PTP-1b, carnitine palmitoyltransferase 1 (CPT1, muscle type), PPARγ, β-adrenoreceptors, the transcriptional coactivator of nuclear receptors (PGC-1), resistin, TNF-α, GLUT-4, superoxide dismutase (SOD), lipoprotein lipase (LPL), adiponectin, leptin, leptin receptor, ciliary neurotrophic factor and its receptor CNTFR, plasminogen activator inhibitor-1 (PAI-1), etc. The list of genes will be continually revised and new genes may be added to our list as new information becomes available about their putative functions. Further to the evaluation of mRNA levels for these candidate genes, the protein levels will also be measured with antibodies, where available. If deemed necessary and important for to the completion of the project, new antibodies will be manufactured.

mRNA and protein levels in the candidate genes are expected to reveal the role of each gene as it may change according to the fat/protein content of various foods or to the exercise stress levels. In the event that there are differences in the expression levels of the candidate genes among individuals, during the same phase of a given experiment, it would suggest that genetic variants in the promoters might be causing these differences. In this case, the promoter sequences of the candidate genes will be examined for the presence of polymorphisms that might affect the expression levels of the candidate genes. Therefore, genomic DNA will be isolated from all the participants. Any polymorphisms we identify will be functionally characterized using transfection constructs to measure promoter activities in the appropriate cell culture systems.

B.1.b. Genome-wide microarray approaches

We will employ two global microarray approaches to identify genes expressing in peripheral tissues (fat and muscle): Genechips and Genefilters. The advantage of using both Genechips and Genefilters is that the two approaches complement each other at the technical level. For example,

the Genechips offer a greater range of genes for screening but at a lower resolution in terms of specificity, while the reverse is the case for the Genefilters.

Genechips are silicon or glass surface chips upon which thousands of genes may be spotted. There are several commercial companies that offer various options in the number of genes and their origin with regard to tissue-specificity. In addition, the PBRC has its own Genomics Core Facility. The Core facility is in the final stages of preparation of glass Genechips containing genes expressing in mouse muscle and fat tissues. It is anticipated that the facility will also develop by the end of the year 2001 Genechips containing equalized genes from human muscle and fat tissues for the study proposed here. These Chips will be used to screen the pooled cDNAs from individuals participating in the diet and exercise regimes in Task 2 Projects 2A and 2B, as described in the above paragraph.

Genefilters are typical nylon membrane filters upon which thousands of genes have been spotted. Such Genefilters are commercially available from Research Genetics (Huntsville, Alabama) and consist of successive releases of cDNA clones of genes spotted on the membranes. Each membrane contains approximately 5,184 genes and there are nine different sets representing a total of 46,656 genes and possibly the entire human genome. The greater advantage of the Genefilters is that they can be stripped and re-used four to five times, which can provide assurance in terms of reproducibility of the data. An additional advantage of the Genefilters is that they are more economical to use while the Genechips could be 10- to 15-fold more expensive if commercially developed. The disadvantage of the Genefilters, on the other hand, is that only an eighth of the genome can be viewed and analyzed while working with a single filter. Software for the analysis and interpretation of the data for both the Genechips and Genefilters will be purchased separately.

B.1.c The Genomics Core Facility at Pennington

The candidate gene and microarray expression studies described in this section will be aided by the Genomics Core Facility at the Pennington Biomedical Research Center (co-directed by Drs. Leslie Kozak and Robert Koza). The facility is equipped with multiple high throughput (HT). Pertinent to this proposal, the Core Facility has possession and the technical expertise of an ABI 7700 and the more HT ABI 7900 sequence detectors that can perform multiple Real-time RT-PCR reactions using the TAQMAN approach. The facility is also equipped with an ABI 3100 (16 capillary) and the more HT ABI 3700 (96 capillary) DNA sequencers, a Biomex FX robotic workstations for automated set up of PCR reactions, a GeneMachine OmniGrid glass microarray spotter (20,000 genes per slide), a GSI Lumonics ScanArray 5000 microarray slide scanner, and it is expected to purchase in the year 2002 an ABI 6100 workstation for HT RNA purification in 96-well plates starting with cell or tissue homogenates.

B.1.d Potential Problems and Alternatives

There are several potential sources that pay present problems. The first one is that our Genechips will require a tedious testing process to ensure reproducibility of the data. This is an inherent problem with all Genechips, commercial or otherwise, because most glass or silicon Genechips can be used only once. Furthermore, microarray measurements are often biased by factors that are not well understood. One way to minimize the bias associated with microarray measurements is to utilize a group of control samples of known concentrations and ratios as internal references. We will develop our in-house Genechips with that in mind and will include multiple controls to ensure that the expression level differences measured are real and not artifacts arising

from the procedure used. Moreover, we will employ the Genefilters that provide more robust and reproducible data, albeit, at a lower scale of genome coverage. The results from the two approaches will be compared and used to validate the data. For example, if the levels of expression of a known gene are consistent between the Genechips and the Genefilters, then the data from both methodologies will be considered acceptable and valid. If not so, both approaches will be reviewed and methodological adjustments to the made.

B.2 Functional characterization of genetic variants in candidate genes for food intake and energy homeostasis.

Candidate genes in central and peripheral control-centers for food intake and energy homeostasis will be studied separately because of the importance of their putative functions. This approach is taken to complement the expression studies in fat and muscle so that specific genes and genetic variants in these genes can be functionally analyzed in greater detail. ***Hypothesis 2:*** *We hypothesize that polymorphic variants in certain genes that express centrally and/or peripherally could predispose individuals to increased appetite and diminished energy expenditure.*

Three genes have been selected for these analytical studies and they are the Agouti Related Protein (*AGRP*), the Protein Tyrosine Phosphatase 1B (PTP-1b), and the Uncoupling Proteins 2 and 3 (UCP2 & UCP3).

AGRP is a powerful appetite effector (stimulant) that has been shown to induce obesity when overexpressed in transgenic animal models or administered intracerebroventricularly (central control of body weight and energy homeostasis). Hormones can affect *AGRP* expression but the mechanism by which this takes place is largely unknown. Gene expression is controlled by promoters that respond to various transactivation factors, which are activated by hormones or hormone receptors. We have identified the promoter of the human *AGRP* and multiple cis-acting elements that are putative recognition sites for transcription factors. We have also identified a region in the non-coding exon of the gene with significant promoter activity in a periphery-derived cell line (but not in a hypothalamic cell line) suggesting that this region may be a separate promoter driving expression of the short transcript in the adrenal. We now propose to identify the hormones that regulate *AGRP* expression levels of both transcripts and to pinpoint the sequence motifs in the promoter that respond to the hormones. This information will elucidate the mechanism that regulates *AGRP* expression and enable us to develop interventional strategies to modify its expression levels. By doing so, appetite could be reduced or induced depending on the requirements of various pathological conditions. Considering that obesity is approaching epidemic proportions, the discovery of mediators of appetite would enable us to, perhaps, decelerate the development of obesity and minimize the associated health risk factors that may be of detriment to military personnel.

PTP-1b is a negative regulator of insulin action and has been shown to prevent obesity and Type 2 diabetes when deleted in animal models (peripheral control of body weight and energy homeostasis). PTP-1b has been shown to dephosphorylate the active (autophosphorylated) form of the insulin receptor and thus it may play a critical role in signal transduction cascades mediated by the insulin receptor. Because of its ability to dephosphorylate the insulin receptor, PTP-1B has also been implicated as a negative regulator of insulin action and as a potential mediator in the pathogenesis of insulin resistance and Type 2 diabetes. Obese individuals have been shown to have elevated PTPase activities in adipose tissue and experiments involving skeletal muscle have provided evidence that PTP-1B has negative regulatory effects in the pathogenesis of obesity, whereas, other

regulatory mechanisms may be operative in the diabetic state. The study proposed here aims to undertake the task to determine the gene structure of the human *PTP-1b* gene so that genetic changes affecting the protein levels or protein activity may be identified. In addition, the minimal promoter of the gene will be isolated to examine for the presence of polymorphisms that may affect the expression levels of the gene in obese and diabetic individuals. We hypothesize that overexpression of this gene might predispose individuals to become obese from the inability to regulate properly the action of insulin.

UCPs express in muscle and fat tissues (both tissues are sites of thermogenesis) and have been shown to result in excess reactive oxygen species production and overfeeding when overexpressed in transgenic animal models (peripheral control of body weight and energy homeostasis). UCP2 and UCP3 are members of the family of uncoupling proteins and are likely to be involved in membrane potential ($\Delta\Psi$) dissipation functions, as defined by the prototypical UCP1. UCP2 is ubiquitously expressed, whereas, UCP3 is predominantly expressed in skeletal muscle. Mutations and polymorphisms in *UCP2* and *UCP3* were identified in our laboratory and a UCP3 polymorphism was significantly associated with reduced ability to oxidize fat and elevated respiratory quotient in the Gullah-speaking African Americans. As an uncoupler of oxidative phosphorylation, UCP3 has the potential to play an important role in energy balance and determination of body weight. Accordingly, UCP3 is regulated by the thyroid hormone, β_3-adrenergic agonists, leptin, and fat feeding in rodents. In humans, significant linkage has been reported between markers at the UCP2/UCP3 gene locus with resting metabolic rate (D11S911, p=0.000002). Recently, transgenic mice overexpressing UCP3 were shown to be hyperphagic but resistant to the development of obesity. Thus, UCP3 is a compelling candidate gene for the control of thermogenesis and fat oxidation. We hypothesize that UCPs play an important role in the ability of individuals to oxidize fat and to adapt in conditions of extreme cold and extreme heat. This could have significant implications on military personnel that need to perform various activities or combat at divergent climatic conditions.

B.2.a Hormonal Regulators of Expression and Genetic Variants in *AGRP.*

We hypothesize that certain hormones (i.e., leptin, glucocorticoids, ghrelin, etc.) might have regulatory effects on *hAGRP* expression in the hypothalamus and the periphery. Little is known about the regulation of the short transcript in peripheral tissues (mostly the adrenal) but the study proposed here will investigate at least a selection of hormonal factors that might affect its expression. To measure hormonal effects on *hAGRP* expression, we will employ two approaches: (a) evaluation of promoter activity as an expression of promoter//luciferase gene constructs, (b) direct measurement of *hAGRP* mRNA by real time RT-PCR using promoter//*AGRP* compound constructs. The gene control region consists of sequences that regulate (up or down) expression of genes and they are components of the minimal promoter. We will identify such control elements in *AGRP* and examine their ability to regulate expression of the gene in the CHO (Chinese Hamster Ovary) and GT1-7 (mouse hypothalamus) cell lines. At present, we intend to perform a thorough examination of the minimal promoter region and identify promoter regulatory domains. We hypothesize that the region –700/+400 contains sequence motifs that regulate *AGRP* expression. To examine for promoter regulatory elements in the –700/+400 region, we will start our screening with a construct comprising the region –628/+412. We have a general understanding of this region from the transfection constructs already made and from the consensus regulatory elements we have identified. In addition, we have identified a polymorphism at position –38 (–38C>T) that alters promoter activity and has been associated with obesity in African populations. The impact of the –38C>T polymorphism will be constantly monitored by reference to the polymorphic constructs we have

already made and the new ones we propose to build. Constructs – 148/+82(*C/C*) and – 148/+82(*T/T*) are already available and will provide the basis for the compound constructs – 148/+82$_{(C/C)or(T/T)}$//–19/+1230 and – 148/+82$_{(C/C)or(T/T)}$// +358/+1230 to measure the impact of the polymorphism on the direct expression of the gene by real-time RT-PCR.

B.2.b Genetic variants in the promoter and coding regions of PTP-1b

Based on the genomic organization of the murine homologue, it is anticipated that the human *PTP-1b* gene consists of at least nine exons. A human BAC library has been screened with the *PTP-1b* cDNA as a probe. A restriction map of the BAC clone has been constructed and it will facilitate the determination of DNA sequences. Screening of genome databases has resulted in the identification of a BAC clone with 100 percent homologies with the known exons of the gene. We will utilize the sequence of this BAC clone as well as sequences determined from the BAC clone we have identified to determine the gene structure of PTP-1b. We have determined the transcription initiation site of the human PTP-1b gene using the known sequence of the full-length cDNA and performing 5' RACE. A 5 kb of genomic fragment upstream of the transcription initiation has been fragmented into smaller overlapping segments and cloned into the pGL3 basic, promoterless, luciferase expression vector. We have identified regions with significant promoter activity and we propose to extend our studies to characterize in detail this promoter. Our approaches to do that are identical to the ones we have employed to characterize the minimal promoter of AGRP, described above. The promoter sequence will be examined for presence of polymorphisms. The impact of genetic variations on promoter activity or the properties of the protein product(s) will be functionally characterized using *in vitro* model systems. In the case of mutations in the promoter, constructs that differ by a single nucleotide change will be evaluated for differences in promoter activities. Furthermore, Electrophoretic Mobility Shift Assays (EMSAs) will be performed to evaluate the impact of mutations on the affinity of promoter constructs to bind transcription factors.

B.2.c Functional characterization of UCP2 and UCP3 Structural Mutations.

We have identified in our laboratory several polymorphisms in the coding regions of both UCP2 and UCP3 (published information) while others have reported polymorphisms in the promoter of the UCP3 gene. We will perform association studies to evaluate the impact of these UCP2 and UCP3 polymorphisms in individuals that will participate in the diet and physical activity studies as described in the previous sections of Task 2. A more detailed description of the use of human material is provided in section "I". Furthermore, the PBRC has a wide collection of over 12,000 plasma and buffy coat samples accompanied with a depth of phenotypic data (i.e., anthropometric measures, height, body weight, percent body fat, skinfolds, fasting glucose and insulin, detailed dietary intake while at the PBRC, repeat measurements after and before exercise, etc.). A selection of these samples will be genotyped for the UCP2/UCP3 polymorphisms and association studies will be carried out to examine for a possible role of the UCPs in energy homeostasis, the ability to oxidize fat. We have functionally characterized UCP2 (A55V) and UCP3 (UCP3 wild type, R70W, V102I, R143X, and IVS6+G>A) polymorphisms in a yeast model system by comparing the abilities of the mutant constructs to alter $\Delta\Psi$ with those of the UCP wild type prototypes. We now propose to expand these studies to a mammalian system and measure the abilities of the mutant constructs to generate heat using a calorimetric approach and evaluating oxygen production. These approaches will provide more proximal measurements for UCP2/UCP3 functions and allow us to perform more accurate association studies with the phenotypic data.

B.2.d Potential Problems and Alternatives

We do not purport that AGRP, PTP-1b and the UCPs are the only candidate genes that merit our attention with relation to this proposal. There is a plethora of other candidate genes that might be involved in energy balance homeostasis, physical endurance, innate ability to oxidize ("burn") fat, perform high activity tasks, and regulate body temperature. In some respects other genes will be examined as a part of the genome-wide approach to identify new genetic pathways for energy homeostasis. However, it is currently not possible to functionally characterize new genes on a large-scale basis as that entails the identification of polymorphisms, the establishment of an organism model, and countless hours of laboratory experimentation for functional characterization of the genetic variants. Other candidate genes that could be included in our study are NPY, the melanocortin receptors, galanin, serotonin, mahogany, cholecystokinin and its receptors, ghrelin, the newly discovered resistin, and other genes involved in central and peripheral control-centers for food intake and energy utilization. The techniques to be used to address our hypotheses are established and performed routinely in our laboratory and no technical difficulties are anticipated.

C. Significance and Uniqueness of the Proposed Research

Here, we propose a range of genetic studies to examine the central and peripheral regulation of food intake and energy expenditure. These studies will enhance and complement the existing physiological studies in Projects 2A-B. The study proposed here is unique in many respects. Firstly, our proposal will investigate the expression levels of candidate genes in fat and muscle and will examine the impact of exercise and diet on gene expression using microarray technologies. Secondly, this study will examine genes that are involved specifically in appetite control (AGRP), insulin action (PTP-1b), and energy production/consumption (UCPs). Thirdly, using the broad spectrum of our resources and expertise, as presented by the other projects in this proposal, we will be able to compare gene functional data with physiological measurements. That will provide complete information of the genetic and environmental factors involved in the regulation of body-weight and energy homeostasis and will allow us to develop intervention and prevention measures for military personnel.

D. Potential Military Relevance of the Proposed Research

The level of fitness in humans can determine the level of performance during active, as well as during, sedentary conditions. The job demands of Army career personnel require mind and body alertness and that is determined by the basic ability of the body to utilize food and energy resources efficiently. Levels of appetite regulate food intake, while sub-cellular processes regulate the conversion of food into energy. The project proposed here will investigate the genetic factors that determine the amount of food intake that it is subsequently converted (or not) into energy by various cellular and physiological mechanisms. This project will also examine the genetic factors that predispose individuals to utilize efficiently (or not) the energy generated by their bodies. We have been investigating all these genetic determinants in our laboratory at the PBRC and the project proposed here will extend these studies to identify genetic pathways specific to the lifestyles of military personnel.

E. Proposed Duration of the Study

The functional genomics laboratory is currently engaged in the functional characterization of

polymorphisms in the AGRP gene that could potentially determine the amount of food intake. In parallel, investigations are continuing with the PTP-1b gene to completely determine its genomic structure and organization that will allow us to screen for genetic variants that may impact the functional properties of the protein product. Functional studies are also under way to further evaluate the polymorphisms we have identified in the UCPs (UCP2 and UCP3). Following the completion of the early phases of the physiological studies, as described in the other projects of this proposal, Genechips and Genefilters will be employed to begin screening for genes expressing during different diets and different lifestyles (active/sedentary). In addition, the expression studies of candidate genes will commence as soon as the first fat and muscle biopsies are taken.

An outline of the timetable of the proposed study is provided in Table 1.

Table 1. Time table for the functional genetic studies.

<table>
<tr><th>Year 1</th><th>Year 2</th><th>Year 3</th><th>Year 4</th><th>Year 5</th></tr>
<tr><td></td><td colspan="3">Expression studies of candidate genes by Real-time RT-PCR</td><td></td></tr>
<tr><td></td><td colspan="4">Screening of Genechips and Genefilters for genes expressing during variable diets and lifestyle activities.</td></tr>
<tr><td></td><td></td><td colspan="3">Functional characterization of newly identified genes.</td></tr>
<tr><td>Determination of frequencies of a structural polymorphism in AGRP</td><td colspan="2">Functional characterization of th structural polymorphism in AGRI</td><td></td><td></td></tr>
<tr><td colspan="2">Functional evaluation of the polymorphism in the promoter of AGRP</td><td colspan="2">Hormonal effects on AGRP expression</td><td>Determination of transcription factors for AGRP expression</td></tr>
<tr><td colspan="3">Identification of cis-acting elements in the promoter of AGRP</td><td></td><td></td></tr>
<tr><td></td><td colspan="3">Determination of frequencies of UCP polymorphisms in the new recruits</td><td></td></tr>
<tr><td colspan="2">Gene structure determination of the PTP-1b gene</td><td colspan="2">Functional analyses of UCP genetic variants in mammalian cells</td><td></td></tr>
<tr><td colspan="2">Screening of the PTP-1b gene for presence of mutations or polymorphisms</td><td colspan="3">Functional characterization of genetic variants in PTP-1b.</td></tr>
</table>

F. Names, Titles, Roles, and Percent Efforts of Participating Personnel

George Argyropoulos, Ph.D., Head, Functional Genomics, will provide overall project leadership (70 percent effort) in this project and will be responsible for the gene function-structure studies and will be actively involved in all experimental procedures. Dr. Argyropoulos will supervise all

operational aspects of this study and will ensure that all the procedures are carried out according to Army Regulations and guidelines, IRBs, and consent forms. Dr. Argyropoulos was first to identify and functionally characterize mutations and polymorphisms in the human UCPs and recently in the AGRP gene. He has a proven publication record in the proposed techniques and methodologies and has ample experience in directing a molecular biology laboratory.

Eric Ravussin, Ph.D., Director, Division of Health and Performance Enhancement (15 percent effort) is responsible for coordination of samples provided to the task and for mentoring Dr. Argyropoulos. He is involved in the study design, the implementation and supervision of protocols, and the supervision of all data analysis and reporting.

Two Post-Doctoral Fellows, Ph.D., (TBN) (100 percent effort), will devote 100% of their time to the proposed project. One molecular biologist fellow will concentrate on the expression studies of candidate genes and the use of microarrays for the genome-wide analyses. The other fellow (already recruited), with expertise in cell culture and molecular biology, will be responsible for the functional characterization of the genetic variants in the promoters of candidate genes. This individual will also be involved in the functional characterization of structural mutations in mammalian cells.

A Research Associate, B.S. (TBN), will devote 100 percent of her/his time to the propose project. Preference for the new recruit will be for an individual with some experience in cell culture. She/he will be responsible for cell culture, general care of laboratory operations, and will participate in the construction of promoter constructs.

G. Itemized List of Major Capital Equipment

Not applicable.

H. Subcontracts

Not applicable.

I. Description of Human Use

There will be no additional recruitment of volunteers for these studies other than the ones recruited for Projects 2A-B. Total mRNA will be extracted from muscle and fat biopsies from individuals participating in the studies described under Task 2, Section B (Influence of Dietary Fat on Training and Performance). In addition, human genomic DNA will be used when screening for mutations and polymorphisms in the proposed genes (AGRP, PTP-1b, UCPs) as well as for the screening of any new genes we identify to be involved in the regulation of food intake or during high or low activity levels. Total genomic DNA will be isolated from peripheral blood using standardized commercial kits. There are no alternative methods to obtaining mRNA and DNA from human subjects to perform the studies presented in this proposal, other than the ones described above (i.e., mRNA from fat and muscle and DNA from blood). There will be no monetary cost to participating individuals. The tissue and blood samples will be stored and labeled with a code number that will be linked to the name of each participant. The laboratory of Functional Genomics will store the mRNA and DNA samples in freezers under the code numbers while the names will be confidentially stored at a different site. All the genotyping and mutation-screening procedures will take place under complete anonymity (using only the code number) and laboratory staff will be unaware of the link

between the code numbers and the linked names. This measure is taken to protect the identity of participating volunteers and to minimize any bias in the data analysis and interpretation. Genetic information stored in databases will not be shared and/or made available to any individuals or organizations unless otherwise requested by each participating volunteer. Individuals will have the right to refuse their inclusion in the genetic studies or chose participation in a limited number of genetic analyses. Genetic information will be made available (if requested) to participants under the understanding that the procedures used were not diagnostic but rather those adapted strictly for research purposes. For future studies, individuals may be contacted with new consent forms if additional genetic analyses were deemed necessary. The functional genomics studies proposed here do not intend to discriminate any individual at any stage during recruitment or during their military careers and no genetic diseases will be determined. The genes to be functionally characterizing or identified during variable states of activity are or will be newly discovered and there are no standardized diagnostic techniques to examine these genes for genetic disorders. The information obtained from these studies will not be used to preclude candidate recruits but could facilitate the decision process when designing exercise and diet programs.

J. Conclusions

The proposed project is designed to examine the genetic pathways for food intake and energy utilization. The expression levels of candidate genes and the gemone-wide gene expression using microarray studies intend to identify not only the impact of physical activity and diet on the expression levels of specific genes but also to identify novel genetic pathways involved in energy homeostasis. In addition, we have selected to study three genes that are involved in the central (AGRP) and peripheral (PTP-1b, UCPs) control centers for food intake and energy balance. These genes could predispose individuals to increased appetite and diminished energy expenditure that may be of detriment to the readiness for physical activity. In conjunction with the other projects described in Task 2, this proposal aims to thematically investigate the genetics of energy homeostasis and gene diet/exercise interaction, as they apply to the lifestyles of military personnel.

TASK 3: NUTRITION, STRESS AND BODY WEIGHT REGULATION

A. Problem to Be Studied

Achieving an ideal body weight and composition is essential for optimal performance and for Army personnel to continue their valuable service to the United States. Of those who lose weight by conventional means (diet and exercise), 95 percent regain the lost weight after five years. Complex interactions of energy intake and expenditure and the partitioning of energy between muscle and adipose tissue compartments regulate body weight. Genetics, nutrition, food intake, stress and physical activity influence energy balance and partitioning of energy. We have shown that rodents behave like humans after weight loss caused by simple diet restriction. They regain the body weight that they lost. Similarly, overfeeding produces like effects on both species. Overfeeding of both humans and rodents leads to weight gain and after the termination of overfeeding, both become hypophagic, lose body weight and return to their initial body weight. Body weight may also be influenced by factors such as physical and psychological stress, which cause a chronic disruption of energy balance. Therefore, **this task uses the rodent as a model to gain new basic information on the neurochemical and physiological mechanisms of weight gain and loss.**

Task 3 incorporates studies that address multiple mechanistic pathways that control food intake. Task 3 is a program of laboratory experiments to explore gut signaling, brain signaling and fat signaling in

satiety/hunger, as well as the food reward system. The task also continues rodent studies on the basic mechanisms whereby stress disrupts homeostasis, which has implications for the military. U.S. military forces must function under conditions of environmental stress.

The objectives of Task 3 are to:

1) identify the neurochemical and physiological mechanisms of stress, hunger, food reward, satiety and spontaneous anorexia associated with maintenance of body weight and composition.
2) identify nutritional interventions that modify or prevent a) weight gain or regain, b) stress-induced and exercised-induced disruption of homeostasis and behavior in experimental animals.

The objectives will be achieved using the following strategies:

1) identification of neurological and physiological mechanisms that cause changes in homeostasis and feeding behavior,
2) identification of genes that modify an individual's responsiveness to stress, weight loss and regain,
3) testing dietary components and supplements for beneficial effects in animals that are subjected to conditions of stress, anorexia and hunger.
4) dissemination of new discoveries will be provided by a) quarterly written summaries to USARIEM describing experimental outcomes and data interpretation, b) submission of manuscripts for publication in peer reviewed journals, c) presentation of data at national and international meetings, and most importantly, d) exchange of information with other scientists working on other Tasks. We expect these laboratory experiments to interact with the human physiologic and behavioral experiments of Tasks 1, 2a, 2b and 2c.

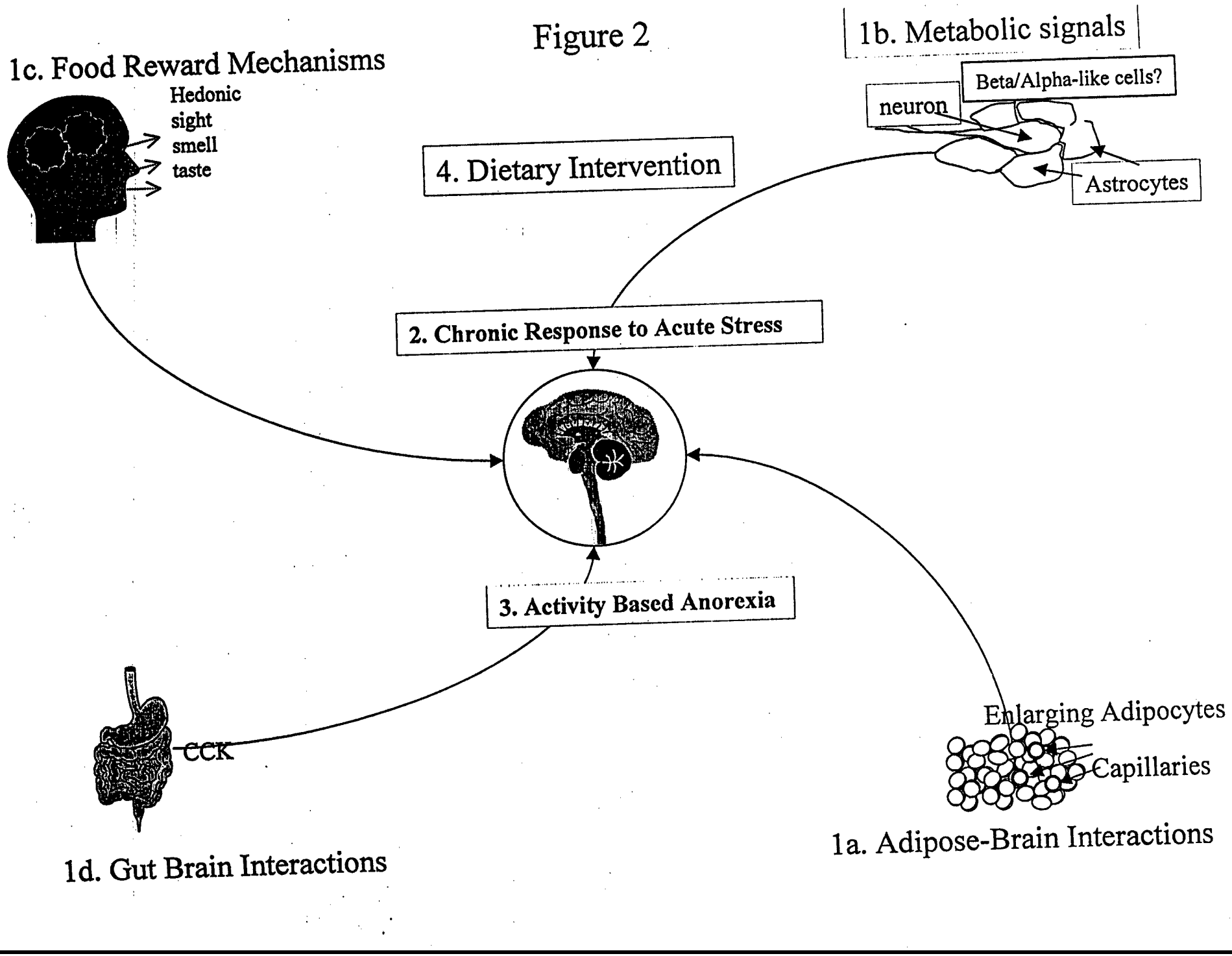
The overall plan for meeting the objectives in Task 3 are shown in Figure 2 (see following page). The first series of experiments (1a, 1b, 1c, and 1d) are focused on the complex control of hunger, satiety and food reward system that are closely linked to feeding behavior and body weight regulation. The brain receives feeding signal from adipose tissue, gut, taste buds, and from blood borne factors. The brain then integrates these various inputs and the net result is a change in feeding behaviors. These studies will advance our knowledge of feeding and body weight regulation. Stress mechanisms will be studied in the second and third series of studies (2,3). It is clear from previous studies that stress has a way of disrupting normal food intake and body weight control including how the brain responds to normal signaling pathways. The proposed studies will examine how stress related genes modify normal physiological controls. The last series of experiment (4) focuses on nutritional intervention approaches that will modify response to stress and improve normal responsiveness to satiety and hunger signals. The approaches will include diets that modify blood glucose profile after a meal. We propose that these diets would activate glucose sensing in the brain and also alter the feeding behavior associated with activity based anorexia. Another example, is to use data from nutrient sensing in the gut to develop diets that contain fatty acids or amino acids that produce the strongest satiety signals (i.e., CCK). These diets could be used to modify feeding behavior in order to control body weight.

The simultaneous use of these approaches will lead to the development of nutritional paradigms that optimize body weight and composition for optimal performance in normal and stressful environments. The energy balance response will be evaluated at levels that range from animal behavior to molecular changes in specific tissues.

The project leader for Task 3 is Roy Martin, Ph.D. His laboratory is at the PBRC in Baton Rouge, Louisiana. Collaborating is Ruth Harris, Ph.D. Dr. Harris conducted Army-sponsored research at the PBRC for six years. Operating as a subcontract with the University of Georgia, Dr. Harris will continue existing and ongoing studies investigating the chronic response to acute stress.

The scope of our research is presented below. It would be difficult to describe all of the experiments in

Figure 2


1c. Food Reward Mechanisms
Hedonic
sight
smell
taste
1b. Metabolic signals
Beta/Alpha-like cells?
neuron
Astrocytes
4. Dietary Intervention
2. Chronic Response to Acute Stress
3. Activity Based Anorexia
CCK
Enlarging Adipocytes
Capillaries
1d. Gut Brain Interactions
1a. Adipose-Brain Interactions

detail. However, details of some of the experiments are included in Section 1 to provide information about how these studies are typically conducted.

B. Outline of Proposed Research

1) Hunger, satiety, and reward mechanisms
   a) Role of brain and adipose cells
   b) Role of metabolic status
   c) Food reward mechanisms
   d) Brain and gut interactions
2) Chronic weight loss response to acute stress
3) Exercise-induced anorexia
4) Exploratory studies in dietary intervention for obesity

1. Hunger and Satiety Mechanisms Related to Body Weight Regulation

Over recent years, certain principles of body weight regulation have been investigated by our group. The regulation of body weight around a "Set Point" has been well established in our, as well as other, laboratories. Under conditions where animals are forced to lose weight by food restriction, body weight is regained when food is made available on an ad libitum basis; these animals over-consume until body weight is reestablished. The opposite experiment is to force feed animals for a period of time so that excessive body weight is attained. When these animals are then allowed to feed to appetite, they spontaneously restrict their intake until the original body weight and body fat levels are attained. We propose to study hunger and satiety mechanisms and genes expressed during the active phases of body weight regain and of weight loss.

Series 1a. The Role of the Brain and Adipose Tissue In Long-Term Regulation Of Body Weight And Food Intake

The brain is the major integrator of information related to body weight, adipose tissue content and energy balance regulation. In addition, the brain initiates changes in food intake, energy expenditure and body fat deposition or mobilization. Therefore, we will utilize gene-array and proteomics technologies to identify novel genes and proteins that are expressed during states of hunger or satiety. Below we describe examples of the studies that will be conducted to answer the following question:

<u>What are the key regulatory factors involved in long-term regulation of food intake and body composition?</u>

<u>Over- and Under-Feeding Model:</u> Rats will be tube-fed a semi-purified diet containing (by weight) 70 percent polycose, 10 percent Casein, 10 percent Corn Oil, three percent Cellulose, and one percent AIN vitamin and mineral mix (or commercial mix). Three levels of tube feeding will be administered to three groups of rats, 50 percent of normal, 100 percent of normal intake, and 200 percent of normal food intake. For tube feeding, a liquid diet is prepared by mixing four parts of the powdered diet with three parts tap water. The rat is restrained by hand and the tube is gently introduced through the mouth to the stomach. The meal (usually 5.0 – 7.0 ml for 100 percent-fed rats) is delivered from the syringe and the tube withdrawn. The amount of food delivered to overfed rats is gradually increased from 100 percent to 200 percent of control intake over a period of six to eight days. Rats are fed via gastric tube three times daily at 0700, 1400 and 2200 hours. Rats will be overfed for approximately 10-25 days. We have used this approach to study novel factors found in adipose tissue. This model has the advantage of having a controlled rapid expansion of adipose tissue mass and the up-regulation of either hunger or satiety signals, thus making it easier to measure changes in gene expression. In addition, since this obesity is produced without changing diet composition and in animals of the same genetic background, there are fewer confounding factors to consider. Furthermore, the exact caloric intake, meal size and timing can be controlled, further reducing variability. At the end of the study, brain and adipose tissues are removed for RNA and protein extraction. Gene array technology will be used to identify genes that are altered in these extreme energy states.

Gene arrays will be used to identify genes expressed in states of hunger and satiety. Rats are sacrificed by cervical dislocation and the hypothalami harvested and frozen immediately in liquid nitrogen for overnight storage. RNA is isolated using Qiagen's RNeasy Mini Kit and Qiagen's RNase-Free DNase Set. Hypothalami are weighed and homogenized individually by rotor-stator using buffer provided by kit, then passed through a 26-gauge needle five to six times. The kit protocol for isolation of total RNA from animal tissues is followed with the modification for on-column DNase treatment. After final elution from the columns, the eluants for each body type are pooled and precipitated by addition of 1/10 vol. 3M sodium acetate and 2.5 vols. ice-cold EtOH. These are cooled at –70C for six hours before recovery, followed by two washes with 70 percent EtOH at room temp. After brief air-drying, pellets are resuspended in 15 ul RNase-free water each and stored at –70C overnight. RNA is quantitated by UV spectrophotometry and by Ribogreen assay, and visualized on a 1.0 percent denaturing MOPS gel. Fluorescently-labeled cDNA probes are prepared from 20 ug total RNA using Clontech's Atlas Glass Fluorescent Labeling Kit, Amersham monofunctional reactive Cy3 and Cy5 dyes, and Clontech's cDNA Synthesis Primer mix for Atlas Rat Glass 1.0 microarray. The kit protocol is followed; cDNA synthesis steps are carried out in a MJ Research PTC-200 DNA Engine thermal cycler. After synthesis, the cDNA is stored under 70 percent EtOH overnight before proceeding. Brief mixing every 5 minutes is done during the dye coupling reaction. After final elution from the kit's NucleoSpin columns, probes are purified through an additional 0.45 um spin filter. An absorbance scan from OD 250-700 is done on total volume of eluted probe, and absorbance ratios are calculated (A260/A550 for Cy3, A260/650 for Cy5). Probes are stored overnight, wrapped in foil at –20C before use. Total volume of probes are combined and hybridized to Clontech's Atlas Rat Glass 1.0 microarray using the hybridization chambers and solution provided, overnight at 50C. After washes are accomplished and slide is air-dried in dark, the slide is scanned in a GS Lumonics fluorescent scanner. Dual-color analysis is performed using Scanalyze2 and Atlas Navigator 1.0 software. This procedure was recently used in our laboratory to demonstrate over-expression and under-expression of hypothalamic genes of genetically obese rats. Approximately 25 genes were over-expressed by four-fold when comparing the obese to the lean rat in this initial study.

In the studies proposed above we expect to begin the identification of genes that are altered in the hunger and satiated states when compared to the normally fed animal. Using this information, we will validate that these genes are normally involved in feeding by selectively altering the expression of targeted genes. Our ultimate goal is to use this information to control feeding behavior and energy balance (see Figure 3 on the following page).

Series 1b. The Role of Metabolic Status In Short-Term Control Of Food Intake

While searching for new genes and proteins involved in body weight regulation, we will focus other studies on known mechanisms that have a high probability of being involved in body weight regulation. We have shown that the pattern of brain uptake of metabolites reflects energy-balance state. During starvation and energy restriction, brain uptake of ketone bodies and fatty acids is increased. The brain uptake index (BUI) for fatty acids is increased 300 percent in food-restricted animals, while the BUI for glucose is decreased 30 percent in the hypothalamus. Others and we have shown that availability of glucose to the brain alters both food intake and diet selection. These observations support the contention that brain uptake and utilization of metabolites reflects energy balance status. In addition, we have demonstrated a role of neuropeptide Y in this selection preference and hyperphagia caused by short-term glucoprivation. Furthermore, glucagon-like peptide1-receptor, corticotropin receptor and leptin have also been identified as modulators of metabolic signaling of food intake. It is apparent from these many different studies that the metabolic changes that occur during positive or negative energy balance lead to a change in short and long term control of feeding behavior.

It is known that high glucose availability suppresses food intake and low glucose availability stimulates food intake. Therefore, metabolic signals received in the brain are in part controlled by glucose sensing cells in the brain. The first step for glucose utilization by cells is its transmembrane transport by a family of proteins called glucose transporters. Among the five isoforms so far identified, GLUT-2 possesses a high Km and is thus capable of sensing glucose over a wide dynamic range. We propose that glucose sensing in the brain is

mediated by GLUT-2 containing neuronal cells and have shown that inhibition of GLUT-2 expression suppresses food intake. This observation supports our contention that GLUT-2 containing cells in the brain are involved in glucose sensing mechanisms and feeding behavior. These unique brain cells are likely to be similar to pancreatic beta and alpha cells that respond to high glucose and low glucose respectively (see Figure 4 on the following page).

Rationale: We have demonstrated that ICV streptozotocin alters the response to 2DG feeding and glucose induced satiety. In addition, the effect of leptin, CRH and GLP-1 on food intake suppression is reduced by streptozotocin treatment. Therefore, we propose that brain cells sensitive to streptozotocin are central to the mechanism of action of these neurochemicals in controlling feeding behavior associated with glucose status (and perhaps energy status). The following is a series of questions which when answered will allow us to determine in a systematic manner the role and nature of streptozotocin sensitive brain cells in glucose sensing, feeding behavior and energy balance.

What brain cells are destroyed by streptozotocin?

Approach: Streptozotocin is known to specifically destroy beta cells of the pancreas. The mechanisms involved appear to include GLUT-2 for specificity of the beta cell (reference) and eventually the process of apoptosis (Morgan et al., 1994; Saini et al., 1996). To identify cells that are influenced by streptozotocin, it is proposed that markers for apoptosis be used to visualize cells and their regional location. Induced apoptosis activity can be monitored by the appearance of poly (ADP-ribose) polymerase (PARP) in individual cells. Furthermore, poly (ADP-ribose) polymerase (PARP) deficient mice are resistant to apoptosis induced by streptozotocin (Pieper et al., 1999). PARP immuno-cytochemistry is likely to be an ideal marker for brain cells damaged by streptozotocin. Therefore, cleaved PARP will be used as a marker for apoptosis induced by streptozotocin (antibody from New England Biolabs). In addition, we will identify the dose at which he feeding response to glucoprivation is eliminated.

Methods: Animals are fasted for 12 hours (food removed at the onset of dark period) prior to streptozotoci (STZ) injections. All injections are performed 30 minutes after the onset of the light period. Each rat is lightly anesthetized with metophane prior to injection, and randomly assigned to a group that either receives centrally ST (Sigma, St. Louis, MO) or 5ul of physiologic saline. A Gilmont micrometer syringe system is used to deliver the cer dosage over a one-minute period. The injector is left in place for one minute after injection to allow for diffusion o treatment solution. The dosage of central STZ is based on previous studies (Woods et al., 1978; Ritter et al., 198 Nitsch and Hoyer, 1991). In order to see the immediate response of apoptotic markers and the loss of regulatory peptides we will have to optimized the time after streptozotocin injection for maximum brain staining for PARP.

Expected Results: Brain cells will stain for PARP in the following areas of the brain: ARC, LH, VMH, PVN, Al and NTS. This expectation is based on the location of cells that are either glucose sensitive or responsive (see background section). Quantification will be made of percentage cells stained in the areas known to be specifically activated by 2-deoyxglucose and or to have cells that are glucose sensitive or glucose responsive.

Pitfalls and Limitations: It may be difficult to quantify the percentage of cells found in each brain region because of variability of penetration of the drug into the brain, the speed at which apoptosis occurs and the loss c cells completely. A pilot time study will be conducted to see if there are regional differences in the percent of cell stained for PARP (destroyed by streptozotocin over time). It is expected that the brain areas closest to the third ventricle will be the ones showing the largest percent staining with PARP initially and then later those further awa show more intensive staining. With the loss of PARP staining we expect that all streptozotocin cells will have bee destroyed in the area. An alternate approach will be to administer streptozotocin directly into areas that show sc PARP staining. This will allow us to determine if specific brain areas are essential for the streptozotocin sensitive to actively participate in feeding behavior.

Are beta cell and alpha like cell markers in brain areas associated with feeding altered, reduced, or elimin by ICV streptozotocin?

Figure 3 on Overfeeding and Underfeeding

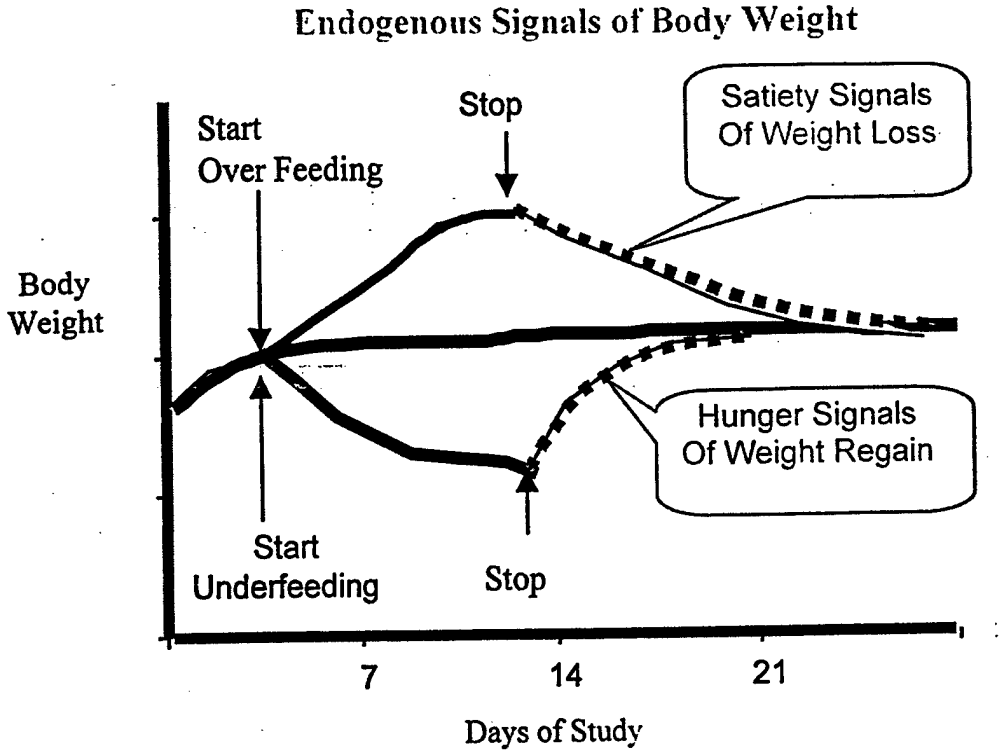


Figure 4. Comparisons between pancreatic islet cells and specialized CNS cells.

	Pancreatic beta cells	Pancreatic alpha cells	Proposed CNS Type I and IV glucose responsive neurons and alpha or beta CNS like cells?
high glucose	increased release of insulin	decreased release glucagon	Type I responsive cells decrease firing rate Type IV responsive cells increase firing rate
low glucose	decreased release of insulin	increased release glucagon	Type I responsive cells increase firing rate Type IV sensitive cells decrease firing rate

Rationale: A number of markers for beta or alpha cells appear in brain areas associated with feeding. In addition, cells in the same areas are shown to be glucose responsive or sensitive by electrophysiological studies. T following have been identified in brain areas associated with feeding or glucoprivic feeding and in alpha and beta c GLUT-2, GK, GLP-IR, Amylin, GAD, preproglucagon, sulfoyurea-receptor. It is propose that streptozotocin sensitive cells identified with PARP staining will show co-localization with markers for alpha and beta cells.

Immunohistochemical assays. Immunohistochemical techniques will be carried out as previously described et al., 1996). Transverse frozen sections (24 mm) will be attached to slides, air-dried at room temperature and fixe with four percent paraformaldehyde in phosphate buffered saline (PBS, pH 7.2) for 15 minutes. Sections are then rinsed once in 3X PBS and twice in 1X PBS and then briefly rinsed with 30, 60, 80 and 95 percent ethanol and store -80°C until use. The reaction sections are incubated in 0.3 percent H_2O_2 in PBS for 30 minutes to reduce endogen peroxidase activity. After being washed with PBS, tissue sections are incubated with four percent normal goat seru in PBS with 0.3 percent Triton X-100 (PBST) for one hour at room temperature. Sections are then blocked with avidin/biotin solution to eliminate binding to endogenous biotin (Vector) and rinsed again in PBST and incubated w primary antibody at the appropriate dilution for one hour at room temperature. Sections are rinsed with PBST and reacted with biotinylated anti-rabbit IgG antiserum (1:2000 dilution) in PBST (Vector, ABC Kit) for one hour at roon temperature and washed with PBST and incubated in avidin and biotinylated horseradish peroxidase (1:50 dilution PBST) complex for one hour at room temperature, and then washed with PBST and rinsed once in PBS. Sections a exposed to 3,3' diaminobenzidine (DAB) for the development of chromogen, washed with water, air-dried and mounted with 60 percent glycerol. For the negative control, primary antibody is omitted in the above procedure. A least 10 sections will be examined for each tissue and a minimum of ten fields will be visualized on each section.

Expected Results: Cells marked with PARP will co-localize with GK, GLUT-2, GLP-IR, Amylin, GAD, preproglucagon, sulfoyurea receptor cells. Some cells not marked with PARP will be labelled with GLP-1 receptor a GAD. It is expected that all GLUT-2 and GK cells will be destroyed by streptozotocin. Predictions are based on Silv Classifications of glucose responsive and sensitive neurons.

Pitfalls and Limitations: The loss of specialized cells within the brain may lead to secondary changes in the content of receptors and enzymes. Therefore, a drop in GK activity may be related to cell loss or a change in the interaction with the other cell types not destroyed by streptoztocin but that are intimately involved in interaction wi glucose sensing cells. A kinetic study of these changes after strep treatment should allow us to see immediate changes in cells destroyed strep vs those that develop more slowly over time. Those that are seen after four hours likely to be those that are related to streptozotocin destruction of cells whereas those that appear later are more lik related to secondary changes in interactions between neuronal networks.

What brain peptides (or receptors) are reduced or eliminated by ICV streptozotocin?

Rationale: Glucagon-like peptide-1 (7-36) amide (GLP-1) decreases food intake when injected centrally. Furthermore, the GLP-1 receptors are found within ependymal cells lining the third ventricle, lateral hypothalamic nucleus, arcuate nucleus, median eminence, areas reported to be responsive to glucose. Finally, mRNAs for GLP-1 receptors, glucokinase, and GLUT-2 are co-localization in brain and beta cells.

There appears to be a neuronal connection between GLP-1 and Corticotropin releasing hormone (CRH). For example, centrally administered GLP-1 activates c-fos in CRH mRNA containing neurons. In addition, our studies have shown that GLP-1 effects on feeding are blocked by an antagonist to CRH. The relationship between CRH and glucoprivic induced feeding is supported by the observation that the expression of c-fos and corticotrophin-releasing hormone genes in the paraventricular nucleus occur during insulin-induced hypoglycaemia.

There is considerable evidence for including leptin in our studies. Leptin decreases food intake primarily via the hypothalamus. Leptin receptors have been found on a population of glucose responsive neurons and these neurons in the hypothalmus are hyperpolarized by actions of leptin. Leptin stimulates the release of corticotropin-releasing hormone (CRH), an inhibitor of food intake.

Another factor we will study is amylin. It is co-secreted with insulin by pancreatic β cells in response to a nutrient stimulus (e.g., during meals). Amylin decreases food intake when injected into the brain. Amylin causes membrane hyperpolarization, in single beta cells exhibiting normal glucose sensing, and reductions in insulin secretion. And finally, lesion of the area postrema/nucleus of the solitary tract (AP/NTS) attenuates the anorectic effects of amylin.

Our studies will determine the nature of brain cells that respond to glucose excess and glucose deficiency. We will also determine the role of neuropeptides and neurotransmitters in glucoprivic stimulation and glucose inhibition of feeding behavior. Our final phase of study will determine how short term signals of feeding behavior are modified by long-term signals of satiety, like leptin and other signals from adipose tissue (see Figure 5 on the following page). Our goal is to use this information to design novel approaches for the control of food intake and maintenance of ideal body weight for optimal performance.

1C. Food Reward Mechanisms

Overeating may be generated by a disruption of normal physiological control mechanisms. This occurs when people and animals are exposed to highly palatable diets. Highly palatable foods leads to overeating, that result in body weight gain. It is proposed that food rewards systems in the brain are powerful stimulus for eating after the physiological state of satiety exists.

Our preliminary data shows that rats exposed to a palatable food (cookies) will consume extra calories in a state that they would not normally eat. It is proposed that brain reward mechanisms are stimulated by the consumption of the more palatable food. The Dopamine system is one of the most studied system related to rewards. Therefore, the dopamine system will be investigated in animals that are over-consuming food.

One of the important components of the dopamine system is dopamine transporter. Dopamine transporter mRNA is up-regulated in rats consuming a palatable diet (preliminary study).

Hypotheses:

Palatable food stimulate eating by activating a reward system, particularly dopamine system. Dopamine related genes affect only palatability-induced hyperphagia, but not the eating behavior associated with normal energy needs.

Research Objectives:

Objective I Determine the time course of response to palatable food.

Objective II Identify over or under expression dopamine related genes after fed with palatable food by gene array. Identify those gene translated protein by Proteomics.

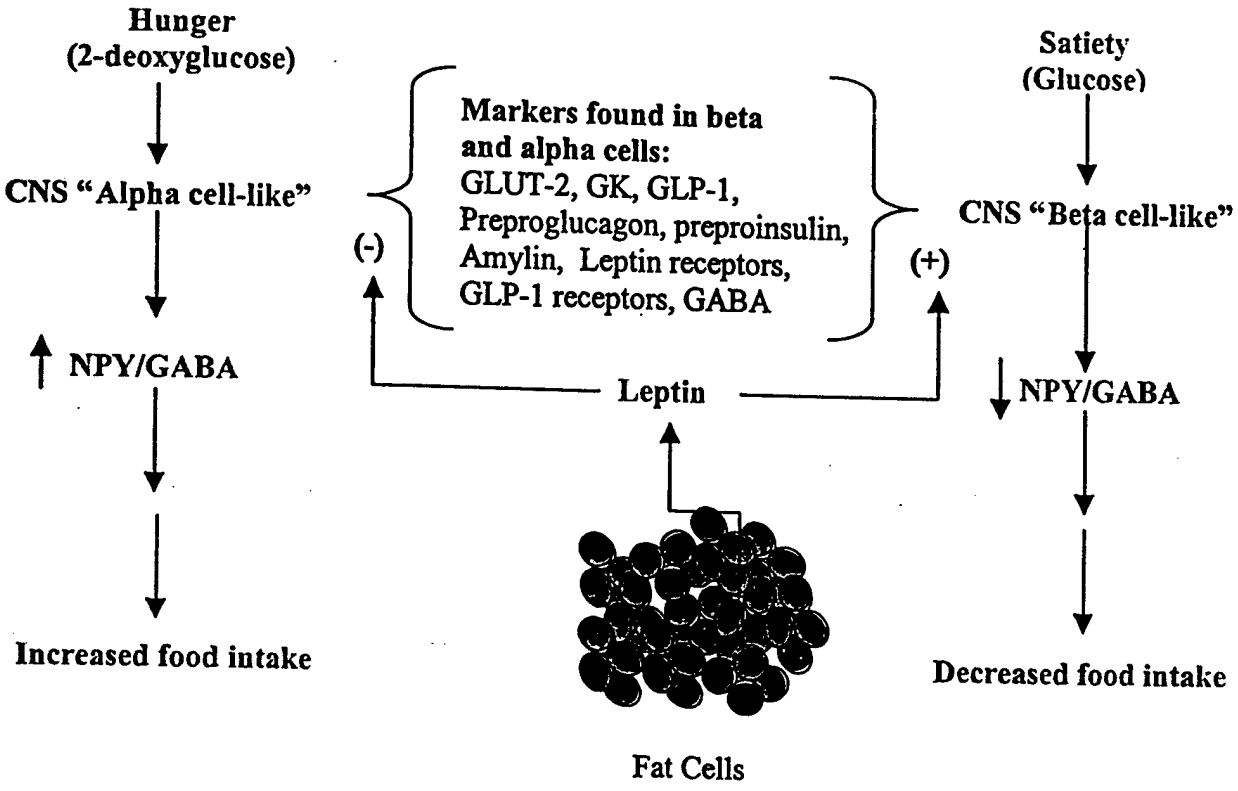
Objective III Localization and semi-quantitation of these gene expression in the brain by in situ hybridization.

Objective IV Use antisense RNA to regulate those related genes expression and observe the feeding behavior stimulated by palatable food.

Summary:

These studies will identify the reward mechanisms and genes controlled by foods of high palatability. It is proposed that these genes are only affected by palatability of the food and not by the energy state. A

Figure 5 showing general hypothesis on the interaction of long- and short-term feeding signals.



dietary approach will be developed to take advantage of both the palatability and the satiating effects of foods.

1D. Brain-gut Interaction in Control of Food Intake and Body Weight.

The satiating effect of food begins with the gut where receptors for different nutrients are found. These chemosensory receptors for nutrients translate information about fatty acids, amino acids and carbohydrate metabolites like glucose to the brain through the vagal afferent system.

One of the most powerful signals generated for the cessation of a meal is cholysestokinin (CCK). The Oletf rat has a CCK receptor defect which leads to overeating and obesity because of the loss of the ability to recognize that nutrients are entering the gut.

Also it has been shown that high fat diets leads to a decrease in satiating effects of CCK. The high fat diet leads to larger meals and obesity.

Experiments will be designed to answer the following questions or issues:

- Role of CCK-a receptors in long term control of food intake.
- Reduced sensitivity to satiation signals in animals maintained on high fat diets.
- Synergy between short term satiety peptides and long term adiposity signals.
- Examine the role of dietary-induced sensitivity to satiation signals in the development of obesity.
- Uncover the mechanism(s) by which high fat diet reduces responsiveness to satiation signals.
- Examine brain and gut neurochemical changes that contribute to reduced sensitivity (melanocortin, NPY and CART).
- Are satiation deficits to peptides or nutrients related to insulin levels or sensitivity?
- Examine the role of reduced leptin or insulin sensitivity in diminished satiation response.
- Investigate potential interaction of CCK with insulin and absorbed nutrients.

2. Chronic Response to Acute Stress

We have established an animal model that provides a reliable and repeatable chronic response to acute stress. Rats which are exposed to restraint stress for three hours/day for three days show a chronic down-regulation of body weight. This loss of weight is independent of the nutritional status of the animal at the time of restraint. Rats that are food restricted before the onset of stress and then allowed to eat ad libitum once stress has ended will not return to the weight of non-stressed rats but only to the weight maintained by stressed animals. We have demonstrated that the chronic change in weight is initiated by central release of corticotrophin releasing factor (CRF) but there is no sustained activation of the CRF system or of other systems that are normally activated during stress. The weight loss during restraint is exclusively lean body mass but during the days immediately following restraint there are shifts in metabolism to make the difference in weight a combination of both lean and fat tissue.

During the post-stress period we have been unable to find any substantial difference in neurochemical or physiological systems of control rats and rats that have been exposed to repeated restraint stress. Recent experiments, however, suggest that the rats that have been restrained are a model for post-traumatic stress disorder (PTSD). Corticosterone release and central catecholamine turnover in response to a mild stress are greater in the restrained rats than in rats that have not been previously stressed. We are in the process of validating repeated restraint as a model for PTSD and, once it is validated, will collaborate with Dr. Charles Morgan, Director of the National Center for PTSD, Health Care System, West Haven, Connecticut. The studies will focus on central and peripheral mechanisms that mediate the increased stress responsiveness of these animals and will examine the processes involved in the initial cascade, during restraint stress, that cause a chronic increase in sensitivity towards subsequent stressors. Once these pathways are identified it will be possible to develop nutritional strategies or supplements that have the potential to counteract the stress-induced changes.

Genetic Markers for Stress Responsiveness

This is the continuation of a project that was initiated five years ago. Potential markers are identified based on published information linking a specific protein with modification of a system that is also influenced by stress. This system may be neurochemical, physiological or behavioral. Transgenic mice that are either deficient in this protein or overexpress the protein are tested for their behavioral response to stress and then the mechanisms underlying any shift in stress-responsiveness are investigated. To date we have investigated two proteins. The first was ApoE. There is substantial literature linking ApoE genotype in humans with risk for Alzheimer's disease. We demonstrated that mice that are deficient in ApoE have an exaggerated behavioral and endocrine response to stress that is associated with increased production of auto-antibodies, including one that is specific to brain peptides. More recently we have been investigating the stress responsiveness of mice that over-express Agouti protein. Agouti and related proteins are endogenous antagonists of melanocortin receptors and are expressed in the brain, skin, adipose tissue, liver and kidneys of humans. Melanocortin proteins include ACTH, which stimulates adrenal glucocorticoid release, and αMSH, which has been shown to suppress the immune responses to stress. Agouti mice that are exposed to restraint stress show increased anxiety behavior, corticosterone release, weight loss and central catecholamine turnover compared with stressed wild-type mice. We will investigate whether the increased responsiveness is due to chronic antagonism of all melanocortin receptors in transgenic mice or whether it can be replicated by acute blockade of specific melanocortin receptors.

Additional candidate proteins for markers for stress responsiveness have been identified from the literature and will be investigated based on strength of the evidence for the protein being involved in modifying stress-related systems and availability of mice in which the gene is either deleted or over-expressed. The candidate genes include prolactin, transforming growth factor (TGF), CRF, and growth hormone.

3. Anorexia Associated with Exercise

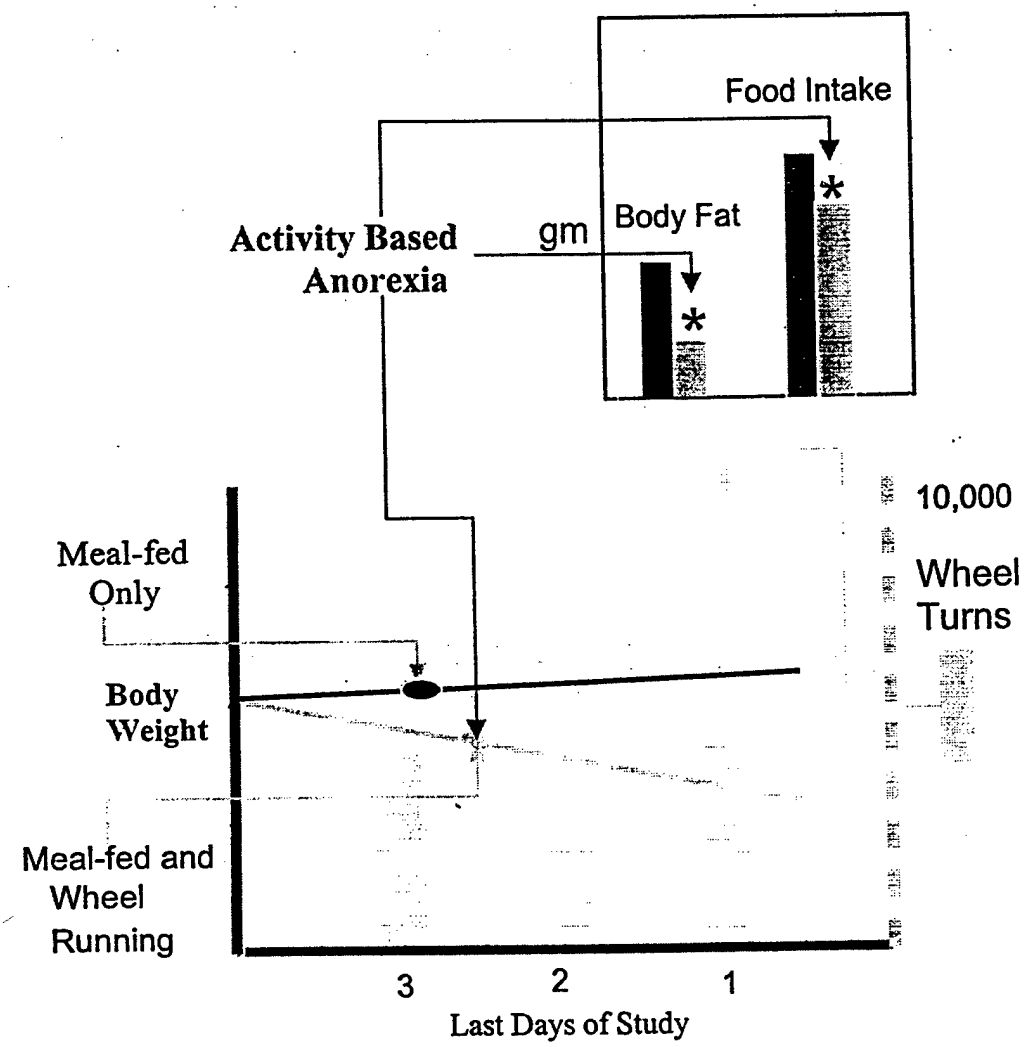
In order to maintain the Army's standard for the ideal-body weight (as well as society's standards) many have become locked into a physiological and psychological situation by which excessive weight loss occurs. "Dieting" and exercise are used by many to reach and maintain a trim and fit standard. We have utilized an animal model of anorexia that involves both diet restriction and exercise. These two manipulations initiate a chain of neurological and behavioral events that cause spontaneous anorexia. The anorexia and running disorder is so severe that if no intervention is applied, the animals will continue to run and reduce food intake until their death. Our studies are not run to that extreme. In just the first few days food intake and body fat content are reduced, while running behavior is stimulated to very high levels (see Figure 6 on following page).

We will use the same experimental approaches, as mentioned above, to identify novel regulatory factors involved in anorexia. For example, we will utilize gene-array and proteomics technologies to identify novel genes and proteins that are expressed during the activity induced anorexic state. In addition, we will investigate known neuronal signals that have a high probability of being involved in anorexia. We have shown that the hypothalamic and pituitary axis is involved and that the brain mechanisms may include those studied above for satiety and stress. For example, the stress hormone, glucocorticoid, is elevated in exercised induced anorexia. As with the studies mentioned above nutritional or functional food approaches will be developed to help reverse this dangerous loss of body weight (see #4 below).

4. Nutritional Intervention

The new information generated in sections 1-3 above will be used to develop unique strategies to prevent undesirable weight regain or to enhance reversal of stress induced weight loss to achieve the ideal body weight for optimal performance. Knowing what metabolic signals are translated into feeding behavior signals will allow us to design diets, supplements, or functional foods that modify these metabolic signals in the desired direction. For example, we have already shown that the type of carbohydrate can influence brain

Figure 6 General Characteristics of Activity Based Wheel Running Model



neuropeptides and the feeding response to glucoprivation. In addition, we have found that the type of fatty acid fed will influence brain uptake and metabolism of fatty acids and alter the rat's preference for high and low protein diets. More recently we have shown that ketones chronically infused directly into the brain will cause weight loss. Thus, we have already generated information that will allow us to begin testing new nutritional approaches for preventing weight gain after periods of weight loss through caloric restriction, running induce or stress induced anorexia.

Does the type of dietary fat alter brain metabolism and effect feeding behavior? Medium chain fatty acids and conjugated linoleic acid (CLA) alter metabolic profiles and total energy expenditure. In addition, CLA has been shown to reduce body fat and body weight. Very little has been done on the action of different fatty acids in the brain. We have shown that direct infusion of palmitate into the brain will cause a loss of body weight. We also have shown that unsaturated fatty acids are taken up by the brain more readily and alter the preference for dietary protein. While many diets have been tested to determine if body weight or composition is effected, we will propose new diets based on the information generated from the basic studies described above.

Does the type of carbohydrate alter the satiating effects of a diet? One current interest of our group is that of carbohydrate diets with different glycemic indexes. It is hypothesized that diets that have both high and low glycemic indexes would be ideal for early termination of a meal and reducing hunger signals that occur in between meals.

Are there functional foods that would decrease hunger signals generated after weight loss? Another approach is to utilize herbs or functional foods as agents to alter food intake and obesity. Evidence is lacking for the use of functional foods in effective body weight control. But new ideas are being developed that may be useful. Developing functional foods that contain structured lipids is an approach that would allow us to deliver foods with unique fatty acid profiles. We will pattern these profiles based on the studies in Section 1 above. The studies of gut and brain interactions will provide further ideas for developing structured lipids for control of CCK and increasing the satiating qualities of foods.

Conclusions: We will identify nutritional approaches that are effective in preventing weight regain, stress-induced weight loss and exercise-induced anorexia and body weight loss. The approaches will include diet composition and/or functional foods.

C. Significance and Uniqueness of the Proposed Effort

Accomplishing the goal of maintaining an ideal body weight and composition is essential for optimal physical and mental performance and for Army personnel to continue their valuable service to the United States. The high rate of failure to maintain body weight for optimal performance has been related to the lack of understanding of the endogenous mechanisms for long- and short-term regulation of body weight. The studies described here will provide new information about endogenous signals of body weight control and behavior. In addition, this new information will be used to develop nutritional approaches that are effective in preventing weight regain, stress induced weight loss and exercise induced anorexia. Because the experiments proposed herein utilize rodents, the validity of our findings will be tested by collaborating with others doing human studies, particularly the investigators in Tasks 1 and 2. The most effective interventions found in the rodent studies will be reviewed for consideration of potential human trials for prevention of weight regain and maintenance of ideal weight for optimal performance.

In addition, our recent experiments suggest that rodents that have been restrained, are a model for post-traumatic stress disorder (PTSD). The use of experimental animals to investigate mechanisms underlying PTSD and to identify potential markers for stress responsiveness facilitates the examination of a stress response ranging from animal behavior to changes in activity of a neurochemical pathway or expression of a particular gene in a specific tissue. Only some of the measurements can be made in human subjects. Once we have validated repeated restraint as a model for PTSD then we will collaborate with Dr Morgan, who

conducts human trials on PTSD, and this will allow the basic studies to complement human trials that are sponsored by the DOD. If we identify proteins that are markers for stress responsiveness in animal models the information will be reported to the Army and will be published, to encourage follow-up investigation in humans.

D. Potential Military Relevance

In the overall context of this research proposal whose major theme addresses body weight regulation, a basic laboratory component is appropriate. Each year the military loses a large number of experienced personnel because of failure to maintain standards of body weight. We know that maintaining an ideal body weight is extremely important to fulfill the missions of soldiers. To investigate these issues in a basic science approach, we have described experiments of neuronal mechanisms for hunger, satiety and energy balance. In addition, we propose to use this new information to develop practical means of controlling hunger signals and body weight.

Identifying mechanisms that either initiate PTSD or are responsible for the increased responsiveness to subsequent stressors, will provide information that can be used either to prepare soldiers about to enter a potentially traumatic situation, or to treat individuals following exposure to such an event.

E. Proposed Duration in Years

Five years.

F. Names, Titles, Roles and Percent Effort of Participating Personnel

Roy Martin, Ph.D. will serve as project leader for Task 3. He has overall responsibility for budget and hiring personnel, overseeing that the objectives of Task 3 are met. In addition, he will provide leadership and mentoring for personnel on the project. Dr. Martin has managed a research program on nutrition and obesity for over 20 years and served as Department Head of a Foods and Nutrition Department at the University of Georgia for 11 years.

An Associate Professor (TBN) will be hired at 100 percent effort to manage the day to day operations in the laboratory. This person will be responsible for coordination of studies, design of experiments and management of resources. The nature of these studies requires a team of experts with different skills and expertise. In collaboration, the team members organize experiments, modify procedures and protocols as necessary, summarize and interpret data collected.

The following is a listing of the other team members and their responsibilities on this project.

Jun Zhou is a Post-Doctoral Fellow and will work 100 percent effort on this task. She has considerable expertise in obesity studies, in vivo and in vitro isotope tracer studies, and analysis of neurotransmitters. She will develop her research interest in the neurobiology of food intake within the objectives of this task.

Drs. Maren Hegsted, Karen O'Neil and Mike Keenan will each work 25 percent effort on this Task. They have considerable experience in nutrition and will provide support for project # 4. They will identify nutritional approaches that are effective in preventing undesirable weight change. The approaches will include diet composition, herbs, supplements and/or functional foods.

Xiaochun Xi is a Research Technician and will work 100 percent effort on this task. Her expertise is in molecular biology. She will develop probes and primers for in situ hybridization and PCR. In addition, Xiaochun will support our efforts for identifying differential expression of genes using DNA array technology.

Two Ph.D. graduate students will work 50 percent time on this Task. They will be assigned to work on

projects #1 and #3 of this Task. Senior personnel will directly mentor them.

A Postdoctoral Fellow (TBN) will be assigned at 100 percent effort to work on studies of brain glucose sensing mechanism. We will identify specialists in this area and will hopefully be able to recruit the very best to fill this need.

A Research Associate (TBN) will work 100 percent on his Task. This person is needed to assist with the behavioral measures, daily monitoring of animals and laboratory assays. It is important that the person who fills this position have prior experience in proper animal care and use procedures.

Jeffrey Harris (50 percent effort) is a student worker who assists in behavioral measures and laboratory assays and is responsible for routine laboratory tasks, such as dish washing and autoclaving.

Steven Lesham, D.V.M is a Post-Doctoral Fellow and will work 100 percent effort on the subcontract. He has expertise in immunohistochemistry, in situ hybridization and working with experimental animals. His research will focus on defining central pathways that mediate stress-induced weight-loss and in neurological characteristics of animals that are genetically pre-disposed to give an exaggerated response to stress. He will also supervise the student worker.

Tiffany Mitchell is a Research Technician (100 percent effort) who will assist with the behavioral measures, daily monitoring of animals and laboratory assays. Tiffany worked on this project previously and is experienced in many of the techniques needed to complete the proposed projects. The University of Georgia pays her salary.

Two graduate students, one Masters and one Ph.D. (50 percent effort), will be recruited through the graduate program of the Department of Foods and Nutrition, University of Georgia. The Masters student will conduct research to evaluate whether animals exposed to repeated restraint are a model for post-traumatic stress disorder and the mechanisms underlying this response. The Ph.D. student will evaluate animals in which the expression of specific genes, related to cognitive function or behavior, have been knocked-out or over-expressed, in order to identify genetic markers for stress-responsiveness.

G. Itemized List of Major Capital Equipment

None.

H. Subcontracts

The leader for the subcontract, Ruth Harris, Ph.D. (25 percent), has overall responsibility for determining the experimental design, organization and completion of studies and timely publication of results for studies and investigating the stress/ cognitive function interaction. Dr. Harris was a project leader for a task in a previous Army grant for six years and is experienced in the methodology needed to complete the studies proposed in the subcontract. She will supervise and mentor a Post-Doctoral Fellow and two graduate students. Ruth Harris has a nine-month faculty appointment at the University of Georgia in the Department of Foods and Nutrition and the sub-contract provides summer salary to ensure 12-month employment.

I. Brief Description of Animal Use

The procedures describe here do not involve significant pain or distress to animals. Only minor pain or distress of short duration are involved and these are alleviated through the use of anesthetics.

To determine that there are no alternatives to the procedures of this proposal the following were used: Medline searches were conducted use using search terms: obesity, food intake, satiety peptides, streptozotocin, glucose signaling, brain, central nervous system, brain metabolism. Attendance at scientific

meetings where discussions of regulation of food intake and obesity are held were also used to obtain information about procedures from others conducting similar studies.

Rationale for Using Animals

Basic mechanisms of brain regulation of body weight and energy balance are studied in animals. In addition, it is more economical to use the smaller species of animals at this level of investigation and because most of the research procedures used could not be ethically applied to human subjects. Once a critical factor is identified for body weight regulation it will be tested in larger species and eventually in human trials after safety concerns are addressed.

Species Identification and Rationale

Most of the literature on basic brain mechanisms involved in the control of food intake and energy expenditure has been done in laboratory rats and mice. Rats and mice have been proven to be a good model for food intake studies. We have shown that rodents behave like humans after weight loss caused by simple diet restriction. They regain the body weight that they lost. On the other hand, overfeeding of humans and rodents lead to weight gain and after the termination of overfeeding both become hypophagic, lose body weight and return to their initial body weight. Body weight may also be influenced by factors such as physical and psychological stress which cause a chronic disruption of homeostasis. Therefore, rodents will be used to gain new basic information on the neurochemical and physiological mechanisms of weight gain and loss.

It is proposed that the initiation and termination of a meal involve (but are not limited to) glucose sensing cells in the brain. These glucose-sensing cells use neurotransmitters, neuropeptides and peripheral signals of energy status (such as leptin) to influence feeding behavior and energy expenditure. Rats and mice will be used to answer the following questions: What is the role of neuropeptides and neurotransmitters in glucoprivic stimulation and glucose inhibition of feeding behavior and energy expenditure. What is the nature of brain cells responding to glucose excess and glucose deficiency? Are beta cell and alpha like cell markers in brain areas associated with feeding altered, reduced, or eliminated by intraventricular injection of streptozotocin ? What brain peptides (or receptors) are reduced or eliminated by ICV streptozotocin.

Rationale for the Number of Animals Required

The number of animals used in each experiment is based on a power analysis. Since the standard deviation of the different measurements determines the number of rats to be used in the above considerations and the standard deviation varies with different measurements, it is necessary that adjustments be made in the estimate of animals needed on each trial as we gain experience with some of these measurements. Usually it requires 8-10 rats per treatment group with 3 to 4 treatment groups per study.

Experimental Design

These studies will provide new information on the control of body weight and obesity. More specifically they will identify key regulatory mechanisms that control both food intake and energy expenditure.

Surgical Procedures will be supervised by Dr. June Zhou and Mihai Covasa. The procedures to be used include:

Brain cannulation and direct brain injection
Placement of microdialysis probes
Placement of Alzet pumps in the peritoneal cavity.
Gastric and intestinal cannulation

Measurements taken in live animals: Food and water intake, energy expenditure (calorimetry), body weight, behavioral assessment for motivation for food (bar pressing).

Feeding and diets: Normally animals will be provided ad libitum access to Purina chow (#5001; 3.4 kcal/g) and tap water. Some specialized diets will be used to alter feeding behavior. Some experiments will use a semi-purified containing (by weight) 70% polycose (or fat) 10% Casein, 10% Corn Oil, 3% Cellulose, and 1% AIN vitamin and mineral mix (or commercial mix). Some rats will be fasted overnight or restricted fed (50% for 4-6 days) to determine if short or long term energy deficiencies are recognized in animals with impaired glucose sensing mechanisms. Additionally, it may be necessary to pair-feed rats by gavaging animals to achieve a controlled intake of a semi-purified diet. In these cases the animals will be tube fed a quantity of diet paired to the intake of another animal.

Anesthesia/Analgesia/Tranquilization

During surgical procedures rats/mouse will be administer methoxyflurane as an inhalant at a dose that puts the animal into a light sleep. Then animals will be injected subcutaneously with ketamine/ acepromazine/zyl (50/3.3/3.3 mg/Kg body weight).

Study Endpoint

Animals will be euthanized at the end of the experiment by rapid decapitation. Due to the nature of the biochemical measurements to be performed, this must be carried out without anesthesia. Decapitation will be carried out by individuals who have had previous training and experience with the procedure and can minimize stress to the animal.

Euthanasia or Final Disposition

If the animals are not used for brain chemistry studies, euthanasia procedures for rats/mice will be done by either carbon dioxide inhalation or barbiturate overdose.

Institutional Animal Care and Use Committee(s) (IACUC) Approval

These procedures have been approved by the UGA IACUC on NOV 1, 2000. The same procedures will be presented for approval by the LSU IACUC as soon as possible.

U.S. Department of Agriculture (USDA) Animal and Plant Health

Inspection Service Animal Care Inspection Report: We will include a copy of the most recent USDA Inspection Report for any and all facilities where animal research will be performed.

Qualifications

Dr. Roy Martin has conducted animal research funded by the federal government for over 20 years. He bears the primary responsibility for insuring that new personnel receive formal training in the proper use and care of animals used on this project. His assistant Dr. Zhou has conducted animal studies involving the surgery and euthanasia.

Accreditation

LSU/PBRC is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC).

J. Conclusion

The goal of the studies proposed here is to identify key metabolic pathways involved in the regulation of body weight. This information will be used to develop dietary interventions or behavioral modification tools that can be used by soldiers to achieve their ideal body weight and composition. As maintenance of a healthy body weight is essential for optimal performance, Army personnel will be better equipped to continue their valuable service to the United States. We will investigate the complex interactions between energy intake and expenditure and the partitioning of energy between muscle and adipose tissue compartments in the body. We will also study how body weight is influenced by factors such as physical and psychological stress, which cause a chronic disruption of homeostasis. Upon completion of this project, we will have identified the neurochemical and physiological mechanisms that are activated by stress, hunger, satiety and spontaneous anorexia and are associated with maintenance of body weight and composition. We also will have identified nutritional interventions that modify or prevent a) weight gain or regain, b) stress-induced and exercised-induced disruption of homeostasis and cognitive function in experimental animals.

TASK 4: LABORATORY FOR HUMAN AND FOOD SAMPLES

A. Problem to Be Studied

We propose to continue as a support reference laboratory for USARIEM, as well as support Tasks 1, 2 and 7 for this cooperative agreement. Over the duration of the last grant, the Clinical and Food Analysis Laboratories have supported 17 studies in collaboration with USARIEM and approximately 43,000 tests performed in 1999-2000 alone. Over the duration of all the previous grants the laboratories have supported a total of approximately 52 studies. For these studies to date, a total of approximately 260,000 tests were performed. The number of different analytes procedures performed for army studies was in excess of 70 different tests. Of these, nearly 30 were developed specifically for Army projects. These include the following:

Vitamin C
NEFA
Glycerol
Lactate
Salivary Caffeine
Salivary Melatonin
CRP
IL-1B
IL-6
Myoglobin
TNF-a
Urine Nitrogen
BHBA
EAST
EGR
ETK

Amino acids (Ala, Glu, GLn, Leu, Trp)
RBC & serum selenium
Retinol Binding Protein
Prealbumin
Transferrin
Haptoglobin
25 OH Vitamin D
1,25 diOH Vitamin D
Osteocalcin
Homocysteine
RBC Transketolase with activation by thiamine pyrophosphate
RBC Glutathione Reductase with activation by riboflavin
RBC AST with activation by vitamin B6

Completed Studies

We outline below some of the projects supported by this laboratory.

75th Ranger Regiment Study. The Ranger Regiment Study consisted of a health assessment panel (chemistry 12 panel including iron, TIBC, and lipids) plus a vitamin C on 144 individuals from the 75th Ranger Regiment in

Savannah, Georgia. Personnel from the laboratory were present on site for specimen processing and preparation for analysis.

Sergeant Major's Academy Nutritional Survey. This study was conducted at the Sergeant Major's Academy in El Paso Texas on multiple occasions. A general health screen and nutritional marker screen were performed on 465 officers at three time points for a chemistry 26 panel including lipids, iron, T3, T4, TSH, ferritin, apolipoprotein A1, apolipoprotein B, C Reactive Protein, vitamin B12, folate, NEFA, vitamin C, retinol binding protein, and homocysteine. Personnel from the laboratory attended the study to help with processing of samples.

Combat Army Support Hospital. This nutritional survey was performed at Fort Bragg. A chemistry 26 panel including lipids, vitamin C, B12, folate, ferritin, C reactive protein, retinol binding protein, RBC folate, Apo A1, Apo B, RBC transketolase, RBC AST, and RBC glutathione reductase, homocysteine, and free erythrocyte protoporphyrin were analyzed on 52 soldiers.

Ranger 3. A chemistry 26 panel including lipids, iron, TIBC, BHBA, lactate, NEFA, amino acids, vitamin C, RBC folate, vitamin B12, 25 hydroxy vitamin D, vitamin A, vitamin E, cortisol, growth hormone, LH, T3, T4, TSH, testosterone, and SHBG were analyzed on 306 soldiers.

Ranger 4. The Ranger 4 study examined Ranger school soldiers with a chemistry panel, BHBA, NEFA, lactate, thyroids, TSH, free T3, T3, free T4, total T4, cortisol, testosterone, folate, growth hormone, free and total, testosterone, and DHEAS.

Caffeine and Sleep Deprivation (Effect of Repeated Dosings of Caffeine on Vigilence). This study examined salivary melatonin and caffeine on 239 soldiers receiving caffeine dosing.

Assessment of Energy Expenditure and Nutritional Status of Female Navy Personnel Onboard Ship (Navy Study). The Navy study, Assessment of Energy Expenditure and Nutritional Status of Female Navy Personnel Onboard Ship, included testing for homocysteine, erythrocyte glutathione reductase, erythrocyte transketolase, erythrocyte aspartate aminotransferase, apolipoprotein A1, apolipoprotein B, transferrin, 1,25 dihydroxy vitamin D, osteocalcin, total iron binding capacity, chemistry and lipid panel, vitamin B12, and serum folate.

West Point Follow-Up. This recent study was assessment of West Point Cadets involved in the original West Point Study for markers of bone health (25 hydroxy vitamin D and 1,25 dihyroxy vitamin D).

Mangoday. Salivary melatonin analyses were completed for the Mangoday study on 259 volunteers.

Special Forces 3. One hundred samples from the Special Forces 3 study were analyzed for a chemistry 26 panel, BHBA, lactate, iron, TIBC, NEFA, vitamin C, RBC folate, vitamin B12, 25 hydroxy vitamin D, vitamin A, vitamin E, cortisol, growth hormone, LH, T3, T4, TSH, testosterone, and SHBG, and amino acids (threonine, histidine, tyrosine, methionine, valine, tryptophan, phenylalanine, isoleucine, and leucine).

Sleep Deprivation Study. This in-house PBRC study looked at the effects of supplements of tryosine, caffeine, phentermine, or amphetamine on markers of cognitive performance after 40 hour sleep deprivation. Analyses were performed for insulin, cortisol, growth hormone, tyrosine, urine creatinine, catecholamines on 75 subjects.

Hot Weather Feeding Study. This study which previously examined chemistry and nutritional assessment measured RBC transketolase, glutathione peroxidase, and AST (analyzed with in vitro activation by the respective cofactors).

Carbohydrate and Performance Study. Glucose, lactate, NEFA, and insulin were analyzed for this study examining the effect of carbohydrate on performance. A total of 255 samples were analyzed.

Special Forces 6. The SFAS-6 study examined chemistries, T3, T4, TSH, free T3, free T4, cortisol, testosterone, folate, growth hormone, and IGF-1.

Post Exercise Nutrient Supplementation Study (PENS). PENS tests included a 46 chemistry 26 panels including glucose and CK, cortisol, insulin, glucose, urine nitrogens on 70 samples, and IGF-1. Tests were also run for alanine, glutamic acid, glutamine, and leucine by HPLC.

Ongoing Studies

We outline below studies which are currently being supported by the laboratory.

Arctic Norwegian Soldier Study. Samples were received for analysis on two Norwegian soldiers before and at two time points after their trek across the Arctic Ocean. Tests to be performed include red cell transketolase, glutathione reductase, and AST before and after activation by their respective cofactors, vitamin C, Chemistry and lipid panel, 25 hydroxy vitamin D, osteocalcin, B12, folate, ferritin, transferrin, homocysteine, vitamin E, vitamin A, and carotenoids.

Combat Army Surgical Hospital (CASH). Analyses are pending for this study for vitamin A, vitamin E, and carotenoids.

Navy Women Study. Marine Women Weight Gain Study (samples to arrive in Oct/Nov, LTC Bathalon is the PI).
EES Effects of Immune Egg Protein and Antioxidants on Muscle Soreness and Strength after Eccentric Exercise" (EES). Analyses of chemistry panel including CK, IL-1, IL-6, TNF-α, CRP, and myoglobin were completed for the Eccentric Exercise Study (EES). Other tests performed included total antioxidant capacity (FRAP), and vitamin C. In this study we provided fast turnaround (2 days) of results for CK.

B. Outline of Proposed Research

The plan for the Clinical Laboratory and Food Analysis Laboratory is to continue receiving samples for analyses for projects conducted by USARIEM, as well as support Army sponsored studies in-house at the PBRC. **The laboratory supports Tasks 1, 2A, 2B, 2C and 7.** In certain cases, personnel from the lab will travel on-site to field studies for processing of samples. We will work closely with USARIEM to determine testing protocols and to perform testing for studies that are selected after consultation. A summary of USARIEM studies which we know about at this time are included below:

Marine Women Study. In this study, which will be conducted at Parris Island, we will collect samples for the analysis of NPY, CRF, cortisol, testosterone, IL-6, glycosylated hemoglobin, ACTH, leptin, NEFA, TSH and fructosamine. Personnel from the lab will help process samples on site.
Dr. Kellogg's Filter Paper Study. The clinical lab at the PBRC will aid in a study by Dr. Mark Kellogg to try to elucidate methods of sample collection onto filter paper from saliva and subsequent analysis for various metabolic and hormone assays.

Fort Bragg Weight Loss Study. This proposed study forms the basis for Task 1. This proposed study is an in depth weight loss study at Fort Bragg, designed to evaluate a weight management program. Planning for this study is underway, and all investigators have met at the PBRC to discuss protocol development. The clinical lab and food analysis labs will function as a resource for this study.

C. Significance and Uniqueness of the Proposed Effort

The Clinical Research Laboratory and Food Analysis Laboratories will continue to serve as an analytical repository for research studies conducted at USARIEM and for PBRC in-house Army supported research. This is a unique service provided by our laboratories specifically for the army. Important aspects of the services

provided to the Army include: 1) over $1 million of in-house equipment available for routine and esoteric testing; 2) intensive monitoring of quality control and external monitoring to insure quality results; 3) specialized and custom test method development; 4) compilation of results in a database or spreadsheet; 5) working with investigators from USARIEM to determine optimal testing schemes, collection protocols, and sample requirements 6) provide help in processing of samples in field studies; 7) provide help in writing of protocols and results for publication; and 8) collaboration with investigators at the PBRC for design, implementation, and testing for army funded in-house studies conducted at the PBRC.

Equipment in the clinical laboratory includes: a Beckman CX7 for routine chemistry analyses, a Beckman CX5 for specialized automated chemistry testing, a Coulter STKS for automated CBC and differential counting, an Abbott IMx for homocysteine analysis, a DPC Immulite Immunochemistry analyzer, a Bio Rad HPLC system for catecholamines, an Antek nitrogen analyzer, a Packard multi-well gamma counter for RIA analyses, a Perkin Elmer P1000 ICP for mineral analyses, a Perkin Elmer Z5100 Zeeman effect graphite furnace atomic absorption spectrophotometer for trace elements, a Bio Rad ELISA reader for microplate based ELISA procedures, two Hewlett Packard 1090 HPLCs, and a Hewlett Packard capillary electrophoresis system. In addition a DPC Immulite 2000 immunochemistry analyzer has been ordered. The Food Analysis Lab has a CEM microwave for moisture determination, a CEM microwave system for ash, a Perkin Elmer nitrogen analyzer for protein, a Soxtec extraction system for total fat, a Fibertec system for fiber analysis, two Hewlett Packard gas chromatographs for fatty acids and cholesterol, and two Waters HPLC systems.

The clinical lab is accredited by the College of American Pathologists, the Health Care Financing Agency, and certified by the Centers for Disease Control for lipid analyses. These accreditations require daily monitoring of performance and stringent record keeping. In-house quality control procedures are in place to monitor daily checks of refrigerator and freezer temperatures, water bath temperatures, water quality, reagent stability and quality, and result quality. In addition to our daily quality control, which is purchased for available constituents or made in-house for those not available commercially, there are also periodic external surveys in which the lab participates to compare our results to those of similar users across the country. The laboratory also works with Bio Rad Laboratories to establish value assignments for their quality control samples. In addition, collaborative work we performed with AOAC International resulted in the interlaboratory validation of a reference method for the analysis of total nitrogen in urine (AOAC Official Method 994.19) in 1995.

Custom test development has long been a unique and strong point of our laboratories. Many new methods have been specifically developed and/or automated for studies being performed by USARIEM. All methods developed by the clinical and food analysis labs must meet stringent method development protocols which include measures of linearity, accuracy, sensitivity, precision, correlation with other known methods if possible, recovery, and interference. All references ranges must be either established or verified.

Laboratory personnel have in the past traveled on-site to USARIEM field studies to process samples for subsequent analyses at the PBRC. Personnel have traveled to many sites to perform processing for the Ranger 2 study, Metabolic Variation study, IDNS study, Ranger 3 study, Combat Army Support Hospital study, Sergeant Major's Academy study, and the Ranger Regiment Nutritional Assessment Study. Continuing efforts in this regard will be a plan of this grant proposal. Currently, trips are planned to Parris Island South Carolina, and Natick, Massachusetts.

D. The Potential Military Relevance

Potential military relevance of the clinical and food lab participation will be to enable the military to have specialized laboratory services available to the Army at USARIEM which provides a service of lab testing without the burden of equipment, personnel, additional funding or detail. Because the laboratory has a nutrition focus and a research support mission, the focus of testing is relevant to USARIEM's mission. Because the services provided by the PBRC are under one roof, there will be less need to farm samples out to many multiple laboratories. All lab-testing services will be provided by the PBRC with additional services as mentioned above. This includes custom test development, quality results, working with military investigators

from the design to the final reporting of results, help in processing of samples in field studies, and a laboratory for PBRC in-house army supported research. Our experience in working with the army and the trust and working relationship we have developed will help us to continue to provide an exemplary service to the army.

E. Proposed Duration of Effort

Five years.

F. Names, Titles, Roles and Percent Effort of Participating Personnel

Jennifer Rood, Ph.D. will oversee and direct the clinical laboratory analyses that will be conducted for USARIEM and for PBRC projects. This will include management activities, reporting quarterly and annually, as well as research and development activities of the laboratory. Richard Tulley, Ph.D. will assist Dr. Rood in these activities. Dr. Rood is a certified by the American Board of Clinical Chemistry and she has worked on the previous army grants since 1993. Dr. Rood will devote 75 percent of her time to this project. Richard Tulley, Ph.D. is a board certified clinical chemist (American Board of Clinical Chemistry) with 20 years experience and 12 years experience working on army-related lab testing for the previous three grants. Dr. Tulley will devote 60 percent of his time to this project. A total of 1.35 FTEs will be employed in the management of the project, beginning in Year 1.

The duties of the Research Associates will be to organize samples and perform analyses on samples submitted by the Army in support of nutritional research conducted through the Military Nutrition Division. Research associates will maintain instruments, perform troubleshooting on those instruments, perform routine quality performance checks, perform analyses, enter and verify results of those analyses into the laboratory computer, and print reports. There are 4.75 FTEs allocated for these purposes.

Laboratory assistants will receive and unpack samples, log them into the laboratory computer, prepare samples for analysis, aliquot samples, deliver samples to appropriate work stations, and compile results obtained from the appropriate work stations. There is 1.0 FTE allocated for this work.

In some field projects, laboratory personnel will travel on-site to process samples for analysis. These personnel are pulled from the 7.7 FTE allocated for this task.

Food Analysis Laboratory

The Food Analysis Laboratory will employ one research associate and a lab technician to perform food analyses for the Army in support of nutritional research conducted by the Military Nutrition Division. This is allocated at 0.7 FTE. The laboratory supports Tasks 2 and 7. Analyses offered by this lab include proximate analysis, cholesterol, fatty acids, total dietary fiber, vitamins A, E, and minerals on military food samples. This service has proven invaluable in food design and diet determination in the past.

Students

Three students will be employed to perform such activities as wash glassware, autoclave waste, file, and other miscellaneous duties as assigned.

G. Itemized List of Major Capital Equipment

None.

H. Subcontracts

None.

I. Brief Description of Human Use

Not applicable.

J. Conclusion

The Clinical Research Laboratory and Food Analysis Laboratories at the PBRC have, in the past, developed new methods of analysis specifically for the Army, analyzed samples for 52 studies and performed analysis of greater than 250,000 tests in collaboration with military researchers. The lab developed 30 tests specifically for the Army and has in excess of 100 tests on-line and available for use. Personnel from the lab have processed samples on-site for seven Army field studies for at least 10 trips involving in excess of 15 people.

Tests performed by the Clinical Research Lab for the Army, since the inception of previous grants include the following:

General Chemistry
Chem 26 panel
urine creatinine
albumin
iron
TIBC
glucose
HDL
cholesterol
triglyceride

Metabolites
ammonia
lactate
free fatty acids
BHBA
amino acids
homocysteine
catecholamines
glycerol

Minerals/Trace Elements
calcium
phosphorus
selenium
copper
zinc
magnesium
sodium
potassium

Hormones
PTH
growth hormone
melatonin
cortisol
insulin
ACTH
SHBG
Testosterone
osteocalcin

Vitamins
25 OH vitamin D
1,25 dihydroxy vitamin D
folate
RBC folate
vitamin B12
vitamin A
vitamin E
vitamin C
RBC transketolase with activation by thiamine pyrophosphate
RBC glutathione reductase with activation by riboflavin
RBC AST with activation by vitamin B6

Proteins
transferrin
ferritin
haptoglobin
retinol binding protein
prealbumin
IL-1B
IL-6
Myoglobin
TNF-a
Sensitive CRP

Miscellaneous
CBC
urinalysis
caffeine
bromide
deoxypyridinoline
nitrogen

fecal Polyethylene Glycol (fecal marker)
free erythrocyte protoporphyrin

Food Analysis
Total fat
Protein
Carbohydrate (calculation)
Ash
Moisture
Fatty acids
Cholesterol
Vitamin A
Vitamin E

Services which the laboratory will provide include:
- Help in design and implementation of studies
- Consultation on sample processing/storage/shipping
- Help in processing of samples
- Reporting of results
    -hard copy
    -compiled data in database
- Help in manuscript writing
- Original method development
- Laboratory support of PBRC in-house Tasks 1, 2A, 2B, 2C and 7
- Laboratory support for USARIEM research projects.
- Laboratory support of Task 1 in collaboration with USARIEM

TASK 5: STABLE ISOTOPE LABORATORY

A. Problem to Be Studied

Over the duration of the grant, the Stable Isotope Laboratory has supported eight studies in collaboration with USARIEM. For these studies to date, a total of 167 doubly labeled water measures of energy expenditure, 167 measures of water turnover, and 167 measures of total body water have been conducted. For these measures, a total of 3,812 deuterium and 3,057 ^{18}O isotope analyses have been performed.

Background

Completed studies supported by this laboratory are listed below.

75th Ranger Regiment Study. The first study completed was a DLW study for the 75th Ranger Regiment Study in Savannah, in which there were two phases, the second phase being a Field Training Exercise (FTX). The mean energy expenditures in barracks and in the field were 4200±800 kcal/d and 4800±900 kcal/d.

Ranger Study. The second study completed was a large, long-term study in Rangers which included four separate DLW dosing periods. The Ranger Study completed this year is different from our past Ranger studies in that we obtained energy expenditure data for the entire period, from 12 Feb, through 10 Apr, by doubly labeled water (DLW). Four DLW doses were administered, 12 Feb, 25 Feb, 9 Mar and 24 Mar, to cover the entire period. The isotope analyses for the Ranger 96 study were completed, and final calculations been made. The raw data, as well as the summary sheet for energy expenditure calculations from repeat analyses are given in the Appendix (22nd Quarterly Report). Energy expenditure during each period was quite high, nearly 5500 kcal/d for periods 1-3, and just under 5000 kcal/d for the final period (See 22nd Quarterly Report).

Norwegian Rangers (II). The 3rd study completed was a study in which Norwegian Rangers underwent an intense training exercise under harsh environmental conditions. Since these soldiers received very little food, and hence relied largely on their fat stores for energy, we used an assumed RQ of 0.75 for conversion of CO_2 elimination to energy expenditure. The mean energy expenditure in this study was very high, 5650±800

kcal/d.

8th Engineer Support Battalion. A three-day study of the proposed go-to-war ration for the Marines, the Tray Pack Ration, compared to B-rations. Participants in this study were 19 Marines from the 8th Engineer Support Battalion during a construction mission. Energy expenditure was measured three times during the mission, requiring 870 deuterium analyses and 849 ^{18}Oxygen analyses. Average total daily energy expenditure during the Marine construction mission was 3330 kcal/d. The energy expenditure was highest during the first phase, most likely because of the physically demanding tasks of unloading equipment and supplies coupled with the digging of the foundation during the first week. There was no difference between the two ration groups. The Average energy expenditure of 3330 kcal/d was less than the 3600 kcal/d Nutritional Standard for Operation Rations, suggesting that this standard is adequate for moderately active Marines in normal field missions. Those consuming the T Ration were in greater negative energy balance (-784 kcal/d) compared to those consuming the B Ration (-448 kcal/d) over the course of the study. The overall water turnover calculated from the deuterium turnover rate was 5.7±1.0 L/d. The more active construction engineers had a significantly greater water turnover rate than administrative and support personnel (6.0±0.9 vs 5.0 ± 0.8 L/d). While the Marine leadership continually stressed the importance of fluid consumption, the difficulty of individuals obtaining and consuming sufficient fluids while away from base camp was a problem. The education of every soldier and marine on the importance of fluid consumption and the consequences of hypohydration needs to continue.

Norwegian Rangers (III). The energy expenditures reported for the Norwegian Ranger Training studies are some of the highest reported energy expenditures we have observed in military personnel, 6250±770 kcal/d this year, 500 kcal/d higher than was observed in the previous Norway study of 5650±800 kcal/d. The only energy expenditures approaching this level were those observed in the Marine Crucible event, the "IOC war" filed exercise (see below) and the "Mountain Class" phase of the Ranger Training studies. This study required 226 deuterium analyses and 169 ^{18}Oxygen analyses.

Infantry Officer Course (Spring). Metabolic energy balance and thermal status was assessed in 14 male USMC volunteers during a 10-day field exercise (FEX) at Quantico, Virginia. The USMC Infantry Officer Course (IOC) is a 10-week, MOS-producing school, required of all Infantry Officers before assignment as platoon commanders within the Fleet Marine Forces. The bulk of the training takes place in the elements, much in simulated combat and tactical field exercises. Upon completion of the Infantry Officer Course, the lieutenants are prepared for assignments as Marine 0302 infantry officers in the Fleet Marine Force. The culminating exercise for the IOC students is the "IOC war" or field exercise (FEX). The nine to 10 day FEX is designed to test the students' physical and mental endurance while applying the tactical lessons learned from earlier instruction. The students involved in the FEX operate on one and a half meals per day and very limited sleep, adding to physical and mental stress. The "IOC war" is the final evaluation of what the students at the IOC have learned and how well they can apply it on a simulated battlefield with the stress of a battlefield environment. The Basic School requested the support of the USARIEM/PBRC in determining nutritional and thermal states of trainees conducting field exercises with the Infantry Officer Course during winter months. The question we were asked to answer was whether the intense physical activity, limited sleep, and restricted rations of the FEX, when combined with cold, damp weather, result in excessively negative energy balance and evidence of hypothermia. Total daily energy expenditure was measured by the doubly labeled water method (281 deuterium analyses and 407 ^{18}Oxygen analyses); food intake was assessed through daily collections of ration wrappers; daily activity logs were provided by each volunteer; local weather data were collected with an automated weather station. Each volunteer wore a prototype Warfighter Physiologic Status Monitor (WPSM) system consisting of a network of wearable WPSM sensors that collected synchronous, time series measurements of body core temperature (telemetry pill), heart rate (HR), activity (sleep/wake) patterns, and geolocation (GPS). The energy expenditure during the exercise, was 6100 ± 840 Kcal/d while energy intake was only 1334 ± 667 Kcal/d. Water turnover measured by deuterium turnover was 3.20 ± 0.37 L/d. Carbohydrate intake was inadequate, and transient cold stress (core temperatures around 35.50C) was evident during inactivity/sleep. It was concluded from these results that dismounted warfighter nutrition should be improved by providing an easy-to-eat source of carbohydrate. In addition, it was suggested that the ability of

the modular sleeping bag should be tested to prevent cold stress during periods of inactivity/sleep while in the "travel light, freeze at night" phase of an assault. Energy expenditure during the Infantry Officer Training Course, conducted at Quantico, March 1999 was also high. This was expected since they were carrying around 100-150 lbs. and were getting very little sleep.

Infantry Officer Course (Summer). Isotope analyses were completed for the doubly labeled water study for the Summer Quantico Study. There were no shifts in baseline isotope abundance in the five subjects who received tap water. Therefore, no adjustments were necessary in the ten subjects who received the DLW dose. The subjects received only one MRE and five packs of a carbohydrate beverage during the study. Therefore they only received just under 2300 kcal per day, and were in substantial negative energy balance. To calculate the energy equivalent of CO_2, the macronutrient content of the MRE, the carbohydrate beverage, plus available glycogen stores were calculated, and body stores of protein and fat (assumed 20 percent protein and 80 percent fat) were estimated. This led to an RQ of 0.804 (including protein), giving an energy equivalent of CO_2 of 5.769 kcal/L CO_2.

Disabled Submarine. Isotope analyses and calculations for the nine-day simulation of a disabled submarine (DISSUB) with eight volunteer "survivors" conducted in the NATICK chamber facility are complete. The purpose of this study was to examine the physiological effects of resting (surviving crew would be confined to their bunks) exposure to mild hypoxia (16.75 percent O2), hypercapnia (2.5 percent CO2), and cold (submarines cool to water temperature of about 4 deg C several hundred feet below the surface) and high humidity. The primary objective is to document average metabolic rate and CO2 production in crew. Estimates currently available are based on measurements thought inaccurate.

There was a mean decrease in total body water of 0.4kg during the study, as assessed by total body water (TBW) measured with deuterium oxide at the beginning and end of the study. Water turnover was approximately three liters/day over the entire period, but was higher during the first four days of the study. Energy expenditure increased throughout the study, averaging 3559 kcal/d during the first three days, 3813 kcal/d from days 0-5, and 4476 kcal/d throughout the whole seven days. Energy expenditure calculated using the two point and regression analysis gave very similar results for days 0-5 and days 0-7.

Body composition data was used to calculate body energy stores used for energy, which, combined with dietary intake, was used to estimate the average respiratory quotient to be used in calculating energy expenditure by the doubly labeled water method. The average fat free mass and fat mass lost during the study was 0.92 kg and 0.63 kg. Assuming that 300 g of glycogen was utilized, and combined with the dietary intake, this gave an RQ of 0.82 and an energy equivalent of 5.794. However, the body stores used, or the estimated energy intake were significantly underestimated, as the Energy Expenditure obtained from this Intake/Balance method was 955 kcal/d lower than that obtained from the DLW method. When an FQ was estimated based on the energy expenditure and body stores necessary to make up the difference, a lower FQ of 0.799 was obtained. But the energy equivalent for CO_2 was quite similar at 5.883. (For an in depth analysis of the FQ and Intake balance calculates see Table 3 in the 12th Quarterly report.) That the intake balance method would not give an accurate measure of energy expenditure over such a short time is not surprising, as the error in body composition and energy intake are quite high for these calculations. However, the major reason for this discrepancy was that the final body composition measurement was obtained two days after subjects resumed ad lib feeding.

Ongoing studies supported by this laboratory are described below.

Ft. Carson Study. For the Ft. Carson study of energy balance with the 10th SFG, 20 subjects were dosed with doubly labeled water and three undosed subjects will serve a placebo subjects to examine any shifts in baseline isotope shifts. The ^{18}O isotope analyses have been completed on all samples. The deuterium isotope analyses have been completed on approximately 50 percent of the samples.

Sustained Operations. For the sustained operations study, 10 subjects were dosed with deuterated water to

study TBW and water turnover. Additionally, four subjects were undosed, to serve a placebo subjects to examine any shifts in baseline isotope shifts. All samples have been logged in and sample cleanup completed.

B. Outline of Proposed Studies

In discussions with Dr. Andrew Young, Chief, Military Nutrition Division at USARIEM, the goals for the Stable Isotope Lab include: continuing support for energy expenditure measurements during the various field studies to evaluate new rations and examine interactions between nutrition, performance and the environment, and a basic research program to identify and elucidate the etiology and pathophysiological mechanisms of heat, cold, and altitude-induced injuries and illnesses.

There are, at present, two studies that are in fairly advanced planning. One is a joint effort between Dr. DeLany at the PBRC and USARIEM to look at attrition rates of overweight female recruits. The recruits - male and female - are allowed to come in above the screening weight, but DO MEET Marine body fat requirements. Thus, while above weight, they are not above body fat requirements. These individuals participate in all aspects of basic training - unless injured. The goal is, of course, to facilitate adequate weight reduction to meet BOTH the Marine body fat and weight standards by the time the recruit gets to the Crucible. All the recruits are weighed on schedule, and only those overweight are monitored for overweight status. The current commander of the 4th battalion (the female battalion) has made major changes in the dining facility already-the entire bn gets fat modified foods, and those on weight control get a further modified diet (pre-prepared low fat meals). This study will combine the measures of energy expenditure in men and women undergoing basic training art Parris Island, as well as a study of overweight women undergoing the same training regimen.

The second proposed study is a large collaborative study with USARIEM personnel, which will be headed by LTC Gaston Bathalon. This proposed study is an in depth weight loss study at Fort Bragg, designed to investigate methods by which the number of recruits lost due to problems remaining under the military weight standards. Planning for this study is underway, and all investigators have met at the PBRC to discuss protocol development.

In addition, we plan to begin two new areas of research. The first area is an extension of ongoing studies to "resolve function fuels," which involves using doubly labeled water to validate other methods of measuring energy expenditure, such as foot contact and activity monitors and vigilance monitors. The second area is the study of "Maintenance of Body Matrix," or the study of build up and catabolism of body tissues. This would include stable isotope studies of protein, fat and carbohydrate metabolism.

Finally, we have had preliminary discussions with Dr. Young and tentatively propose two additional projects for investigation. First, we will consider a validation of respiratory chambers using DLW and other energy expenditure methods. Second, we will consider a Marine Corps study. We would provide guidance to the Marine Corps regarding the maximum allowable duration that Marines should be allowed to subsist solely on the Meal, Cold Weather (MCW replaces RCW). It is likely that such a project would provide an opportunity to extend the database on TDEE of different military populations to a Marine Corp cold-weather combat training exercise. We would use different exercise than Crucible or the IOC FTX.

C. Significance and Uniqueness of the Proposed Effort and
D. Military Relevance of the Proposed Effort

The DLW method is well suited for military nutrition studies because it is a true field technique for accurate determination of energy expenditure. There are no requirements for subject compliance (except giving spot urine and/or saliva samples) such as filling out logs, there is no equipment to break, and it can be used to validate other field techniques. In addition to providing measurement of integrated energy expenditure, the DLW method provides simultaneous measures of total body water (for hydration status changes and for calculation of body composition) and water turnover, key measurements for many military

nutrition studies. Hydration status is perhaps more critical to the sustained performance of soldiers than energy balance, and water requirements have been studied during many field studies.

This cooperative agreement provides a valuable service in making this technology available for USARIEM studies of military relevance. The Task has in the past led to greater knowledge of warfighter energy and water needs.

E. Proposed Duration

Five years.

F. Names, Titles, Roles and Percent Effort of Participating Personnel

James DeLany, Ph.D. (70 percent effort) serves as director of the Stable Isotope Laboratory and project leader. He will coordinate the activities associated with the measurements of energy expenditure and laboratory function.

The Assistant Director, Laurie Byerley, Ph.D. (50 percent effort), will be responsible for the day to day management of the stable isotope lab, including mass spectrometer operations and quality control. She will also coordinate the effort of the student workers involved in sample cleanup, glassblowing and data entry.

The Research Associates (3.4 FTE) shown in the budget will perform the isotopic measurements and calculations of ^{18}O and/or deuterium for the DLW method, and total body water and water turnover measurements.

The student workers are responsible for: sorting and logging all Army samples that come into the lab, sample cleanup and alliquoting for isotope analysis, making the Vycor tubes used for the deuterium analysis, cleaning and drying bottles used for ^{18}O analyses, entering data in the mass spec log books and sequence tables, and cleaning up the isotope data that is dumped into a Lotus spreadsheet to maintain an electronic log of the isotope analyses.

G. Itemized List of Major Capital Equipment

None.

H. Subcontracts

None.

I. Brief Description of Animal and Human Use

Not applicable.

J. Conclusion

The application of stable isotope techniques provides true field measurements of energy expenditure and/or total body water shifts and water turnover measurements under a variety of environment conditions which military personnel would be expected to encounter in their duties. These studies allow us to examine interactions between nutrition, performance and the environment to better support our military personnel in the conduct of their duties.

TASK 6: NUTRIENT DATABASE LABORATORY

A. Problem to Be Studied

Since 1996, the Nutrient Database Laboratory has supported 10 studies in collaboration with USARIEM. Timely receipt of dietary data via computerized nutrient analyses of recipes, menus, and dietary intakes of soldiers is critical to assessment of the soldiers' needs and interrelationships with other aspects of military life. We can offer programming expertise, personnel stability, and staff who can be involved in precise data collection, both in garrison and field settings. In our previous efforts, we assisted effectively in dietary collection protocols, data entry, and analysis of dietary data collected in a variety of USARIEM projects.

Completed Studies

1. Hunter Army Airfield/Fort Stewart, Savannah Georgia, Summer 1996
Role: Nutrition support for collection and analysis of dietary data in garrison setting and via MRE consumption and food records kept by the Rangers from the 75th Ranger Regiment.
Dates: 13 July - 5 August, 1996
Number of subjects participating in the study: 241
Approximate # days dietary data collected on each subject: 8
PBRC Participants in Study: Catherine Champagne, Alice Hunt, Ray Allen, Mary Baldwin Sanders, Barbara Eberhardt, Anyce Griffon, Philippe Hebert. Stacy Heilman, Leslie Favie

2. Sargeants Major Academy, Biggs Army Airfield, Fort Bliss, El Paso, Texas
Role: Nutrition support for collection and analysis of dietary data collected via self-reported food records.
Dates: 19 - 26 September, 1996; 7 - 13 December, 1996; 1 - 7 March 1997
Number of subjects participating in the study: 110
Approximate # days dietary data collected on each subject: nine total (three days/time period)
PBRC Participants in Study: Catherine Champagne, Ray Allen, Barbara Eberhardt, Anyce Griffon, Philippe Hebert, Kelly Patrick, Jacqueline Bowman, Rowena Santillan, April Hebert, Anita Sawyer, Fatemeh Malekian, Regina Louviere
Students recruited: Jennifer Fisher, Louisiana Tech University, Ruston, LA; Melanie Taylor, North Oaks Hospital, Hammond, LA; Robyn Guillot, North Oaks Hospital, Hammond, LA; Bethany Donaldson, North Oaks Hospital, Hammond, LA

3. Camp Mackall, North Carolina
Role: Assistance in the collection and data entry of food consumption data from the consumption of MREs.
Dates: 29 April 1997 to 13 May 1997
Number of subjects participating in the study: 263
Approximate # days dietary data collected on each subject: 5
PBRC Participants in Study: Catherine Champagne, Ray Allen, Bill Glover, Barbara Eberhardt, April Hebert, Troy Fontenot, Kelly Patrick, Fatemeh Malekian, Regina Louviere, Alan Pesch

4. Fort Bragg, North Carolina
Role: Nutrition support in the collection and analysis of data collected via self-reported food records.
Dates: 5 - 24 September, 1997
Number of subjects participating in the study: 27 Rangers
Approximate # days dietary data collected on each subject: nine total (three days/time period)
PBRC Participants in Study: Catherine Champagne, Ray Allen, Barbara Eberhardt, April Hebert

5. Fort Lewis, Washington
Role: Assistance in the collection and data analysis of self-reported food records.
Dates: 8- 13 February, 1998
Number of subjects participating in the study: 146

Approximate # days dietary data collected on each subject: 3
PBRC Participants in Study: Catherine Champagne, Ray Allen, Eric LeBlanc, Barbara Eberhardt, Bradley Prather, Alana Cline

6. Bahamas
Role: Nutrition support in data collection of meal preparation, food consumption, and final data analysis for subjects in a remote field setting.
Dates: 2-10 April, 30 April - 8 May; 21-29 May, 1998
Number of subjects participating in the study: 241
Approximate # days dietary data collected on each subject: 18 total (6 days/time period)
PBRC Participants in Study: Catherine Champagne, Barbara Eberhardt, April Hebert, Ray Allen (for off-site data analysis)

7. Fort Drum, New York
Role: Assistance in data collection efforts.
Dates: 26 July – 7 August 1998
PBRC Participants in Study (did not process data for this study, only sent personnel for data collection): Melanie Taylor, Chris Guidry, Leslie Dupont

8. Navy Ship Study, San Diego, California
Role: Assistance in on-site (shipboard) study of food consumption of female military personnel who were consuming foods prepared on ship and data entry and final data processing of those foods consumed.
Dates: 21 February – 3 March 2000
Number of subjects participating in the study: 31
Approximate # days dietary data collected on each subject: 8
PBRC Participants in Study: Eric LeBlanc, Dawn Turner, Catherine Champagne, Ray Allen, Jarret Keller (assistance in close-out efforts)

9. Fort Carson Study, Colorado Springs, Colorado
Role: Nutrition support in collection of recipe preparation data, food consumption data, and data entry/analysis of all food consumption data.
Dates: June – July 2000
Number of subjects participating in the study: 45
Approximate # days dietary data collected on each subject: 8
PBRC Participants in Study: Eric LeBlanc, Jarrett Keller, Claire Fontenot, Calynn Davis, Catherine Champagne, Ray Allen

10. Ft. Drum Study, New York
Role: Assistance in data entry efforts in an MRE study conducted by Natick Labs with our participation approved by USARIEM.
Dates: 30 October – 8 November 2000
Number of subjects participating in the study: 235
Approximate # days dietary data collected on each subject:
PBRC Participant in Study: Eric LeBlanc

Ongoing Data Analysis

1. Navy Ship Study, San Diego, California
2. Fort Carson Study, Colorado Springs, Colorado

We expect data to be completed for study 1 by January 28, 2001 and for study 2 by February 15, 2001.

Another study has been proposed for the current grant period by USARIEM investigators who have indicated that dietary intake data will need to be acquired. This is a study of energy expenditure in men and women undergoing basic training at Parris Island for the Marines.

B. Outline of Proposed Studies

In discussions with Dr. Andrew Young, Chief, Military Nutrition Division at USARIEM, the goals for the Nutrient Database Laboratory include continuing support for dietary intake measurements during the various field studies to evaluate nutrient intakes from garrison dining facilities, current field rations and new rations, and examine interactions between nutrition, performance and the environment under many different conditions.

A particular objective of the proposed cooperative agreement period is to translate nutrient database expertise to in-house USARIEM personnel. The PBRC can assist USARIEM in identifying computer support personnel and assist in training for greater independence of USARIEM.

The Fort Bragg collaborative study offers an opportunity for use of Task 6 in analysis of food intake, as well as advising in dietary counseling. We plan to be involved in both aspects. Similarly, Task 6 supports food intake analysis and dietary counseling for Tasks 2A, 2B, 2C and 7.

C. Significance and/or Uniqueness of the Proposed Effort

USARIEM has utilized the expertise of the Dietary Assessment & Counseling/ Nutrient Data Systems Section to process dietary intake data collaboration is one which works effectively. In this proposal, with its central theme of weight management, dietary intake assessment and dietary counseling are key services.

D. The Potential Military Relevance

Obviously, there will be ongoing interest in dietary intake data of soldiers in helping to evaluate nutrient adequacy in a variety of situations of interest to the military. It is necessary to look at dietary intakes in all stages of a soldier's military career, and in a variety of circumstances, ranging from garrison dining facilities to consumption of field rations (B-rations, T-rations, Meals-Ready-to-Eat, etc.). A long-term collaboration such as the task revolving around the Nutrient Database Laboratory at the PBRC appears to be an ideal mechanism by which to address the dietary intake questions of interest. We can provide the computer and programming support very effectively in field situations and the presence of data collection and data entry personnel assures that there is continuity between USARIEM and PBRC investigational and support team members. Data is sent to USARIEM in a timely fashion following completion of field studies, especially when staffed by several PBRC personnel at the study site. This results in the presentation of finalized nutrient consumption data to USARIEM, which allows for statistical analyses and report/manuscript generation in an expeditious manner.

E. Proposed Duration of Effort

Five years.

F. Names, Titles, Roles and Percent Effort of Participating Personnel

Catherine M. Champagne, Ph.D., R.D., Chief, Dietary Assessment, Counseling, and Nutrient Data Systems, at the PBRC serves as project leader at 40 percent effort. Dr. Champagne is experienced in both nutrient analysis and dietary counseling. She has been a Co-PI on the Premiere dietary counseling trial and participated on DASH, DASH-NA, DPP, SHOW and Delta as an Investigator.

Raymond Allen, Ph.D., is the Information Systems Administration/Programmer of the PBRC's Nutrient Data Systems, and is assigned 40 percent to this task.

Three Research Associates are assigned to this Task. They provide dietary counseling and food intake assessment on an as-needed basis. They are Barbara E. Cerniauksas, R.D., who is assigned five percent to the task; April H. Kurtz, R.D., who is assigned five percent to Task 6; and Dawn Turner, B.S.-Nutrition, who is assigned 10 percent to Task 6.

In addition, Eric LeBlanc, B.S.-Computer Science, is employed as a Research Associate-Computer Programming Operations at 100 percent on Task 6.

G. Itemized List of Major Capital Equipment

Computer and software upgrades as needed will be necessary to continue the efficiency of data entry to support field studies. We are currently in the process of upgrading our laptop computers as plans are finalized for studies to be completed during the initial portion of this grant period. These equipment purchases are budgeted at $20,000.

H. Subcontracts

No subcontracts are anticipated.

I. Brief Description of Animal and Human Use

As we continue to support USARIEM's efforts in planning for additional field studies, USARIEM investigators will address all human use concerns in the drafting of protocols for specific projects. Similarly, PBRC-initiated studies are reviewed and approved by PBRC IRB and HSRRB. We do not anticipate additional human use issues as a result of our involvement in data entry and subsequent nutrient analysis of intakes of individuals participating in these projects.

J. Conclusion

Continued support for USARIEM projects and for Tasks 1, 2A, 2B, 2C and 7 of data collection, entry, and analysis is the mission of this Nutrient Database Laboratory task. Efficient and accurate information delivery on the nutritional intakes of the study population is a goal.

Additionally, this task can provide assistance in dietary counseling for Tasks 1, 2A, 2B, 2C and 7. The 12-year history of successful collaboration demonstrates the flexibility and interest of PBRC personnel in the success of Task 6.

TASK 7: METABOLIC UNIT PROJECT

A. Problem to Be Studied

The Metabolic Unit project is a device to allow core support in the PBRC's Metabolic Unit for Task 2, as well as to allow the support of new studies originating in USARIEM to be supported in the PBRC's Metabolic Unit. Thus, this task allows for support of Clinical Studies in Health and Performance Enhancement: Project 2A, Metabolic Understanding of Energy Balance and Project 2B, Influence of Dietary Fat on Training and Performance. Additionally, problems of military significance can be identified and, with collaboration from USARIEM, addressed through studies in the PBRC Inpatient Metabolic Unit. We do not have inpatient projects planned at this time. We use this Task as a "place holder" to allow for projects to be executed in the PBRC's

Metabolic Unit should the need arise.

B. Outline of Proposed Research

In 1993 the PBRC Metabolic Unit was used for a special inpatient study. A description of this experience serves to illustrate how other studies might be developed to address military problems.

The PBRC served as the site for two research cohorts of U.S. Army Rangers. That project was, "Assessment of Intra- and Inter- Individual Metabolic Variation in Special Operations Forces Soldiers." The PI for the project was Ms. T. E. Jones, affiliated with the Military Nutrition Division at USARIEM. Co-Investigators were C. Gabaree, Lt. Col. T. C. Murphy, Donna Ryan, M.D., and E. Brooks, R.N., M.N.

The purpose of the study was to evaluate a group of Special Operations Forces (SOF) volunteers to determine the metabolic variation during rest, exercise and post-exercise recovery of the individual soldiers. On June 11, 1993, 10 SOF soldiers arrived to serve as the first cohort for testing. Army personnel at the PBRC included Tanya Jones (PI), Sven Ljamo, M.D. (Medical Monitor), Catherine Gabaree (Exercise Physiologist), Lt. Col. Cliff Murphy (Dietitian) and three civilian spotters for exercise testing. The first cohort of military volunteers and civilians left the PBRC on July 1, 1993. There were minimal complications that occurred in the SOF volunteers (subungual hematomas, muscle soreness, and poison ivy dermatitis). All procedures were carried out safely and satisfactorily. A mid-course correction session at the end of the first cohort stay resulted in minor procedure adjustments. From July 9-24, 1993, 10 members of the SOF from the 10th SFG at Fort Devens, Massachusetts participated in the study. All procedures were carried out safely and satisfactorily.

The Metabolic Unit project demonstrated that carbohydrate loading produced increments in physical performance in SOF soldiers. However, the variation between individual soldiers was not great enough to support developing individualized carbohydrate supplements. As a result of this work, the SOF did not pursue a plan to develop individualized soldier supplements for SOF. Therefore, this lack of metabolic variation does not mean that carbohydrate loading would not be effective and the military will pursue carbohydrate loadings for high intensity exercise operations for our SOF soldiers.

We would offer the Metabolic Unit at the PBRC for additional inpatient studies on an *ad hoc* basis.

This task also allows for budgeting of core support for Task 2A and 2B.

C. Significance and/or Uniqueness of the Proposed Effort and
D. The Potential Military Relevance

The PBRC's Inpatient Metabolic Unit contains 14 beds and two indirect calorimeters (one of <15 such units in the United States). The unit is served by a Metabolic Kitchen capable of producing standardized, controlled diets for up to 110 subjects/day and the Metabolic Unit is staffed by a seasoned clinical trials team. The PBRC's facilities for energy expenditure determination, body composition determination, and food intake assessment are state of the art. The PBRC is capable of a number of specialized metabolic studies (FSIGTT, fatty acid oxidation). The availability of these facilities and trained personnel are an asset to enhance USARIEM's research portfolio.

E. Proposed Duration in Years and Months

Five years.

F. Names, Titles, Roles and Percent Effort of Participating Personnel

Steven Smith, M.D. (five percent effort) is a medical investigator at the PBRC and is also head of the in-patient Clinical Trials Unit. Dr. Smith will serve as medical investigator on this project and will oversee the

medical aspects of the trials if needed. His commitment can be increased as needed.

The Pharmacist, Charles Sides, R.Ph. (five percent effort), is responsible for stable isotope infusate preparation and pyrogen testing. He will also be responsible for Dietary Supplement preparation and administration and will ensure quality assurance for pharmacy services.

A PBRC Recruiter (five percent effort), Betty Kennedy, M.P.A., is assigned to the project and will be responsible for recruiting subjects through personal contacts, newspaper and radio ads, and through circulation of fliers. Recruiters do a brief phone screening of all potential volunteers to determine suitability for participation based on administered criteria. The percent effort can be increased as needed.

Registered Nurses (.90 FTE) and a Licensed Practical Nurse (25 percent effort) are responsible for placement of venous catheters, blood drawing, infusions, and collection of breath samples during the experimental trials. A Nursing Assistant is allocated at 50 percent effort. They also ensure the overall safety of the procedures and monitor each patient's status during the exercise trial. They will play a significant role in medical screening process. In subsequent years, we will need two FTE LPNs to accommodate the subjects.

Metabolic Kitchen Staff: Kitchen Manager (40 percent effort), Dietetic Coordinator (50 percent effort), Technician (50 percent effort) and Student Worker, will be responsible for menu development, implementation, cooking, presentation and other aspects of delivering the research meals.

Another Research Dietitian is to be recruited at 100 percent effort to support Task 2.

Special Clinical Endpoints

Kim Landry is allocated at 50 percent effort for performance of body composition and cardiovascular endpoint techniques.

G. Itemized list of Major Capital Equipment

None.

H. Subcontracts

None.

I. Brief Description of Animal and Human Use

No animal use. All human studies that originate from USARIEM investigators must be approved by the IRB at the PBRC, as well as the HSRRB, prior to study implementation. There are no known independent studies beyond those outlined in Tasks 1, 2A, 2B and 2C.

J. Conclusions

The Metabolic Unit project allows for core support of Task 2 and for use of the PBRC inpatient facilities on an *ad hoc* basis by USARIEM investigators.

B

Previous Recommendations for the Pennington Biomedical Research Center

from the

Committee on Military Nutrition Research

Letter Report:
Plans for the Pennington Biomedical Research Center
June, 1989

INSTITUTE OF MEDICINE
NATIONAL ACADEMY OF SCIENCES
2101 CONSTITUTION AVENUE WASHINGTON, D. C. 20418

FOOD AND NUTRITION BOARD

June 26, 1989

Major General Philip K. Russell
Commander
U.S. Army Medical Research
and Development Command
Fort Detrick, MD 21701-5012

Dear General Russell:

At the request of the Nutrition Research Division of the U.S. Army Research Institute of Environmental Medicine (USARIEM), the Food and Nutrition Board's (FNB) Committee on Military Nutrition (CMNR) of the Institute of Medicine convened at Louisiana State University's (LSU) Pennington Biomedical Research Center at Baton Rouge, Louisiana, on December 12, 1988. The purpose of this meeting was to assist the Army in reviewing and evaluating proposals for research to be conducted at the Center in order to identify those that would provide the most useful data for program planning.

The Pennington Biomedical Research Center will offer opportunities for research on nutrition as it relates to cancer and other chronic diseases, behavior, brain development, and obesity, and to findings at the molecular level. Of particular interest to the Army are issues that affect the nutritional status of Army personnel and their dependents during peacetime because of the overall interactive effects of food, diet, and nutrition on military readiness and preparedness. For this reason, the House Authorization Committee has allocated $3.5 million for the Army to fund nutrition research programs at LSU Pennington Biomedical Research Center (DOD Appropriations Bill, 1988). The Center is at a stage where development of a research agenda that matches LSU's programs with the needs of the Army is appropriate. The research will constitute only part of the research that will be conducted at the Pennington Center.

In evaluating the proposals, the committee considered whether the project could be completed within the time frame of 3 years covered by available funds, because there is no guarantee of further support after 1992. Another criterion of the committee was that the proposed project should include not only basic research but also health promotion. The committee also placed greater importance on projects related to needs with relevance to goals of the LSU's research program and the needs of the Army, e.g., the nutritional status of dependents and of combat and operational personnel. The focus of this research would be a departure from previous Department of the Army programs, which were related more to the soldier under field conditions.

In reviewing the proposals set before them by LSU, it would have been helpful if the committee had available to them a clear understanding of the research agenda within which the proposals were being presented. The committee would be reluctant to consider proposals with the absence of such a set of priorities in the future.

Following is the committee's evaluation of the research proposals presented to them and to Army personnel at the Pennington Center.

A proposal on Cardiovascular Health: Assessment and Intervention at Fort Polk was presented by Dr. Gerald Berenson from Louisiana State University Medical School, New Orleans, La. This proposal seemed to the committee to meet the criteria described above. Dr. Berenson proposes to focus on military service personnel and their families. His group will perform a health risk appraisal on these people upon entry into service at Fort Polk, monitor the effects of dietary intervention strategies, and evaluate the effectiveness of those strategies at the termination of service. The objective is to lower cardiovascular risk for both the soldier and the family. The proposed protocol is similar to the one that Dr. Berenson used in the Bogalusa Heart Study, wherein the effects of dietary intervention strategies on various health status parameters such as body weight, blood lipids, and blood pressure were measured.

The committee believes it would be useful to identify one or two health issues and concentrate on those, for example, to decrease the percentage of fat in the diet from 40% to approximately 25% or 30% of caloric intake and to achieve a 1-to-1 ratio of polyunsaturated fats to saturated fats. The committee believes that several people would need to be hired under the grant to do the analytical work, including laboratory and statistical analyses, at the Pennington Center rather than in New Orleans.

At present, the National Nutrition Monitoring System of DHHS focuses exclusively on the civilian U.S. population. This proposal would provide an excellent opportunity to do a related evaluation within the military community.

For all these reasons, the committee supports this proposal and encourages the U. S. Army Medical Research and Development Command (USAMRDC) to consider it seriously, but is concerned that the usual turnover of military personnel might present follow-up problems during the 3-year limit of the study.

The next presenter was Dr. Alfredo Lopez from LSU's Medical Center in New Orleans. The topic of his presentation was the relationship between vitamin A status and night vision. His research proposal focused on measuring the vitamin A status of the soldier either by monitoring dietary intake levels or by measuring serum levels of vitamin A. To demonstrate by intervention the relationship between increased vitamin A activity and

a decline in night blindness, however, he would need a population that evidenced vitamin A deficiency--a somewhat unlikely occurrence among the U.S. military community. Nevertheless, the committee believes that the proposed study has some relevance to military preparedness with regard to night blindness and dark adaptation. The study could be useful if improved methods to measure vitamin A status in the field would be one of the results. But overall, its relevance to the military mission appears marginal because of the relative absence of vitamin A deficiency. Thus, the committee recommends that this proposal not be considered at this time.

Dr. Marion Hegsted, Associate Professor in the Human Nutrition and Foods Section of LSU's College of Agriculture in New Orleans, talked about the association of diet with cancer, osteoporosis, preeclampsia, and body weight status in female soldiers. Dr. Hegsted also discussed the U.S. military weight standards for women. There may be some distinction between women who exercise and those who do not in relation to osteoporosis. The committee noted that many female soldiers may approach the upper limit of acceptable body weight as determined by military standards. The question arose whether overweight women would resort to bulimia in order to keep their weight down. The committee concluded that these overweight women may constitute a susceptible overweight population, but that bulimia is a complicated illness rather than just a weight control measure. The committee agreed that Dr. Hegsted's comments were meritorious, but that they should be more specifically set forth and reasonable protocols developed.

The committee noted that the Army already had a collaborative effort under way in the area of stable isotopes with Dr. Dale Schoeller, University of Chicago. Therefore, the committee agreed to defer comment on Dr. Pryor's proposal at this time.

Conclusions and Recommendation

The committee concluded that the proposal submitted by Dr. Berenson comes closest to meeting the needs and mission of Army personnel and their dependents. A similar protocol has been used in the Bogalusa Heart Study--a longitudinal study that has been in progress for more than 20 years in Bogalusa, Louisiana. The committee suggests that Dr. Berenson seriously consider the transiency of personnel when he is designing his protocol.

The remainder of the protocols presented (see titles on agenda enclosed) appear to require more thought in design as they relate to military personnel and their dependents. Although several researchers presented "pilot" designs, the descriptions were limited, their directions were not clear, and the usefulness to the Army questionable at this time. Dr. Chandan Prasad of LSU's Medical Center was not able to present his proposal on Dietary Modulation of Mental Performance and Associated

Neurochemical Indices to the committee at this meeting. The committee believes that this proposal may have direct application for military personnel because of its potential significance to cognitive behavior and military preparedness.

The committee sees many interesting opportunities for the Pennington Center to become a center of research supporting military personnel and their dependents, but does not recommend that the program should in any way be intended as a replacement for the excellent ongoing program at USARIEM. For example, the committee suggests that the Pennington Center consider establishing a research laboratory where analytical research can be carried out that can provide nutritional assessment support to the program at Natick. The Pennington facility could be an important adjunct to long-term nutrition research efforts of the military.

Sincerely,

Robert Nesheim, Ph.D.
Chairman
Committee on Military
Nutrition

Enclosures

cc: S. Thier
S. Palmer
D. Schnakenberg
E. Askew
S. Berkow

Letter Report:

Research Progress Review of the

Pennington Biomedical Research Center

May 1992

INSTITUTE OF MEDICINE
NATIONAL ACADEMY OF SCIENCES
2101 CONSTITUTION AVENUE WASHINGTON, D.C. 20418

FOOD AND NUTRITION BOARD

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May 15, 1992

Major General Richard T. Travis
Commanding General
U.S. Army Medical Research and Development Command
Fort Detrick
Frederick, MD 21702-5012

Dear General Travis:

At the specific request of the COL Eldon W. Askew, Ph.D., Chief, Military Nutrition Division, U.S. Army Research Institute of Environmental Medicine (USARIEM) who is Grant Officer Representative of the US Army Medical Research and Development Command (USAMRDC) for Grant no. DAMD17-86-G-6036 to the National Academy of Sciences for support of the Food and Nutrition Board's (FNB) Committee on Military Nutrition Research (CMNR), the CMNR met at the Pennington Biomedical Research Center in Baton Rouge, Louisiana on Sept. 19-20, 1991. The purpose of this meeting was to assist the Army in reviewing and evaluating the progress on work related to the USAMRDC Grant no. 17-88-Z-8023: "Effect of Food, Diet, and Nutrition on Military Readiness and Preparedness of Army Personnel and Dependents in a Peacetime Environment".

This Grant was established to implement this program for which funds were specifically allocated through the House Authorization Committee (DOD Appropriations Bill, 1988). An important consideration in the initiation of the Army funded program was that these funds were allocated for a 3 year period and the Pennington Center was not yet staffed or equipped. Initial proposals for one of these funds were developed by Louisiana State University (LSU) largely drawing on the interests and personnel available from the LSU Medical Center. As discussed in the letter report dated June 26, 1989, to Major General Philip K. Russell (see attachments), the Committee reviewed these proposals

which were quite preliminary in nature. The proposal by Dr. Gerald Berenson came closest to meeting the criteria established in the Appropriations Bill. The Committee also recognized the value of establishing a research laboratory which could provide analytical support to the nutritional assessment program conducted by the Nutrition Research Group at Natick Laboratories.

The Committee on Military Nutrition Research's role in this preliminary review was to assist the Army with identifying research activities that fell within the mandate of the appropriation with the responsibility for the final decisions in program and funding with the Army.

Prior to assembling at the Pennington Biomedical Research Center, the CMNR reviewed: 1) an information paper and background materials, including the Grant Statement of Work, provided by COL Askew, the Grant Officer Representative; 2) the Final Report on USAMRDC Grant to the Pennington Center submitted by the principal investigator, Donna H. Ryan, M.D.; and 3) an earlier report prepared by the CMNR at the request of the USAMRDC reviewing this same research program in 1989. Copies of the information papers, the 1989 report from the CMNR, plus the meeting agenda and list of participants are attached.

On September 19, 1991 the CMNR convened at the Pennington Biomedical Research Center (PBRC) and heard presentations of the research accomplishments during the grant period from the Center staff and a statement from COL Askew. On September 20, 1991 the Committee met in executive session and reviewed the accomplishments of the Pennington Biomedical Research Center over the grant period in relation to the grant Statement of Work, the goals of the principal investigator, and their own previous recommendations. To provide supplemental expertise to the Committee membership in the area of neurotransmitters, the CMNR also submitted a copy of that part of the annual report of the Pennington Biomedical Research Center grant dealing with Project No. 3, Diet, Neurotransmitters, and Behavior, to two scientists currently working in this research area for confidential review. The Committee included the review of this outside team in their deliberations when writing this report. All CMNR members present at the meeting have seen and approved the report. Subsequent to approval of the final draft by the Committee, in accordance with National Research Council guidelines, this report was reviewed in confidence by a separate anonymous scientific review group. The Committee and advisors have reviewed the anonymous comments of this review panel and incorporated their suggestions where appropriate. Staff has then written a letter of response to the reviewers with the final report draft and obtained final approval of the report from the review panel. This report is

thus a thoughtfully developed presentation that incorporates the scientific opinion of the CMNR, and the anonymous National Research Council reviewers.

Following is the Committee on Military Nutrition's evaluation of the research program presented to them and to Army personnel at the Pennington Biomedical Research Center.

General Comments

The Pennington Biomedical Research Center is a very impressive facility having an excellent physical plant for laboratory and clinical research. Considerable progress has been achieved in staffing and development of research activities since the CMNR's last visit on December 12, 1988. This has been made possible by financial support from the U.S. Army, USDA, and grants from NIH and other sources. In addition, the state of Louisiana has provided ongoing support at a level of $4.1 million dollars.

It is worthy of note that the new director for the PBRC, George A. Bray, M.D., has been appointed since the Committee's last visit. Dr. Bray, who is internationally renowned for his research in the fields of obesity and energy metabolism, has provided an important vision for the Pennington Center (which he is moving rapidly to bring to fruition). The progress noted builds effectively on the initial framework established by Dr. Allen Copping, President of the Louisiana State University (LSU) system, and on the ongoing administrative support of Donna H. Ryan, M.D., Project Director for the Military Nutrition Grant.

In general the Committee found that there was effective management support and guidance for the development of activities related to this grant. The progress in each project area was reviewed by the Committee and its assessment follows.

Specific Project Reviews

Project No. 1: Clinical Research Lab. This project is headed by Richard Tully, Ph.D. The major objective of this project was to provide biochemical assessment of nutrition status and to perform food biochemistry analysis. Significant progress has been made in securing necessary analytical equipment, implementing appropriate analytical procedures and most

importantly, in implementing a sound quality assurance program. Dr. Tully has made significant progress in activating an effective clinical laboratory in a short period of time and in utilizing this facility to support requests from USARIEM. It should be noted that all of this has been accomplished with limited staff support.

The Committee is of the opinion that the Clinical Research Lab is a valuable resource to the Pennington Biomedical Research Center as well as being extremely valuable to USARIEM. The nutrition group at USARIEM has previously experienced difficulty in obtaining accurate and timely analytical information from outside contract laboratories. The ability to obtain important analytical data on military nutrition research projects in a timely manner greatly enhances the effectiveness of the nutrition research program. We recommend that the U.S. Army continue to provide partial support for this activity with the understanding that this resource be available on a priority basis to support U.S. Army studies. Further, the Committee supports the provision of additional resources to increase staffing of the Clinical Research Lab.

The staff of the Pennington Center has indicated a desire to develop a food analysis capability. The Committee recognizes the need for food analysis to support the clinical studies which the Center anticipates undertaking. In order to develop this capability effectively it is important to add an experienced food chemist to the Pennington Center staff. A major food analysis program would consume considerable resources both for methods development and actual analysis of various food components. Further, the undertaking of food analysis will require significant equipment additions and the staff should make judicious decisions regarding what analyses need to be performed beyond proximate analysis and inorganic elements. The CMNR believes that the breadth of activity necessary to establish a high quality food analysis laboratory would involve significantly more expertise, resources (equipment, personnel, and supplies), and facilities than is currently projected at the Pennington Biomedical Research Center. The Committee therefore holds that limited analysis on foods, related specifically to electrolyte balance, may be more within the scope of the laboratory's capabilities.

Project No. 2: Stable Isotope Lab. This project is directed by James P. DeLany, Ph.D. who has a good background in the use of stable isotopes to measure energy expenditure and body composition. The stable isotope technique provides a unique approach for use in free-living subjects since it is non-invasive and nondestructive. Consequently, it provides an ideal means of assessing important endpoints in experimentation valuable to the military.

The equipment that has been purchased and installed is state-of-the-art allowing Dr. DeLany to establish his methods and rapidly gear up his laboratory to support multiple studies. The Committee was favorably impressed with the quality and quantity of work completed thus far. In view of the expanding nature of military research projects which utilize stable isotopes in their protocols, the Committee recommends continued funding of the Stable Isotope Laboratory for priority support of military studies.

The staff of the Pennington Center have indicated a desire to increase the staffing of the laboratory by one additional Ph.D. scientist. In view of the importance of this methodology, the Committee would encourage such an addition if possible.

The availability of stable isotopes required for this work is currently limited and could curtail the ability to adequately support this area of research by the military as well as other investigations. The CMNR recommends that the military encourage the development of an adequate supply of the necessary stable isotope through combined efforts of the federal research establishment.

Project No. 3: Diet, Neurotransmitters and Behavior. This project is directed by Chandon Prasad, Ph.D. and has been staffed during the project period with five additional scientists on full or part time basis. In addition nine students have participated part time over the project period. The efforts to date have been devoted to developing the methodology for studying the effect of diet on behavior in animal models.

The CMNR believes that the area of nutrition and behavior is of military relevance, but the current research effort lacks focus and appears to have limited applicability to military concerns. The Committee is of the opinion that there is a need to further explore appropriate, relevant areas of research at the physiological and cellular level that are pertinent to military applications. This would require a reorientation of the current effort with considerably greater focus. It is suggested that the researchers develop more specific hypotheses which then can be investigated to better target the projects and to better determine the relevance to the military. The Committee notes that 25 percent of the funding provided by the Army has been in support of the research program of Dr. Prasad. The military has a major interest in the potential influence of nutrition on behavior particularly in those areas that may improve or maintain cognitive performance under combat stress. With the increasing sophistication of weapons systems there is a need to increase the capability of the individual to maintain mental acuity to function with these systems.

The lack of focus can be illustrated by listing the titles of the 15 projects reviewed. These were: 1) Behavioral neurochemistry of food-derived peptides; 2) Cyclo (His-Pro) and food intake; 3) Determination of tryptophan metabolites using HPLC; 4) Preparation and characterization of dopamine (D_2) receptor protein antibody; 5) Determination of dopamine (D_2) receptor messenger RNA expression; 6) Dopamine (D_2) receptor protein antibody mapping in the rat brain; 7) Dietary protein and behavior in rats; 8) Levels of dietary protein and modification of behavioral responses to CNS acting drugs; 9) Dietary protein and dopamine receptor regulation; 10) Effects of dietary protein on monoamines and monoamine metabolites; 11) Dietary protein and preparatory arousal in rats; 12) Dietary protein and neuronal plasticity; 13) Dietary protein and microtubule-associated proteins; 14) Dietary protein and brain amino acid profiles; and 15) Diet and stress.

Many of the studies involved the effect of dietary protein in brain chemistry neuronal structure and behavior. Most of these studies involved feeding rats diets up to 50% protein. In view of the vast literature involving studies of dietary protein in brain development and behavior in rats, the value of still more rat studies to the military has not been justified. In particular, the use of diets supplying 50% casein is questionable and of little relevance to human feeding in or out of the military. Several of the projects appear to be "fishing expeditions".

It is important that the research conducted under this program be well focused in order that its relevance can be evaluated both in long term and in applications to the near term. The CMNR recommends that a special site review be conducted in which efforts are undertaken to delineate major Army needs and review the Pennington Biomedical Research Center's program in light of responding to those needs with highly focused research. It is recommended that the site review team be composed of individuals who work directly in the area of nutrition, cognition, and behavior with expertise in the field of neurotransmitters.

Project No. 4: Fort Polk Study. The director of this study is Gerald S. Berenson, M.D. who initiated and developed the Bogalusa Heart Study. The project was completed in August 1991. The objective, as presented to the CMNR, was under the general title "Health Promotion Research and Assessment", and is "Assessment of Nutritional Status and Cardiovascular Risk of Military Dependents." While the Committee did not necessarily give this the highest priority rating in 1989, it was a project that could be implemented immediately. The study has achieved the objective of doing an nutritional/health risk appraisal of military dependents.

The second component of the Fort Polk Study was the development of a health promotion/education program for military families. It was unfortunate that the study did not have a larger sample size (n = 70 families) in the three cycles of families involved in the health promotion/education component. In addition, there was not a control group established for this component of the project. The CMNR also noted that the project report did not include any evaluation of the effectiveness of the program either on a short-term or long-term basis. For example, there was no measurement of changes from baseline measurements in behavior or other status indices.

It is the understanding of CMNR that this project has been completed, and future funding is not planned under this program. The CMNR would concur with this position. In the event that future plans might evolve to include implementation of such a health promotion program for military dependents, it is the position of the CMNR that a thorough review of the results of this study and delineation of desired objectives, including inclusion of methodology to evaluate long-term outcomes, should be conducted prior to implementation.

Project No. 5: U.S. Army Menu Modification Project. This project has been carried out by Evelina W. Cross, Ph.D. and Catherine Champagne, Ph.D. The results presented at the CMNR review were very preliminary, and the research team has been granted a no-cost extension to complete the requirements of the contract.

It is the consensus of the CMNR that the investigators were not sensitive to the needs of military garrison feeding program as demonstrated by the preliminary menus provided at the review. The project did not demonstrate any application of menu planning guidelines that would be appropriate in the military menu system, in terms of cost, acceptability, color, etc. Their first phase of menu modification did not meet the objectives of the project; the second phase brought fat down from 40 to 36% (not 30%), but did not appreciably reduce sodium or cholesterol (except when substitutions were made for breakfast eggs). The menus developed initially decreased caloric intake from 3,500 to 3,030 kcal. This lowered caloric intake might be considered a problem in some garrison situations. The menus developed to date and presented to the CMNR did not address cost, appearance, national food preferences, or relevance to the military feeding system.

The CMNR also questions whether the evaluation procedures used (college students consuming a meal as opposed to sensory evaluation panels, etc.) were applicable to the eventual user. Although, the project was incomplete when reviewed, the CMNR was not impressed with some of the

approaches taken. The Committee further viewed the lack of interaction between the menu developers and the military menu system as a serious constraint on the ability of the investigators to achieve their objectives.

Therefore the Committee believes that this project, if continued, should be conducted in a military facility where the staff is more familiar with the military menu and procurement systems in order for a practical program to be developed.

Overall Conclusions and Recommendations

Generally, the Committee was impressed with the quality of the research activities at the Pennington Biomedical Research Center given the constraints of essentially starting from a zero base in equipping the facilities, recruiting staff, and initiating research activities, and felt that the funds provided by the U.S. Army grant had been effectively deployed. The CMNR would encourage continued financial support by the U.S. Army of those activities which have been and can continue to be relevant to the military namely the Clinical Research Laboratory and the stable isotope activity. Further, support of the area of nutrition and behavior should continue with attention to developing a project with greater focus and hence military relevance.

Sincerely,

Robert O. Nesheim, Ph.D.
Chairman, Committee on Military
Nutrition Research (CMNR)

Enclosures

cc: K. Shine
C. Woteki
D. Schnakenberg
E. Askew
B. Marriott

Letter Report:

Review of Three Research Proposals

from the

Pennington Biomedical Research Center

December 1992

INSTITUTE OF MEDICINE
NATIONAL ACADEMY OF SCIENCES
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FOOD AND NUTRITION BOARD

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December 10, 1992

Major General Richard T. Travis
Commanding General
U.S. Army Medical Research and Development Command
Fort Detrick
Frederick, MD 21702-5012

Dear General Travis:

At the specific request of the COL Eldon W. Askew, Ph.D., Chief, Military Nutrition Division, U.S. Army Research Institute of Environmental Medicine (USARIEM) who is Grant Officer Representative of the U.S. Army Medical Research and Development Command (USAMRDC) for Grant no. DAMD17-92-J-2003 to the National Academy of Sciences for support of the Food and Nutrition Board's (FNB) Committee on Military Nutrition Research (CMNR), members of the CMNR met at the Pennington Biomedical Research Center (PBRC) in Baton Rouge, Louisiana on June 3, 1992. The purpose of this meeting was to assist the Army in discussing plans for three projects that were proposed as part of USAMRDC Grant no. 17-92-V-2009 to the PBRC: "Military Nutrition Research: Six Tasks to Address Medical Factors Limiting Soldier Effectiveness."

This grant to the PBRC was established to implement a program for which funds were specifically appropriated through the Department of Defense Appropriations Bill, 1988. The CMNR has on two previous occasions reviewed the work related to this program of research at the PBRC and submitted letter reports with recommendations. The Committee on Military Nutrition Research's role at the meeting on June 3, 1992 was to assist the Army with identifying research activities that fell within the mandate of the appropriation. The responsibility for the final decisions in program remains with the Army. For this visit, the CMNR was asked to focus its attention on projects in the areas of neuroscience and menu modification.

Prior to the meeting, the CMNR reviewed 1) preliminary research proposals prepared by the scientific staff and principal investigator, Dr. Donna Ryan; 2) an information paper and background materials, including the Grant Statement of Work, previously provided by COL Askew; 3) the final report on the previous USAMRDC Grant to the Pennington Center submitted by Dr. Ryan; and 4) two earlier reports prepared by the CMNR at the request of the USAMRDC reviewing this same research program in 1989 and 1992. Copies of the meeting agenda and list of participants are attached (see attachment A).

On June 3, 1992 the CMNR convened at the Pennington Biomedical Research Center (PBRC) where they heard presentations and discussed the program orientations, goals, and preliminary research plans for three projects: basic neuroscience, clinical neuroscience, and menu modification. The role of the CMNR representatives at this meeting was as individuals participating in a discussion of scientific research directions. Since the committee is not in the position of giving real time advice, any comments made by the members of the group were not to be construed as recommendations from the committee.

Following the meeting, PBRC scientists prepared formal proposals describing their goals and research plans. The proposals were received by the committee on July 17, 1992 and the CMNR discussed the materials and drafted their report. The report was then reviewed in accordance with National Research Council (NRC) guidelines by a separate anonymous scientific review panel. This report, based in part on discussions from the meeting, review of formal proposals later developed by the scientists at the PBRC, and on executive session discussions by the committee, is a thoughtfully developed presentation incorporating the scientific opinion of the CMNR and comments of an anonymous peer review committee of the NRC.

Following is the Committee on Military Nutrition's evaluation of the research proposals as submitted (see attachment B in original report).

OVERALL COMMENTS

The CMNR continues to be impressed with the rapid expansion and development of the facilities and staff of the Pennington Biomedical Research Center. In only nine months since their last visit to the PBRC, significant new facilities have become available through the opening of the clinical research section and additional new laboratories. The leadership of Dr. George Bray and

the important contributions of Dr. Donna Ryan to the overall program development on the U.S. Army grant were evident. The Pennington Biomedical Research Center provides an excellent environment for scientific study as well as superb facilities for research support services needed by the Army research programs. Now the challenge is to concentrate efforts in areas in which the PBRC can make a unique contribution to USARIEM's overall research effort, and to foster collaboration with other USARIEM research groups in similar areas.

The CMNR is concerned, however, after reviewing the protocols for the projects proposed by the PBRC, with what appears to be a lack of focus on the nutritional relevance of the projects to the military. The committee is also concerned about the lack of specific details in the protocols including the variables to be tested in most of the studies. There is also a concern that the complexity and practicality of conducting nutritional trials in people may not be adequately appreciated by the project leaders. While the committee realizes that the project proposals were not written as grant requests, it believes that the objectives of the projects should be clear and the protocols sufficiently delineated to clearly define the proposed work.

SPECIFIC PROJECT REVIEWS

Project No. 3: Nutritional Neurosciences Basic Science Laboratory

Project Summary

The purpose of project #3 is to investigate the mechanisms involved in REM (rapid eye movement, sleep phase) deprivation-induced cognitive dysfunction in the rat. Rats subjected to REM deprivation for 96 hours will be tested using behavioral, neurophysiological, and biochemical measurements to characterize the effects of sleep deprivation. Nutritional manipulations will be introduced after the sleep deprivation model is well characterized. These studies will determine if nutritional manipulations can sustain performance under conditions of REM deprivation in rodents.

General Comments

Sleep (specifically, rapid eye movement [REM] sleep phase) deprivation, and its direct and indirect functional consequences, is a common and significant stressor facing a large number of military personnel and is therefore

an appropriate focus for investigation of dietary effects under this grant. A basic and central question is whether the program will focus on chronic changes in nutrition which might "protect" subjects from the negative consequences of sleep deprivation and other relevant stressors, or on acute changes in the diet (i.e., single meal or short-term supplementation). It is of fundamental importance to decide whether the focus of the proposed research will be an acute or chronic nutritional manipulation when developing the protocols for this research. It is suggested that the investigators refer to the many human and animal studies, for example those involving tryptophan and tyrosine supplementation, in planning and in setting priorities among the specific project protocols. A related issue is whether neurotransmitter precursor supplementation will be at physiologic or pharmacologic levels. In addition, while there is an understandable need for initial method development and refinement, the program seems to overemphasize procedural development to the detriment of dietary studies (at least as indicated by the written proposal and on-site discussions).

Notwithstanding, because the basic approach and methods are sound and the available staff and physical resources are adequate, there is a high probability that the program will meet its stated objectives. The neurochemical, histologic, electrophysiologic, and performance measures selected to assess dietary effects on sleep deprivation are appropriate and well-founded in the literature. Responses to comments made by the CMNR during the June visit adequately addressed the basic concerns of the members.

Specific comments, concerns and questions

1. The CMNR is pleased to learn that scientific staff of this project from the PBRC will be meeting with staff of the sleep research unit at the Walter Reed Army Institute of Research to discuss research plans and to develop a dialogue for future interaction. This is of primary importance in order to plan protocols that build on prior relevant research.

2. The committee voiced disappointment that no nutritional relevance was described in the basic project that is titled "nutritional neurosciences." It seems appropriate to expect that the basic studies laboratory should be testing nutritional hypotheses that relate to the clinical studies aspects of the same overall research program. Plans for dietary manipulations, including neurotransmitter precursor supplementation, are poorly described. The protocols for this research should relate to nutritional objectives with the plans for dietary manipulations, including

neurotransmitter precursor supplementation, clearly described. Ongoing advice from a nutritional scientist with expertise in dietary factors that alter behavior and neurotransmitter levels would assist in these efforts.

3. The number of animals proposed per group (8), although probably adequate to determine neurochemical, histologic, and electrophysiologic effects, may be insufficient to reveal proposed behavioral effects (e.g., changes in shuttle box performance).

Recommendations

- The goals of the project should be specifically detailed, clearly related to the nutrition objectives outlined by the Army, and cognizant of the many human studies dealing with nutritional entities or sleep and performance. Also, in the design it should be decided whether the emphasis will be on acute or chronic manipulations in developing the research protocols.

- The CMNR recommends that there be a major emphasis placed on increasing communication between the Basic and Clinical neuroscience groups at PBRC. This aspect of the program is necessary for integration of the research outcomes. There was some feeling among the committee membership that this proposal as presented, did not indicate enough ties to the clinical program and remains not particularly relevant to the military needs. Unless these ties become more evident, the project appears to be more appropriate for a standard individual proposal in a competitive grant arena rather than the present program.

- The research team should seek ongoing advice to strengthen the weak nutritional aspects of their experimental designs from nutritional scientists with expertise on dietary factors that alter behavior and neurotransmitter levels.

- Throughout the project the researchers should remain alert to unexpected changes in subject behavior and performance, as well as other functional effects, and have sufficient flexibility built into their program to pursue these effects experimentally.

- The inclusion of female animals as proposed, is important in future studies.

- The number of animals should be doubled when assessing behavior. In addition, a minimum of 15 animals per group is needed for the micropunch technique.

Project No. 4: Nutritional Neurosciences Clinical Studies

Project Summary

Project #4 involves human clinical studies of sleep deprivation with a mixed inpatient-outpatient design for a 12-day study. Measures of sleep efficiency, sleep stages, sleep onset, and sleep latency periods, neuropsychological tests, attention-demanding cognitive tests, neuroendocrine and immune function testing, and evaluation of the autonomic nervous system are planned. A listing is provided of potential neurotransmitter precursors that may be selected as nutrient loadings to assess their effectiveness in altering the effects of sleep deprivation. The authors state that several details that are missing from the research design will be determined through the planned pilot studies.

General Comments

The above comments regarding the issues of chronic versus acute dietary intervention and physiologic versus pharmacologic doses also apply to this program. Review of the written proposal and on-site discussion indicate that the scientific team has a thorough grasp of neurosciences literature on relevant concepts, methods, and measures. Impressively, the researchers recognize the "Catch-22" inherent in studies of factors which, on the one hand may potentially remedy sleep problems that directly and indirectly result in performance decrements, while on the other hand simultaneously would also likely interfere with attentional processes required for optimal performance during waking. Although there is no current solution to this issue, it is an important one to consider during data interpretation.

More details on the specific protocols are required before a thorough evaluation can be made. At minimum, it is necessary to discriminate among the "shopping list" of "dietary additives" that may be used. It is difficult to understand why the investigators cannot come forth with a short list of nutrients to be tested, and make reasonable judgement calls on dosages, duration of administration, and the timing as related to meals, sleep, stress, etc. In addition, if there is to be any hope of providing nutritional insight, vigorous

efforts must be made to standardize the nutritional behavior of the subjects prior to initiating the protocol.

Little information is provided in the proposal regarding the mechanism of administration for the dietary additives, the amounts to be initially tried, etc., even though a number of possible substances are discussed. For a dietary study, one would expect a great deal more information regarding how the diet will be designed. Even Pilot Study II, which is designed to try out the "nutritional loading" to ascertain whether the protocol is appropriate, does not provide information regarding how the loading will be accomplished. It is obvious that the investigators purposely were trying not to be too specific, but someone needs to decide what substances and or modifications, or both, will be tried.

Specific comments, concerns and questions

1. The addition of a nutrition research scientist trained in conducting human dietary studies to the project team would provide needed expertise in dietary design and subject management.

2. The specific protocols should be better described and more relevant to the clinical objectives of the Army.

3. Manipulating caffeine increases the ecological validity of the results, but introduces the issue of "dietary" versus "nutrient component " intervention. Which, if not both, will be the focus of this program?

4. The investigators refer to the project as a "nutritional" study but, as pointed out, they intend to study substances found in foods at greatly increased levels. Rather than refer to these as dietary "additives," a more conventional term used by the regulatory agencies, industry, and the Congress would be dietary "supplements" if consumed as a tablet, capsule, or liquid, or a "food additive" when added to a food. If the expressed purpose is truly a pharmacological effect, then by law the term used should be "drug" regardless of how it is administered. The confusion over terminology and its ramifications further underscores the need for clarification of this aspect of the protocol.

5. Separating the stresses of caloric deprivation and sleep deprivation may be important. Previous research has shown that weight loss in obese humans is accompanied by marked increases in awakenings, that is, sleep

disturbances. While these findings are not directly relevant to the issue of the effects of sleep deprivation on military performance, they indicate that even short sleep deprivation periods may affect food intake and thus also neurotransmitter levels. In this project, therefore, how will food intake be kept constant to account for issues such as these?

6. The information provided does not indicate if Pilot Study II will test six different dietary additives (based on six subjects) or try one additive on the six subjects for a varying length of time. Since it appears that these will be outpatient studies, how will the investigators "...establish the time required to reach an effective level...?" It would seem that some of the blood work and sleep deprivation would have to be measured periodically as the load was titrated with subject response.

7. If automated (i.e., computerized) assessment of cognitive performance will be employed, it is imperative that subjects be thoroughly familiarized with the equipment and procedures prior to collection of critical data.

8. Instructions for the various cognitive test for the subjects were not addressed in the proposal. Clearly worded instructions are critical to avoid the confounding of speed-accuracy trade-offs frequently made when subjects try to compensate for stress effects.

9. The researchers might consider including a comparison of 1st, 2nd, and 3rd NREM period durations because several studies have shown a disruption of the predictable decline in NREM stage duration over the course of the night.

10. Problems with variability may be encountered when trying to determine urinary catecholamines using 8 or even 12 hour pools; 24-72 hour pools are often needed to reduce variability sufficiently to detect changes in urinary catecholamines.

11. In initially reading the proposal, one might assume that the pilot studies referenced in the first section would be in-house studies, with the larger community study planned as an outpatient study. Given the necessity to develop the methodology (Pilot Study I) and the level of dietary loading needed (Pilot Study II), it would seem extremely difficult to conduct these studies and obtain usable results from them on an outpatient basis. As an example, the number of venipunctures required within the minimum (7 days) or maximum (9 days) protocol length appears to be 56 (14 per day x 4 days during the protocol). This indicates the need for the use of

catheters for the repeated blood draws. Multiple timed urine collections will also be necessary. This really cannot be done on an outpatient basis. It is expected that subjects may try to sleep more (even if instructed not to) during the loading phase in preparation for the sleep deprivation days. Since the investigators are not controlling the subjects' activities or schedules, it seems particularly important, at least in the pilot studies, to be able to control all the variables that may be associated with the response.

12. Will subjects be supine for blood drawings collected for cortisol and catecholamines? Again, very high variability in catecholamine levels can be expected if subjects are not at rest for 15-30 minutes prior to sampling.

13. Despite the questionable reliability of dietary histories and food diaries to assess typical intakes, some such measure should be included to screen for atypical diet histories in potential subjects.

14. Will potential subjects be members of the military or matched to military personnel? It would be more helpful if military personnel were available to serve as subjects at the Pennington Center.

15. It is of concern that the investigators did not consider the venipuncture frequency or the amount of blood drawn as a risk to the subject. No mention was made of human subject panel review procedures, but it is assumed these will meet both LSU and U.S. Army standards and include full informed consent of all participants.

Recommendations

- Prior to initiation of either pilot study, a detailed protocol should be developed that clearly indicates how the concerns described in this review have been addressed. This protocol should include the diet, the specific dietary additives or manipulations that are to be tested, along with the method of administration, subject management (including characteristics that will preclude subject participation), methods for collection of physiological fluids, sleep deprivation routine, and test and performance measures. The protocols should reflect the clinical objectives of the Army.

- Ongoing participation of a nutritional scientist familiar with human dietary studies will provide needed expertise in protocol development and subject management.

- The stress of the repeated blood drawing should be evaluated (assuming that 14 venipunctures per day is what is actually planned), since no mention is made of cannulating the subjects.

- Any possible reactions to some of the neurotransmitter precursors (glutamine, etc.) must be discussed in advance with the subjects.

- It is difficult to understand how this project can be conducted on an outpatient basis and still maintain the level of control needed for data interpretation. It is recommended that serious consideration be given to having the subjects remain at the research facilities. This is routinely done in other research laboratories.

- Pilot studies will need to determine not only loading requirements but also cognitive and neuropsychologic tasks that are sensitive to sleep deprivation yet unaffected by repeated performance/measurement.

- Auditory noise is recommended as an excellent distractor in attention tasks because its parameters are easily controlled and quantified and it is easily administered. In addition, it is a relatively innocuous stimulus that is accepted well by almost all subjects and it is representative of real-world distractors.

- Addressing the neuroendocrine system is important because it may help determine whether stressors alter the metabolism of nutrients and whether nutrients alter the physiologic response to stressors.

- Smoking and exercise habits of potential subjects should be considered as screening factors because of their effects on food intake and sleep behavior.

- Careful consideration should be given to the issue of whether task/test administration is varied from day-to-day or kept constant. Fatigue and reduced motivation are likely given the large number of tests proposed.

- Throughout the project the researchers should remain alert to unexpected changes in subject behavior and performance, as well as other functional effects, and have sufficient flexibility built into their program to pursue these effects experimentally.

- The inclusion of female subjects is important in future studies.

Project No. 5: Menu Modification Project

Project Summary

The Menu Modification Project has evolved to include changes that address some of the concerns expressed in previous discussions. Specifically, during Phase II of the project, modified recipes will be substituted in the standard menu, and acceptability testing will be conducted. These modified recipes will be lower in fat, cholesterol, and sodium. Phase III will include a week in which modified menus will be served and a week of standard menus. Food acceptability will be assessed using computerized score sheets to be handed out at each meal during Phase III. Additionally, during Phase III, surveys regarding nutrition knowledge, practices, etc. will be administered by graduate students. The proposal calls for developing modified menus and testing their acceptability in a "real" situation at Fort Polk. Acceptability will be ascertained by means of a simple 9-point hedonic rating.

General Comments

The key to success of this project is the ability to develop acceptable menu items that achieve the stated objectives. The specific menu items to be altered and the ingredient targets for change are not clear. It appears that this will be a serendipitous process. There is no indication of any study of the major contributors of fat, saturated fat, cholesterol, or sodium in current menus. Such information would permit a better defined approach to menu modifications. For example, it is predictable that eggs are the major contributor to cholesterol intake, therefore, it is reasonable to suggest that removing eggs from the menu by providing alternative/substitute menu items would lower cholesterol intake. Further, since eggs are generally consumed at breakfast it is likely that the major impact would be at that meal. Indeed, this was the reported observation.

It would seem that a project such as this could benefit from a computer analysis of menus and the food menu item contribution of fat, saturated fatty acids, cholesterol, and sodium. The major contributors could then be targeted for modifications. Better computer modeling would provide options for selecting alternative means for evaluating the dietary plans.

The collection of consumption information will be critical in assessing whether compensation for the fat reduction occurs. The proposal indicates that this will be monitored by USARIEM personnel but few details are provided.

The importance of this information should be stressed and every effort made to insure adequacy of these data.

It is essential that closer linkages be developed with Army experts who are in charge of the Master Menu and food service facilities. Without review and critique of ideas earlier in the menu item development process, little that is truly useful for the special circumstances under which Army food services must operate ~~is likely to be~~ accomplished. For example, Army food services must operate within strict financial allocations. Many of the suggested changes would likely be hard to accommodate within current allocations. Furthermore, the basic premise that foods that are developed and taste-tested by college students at Pennington are going to be exportable to the U.S. Army is of doubtful validity. Since all of the proposal is based on that premise, very serious consideration must be given to the whole concept of the project.

The proposed add-on graduate research projects seem of low priority. If these tasks interfere in any way with the major objectives of the project they should be dropped.

Specific comments, concerns, or questions

1. It is unclear whether modified menu <u>items</u> will be substituted in the standard menu in Phase II or modified menu <u>days</u> will be substituted.

2. It is unclear whether the acceptability testing during Phase II will be only on the modified menu items (or days depending on the answer to #1) or also on the standard menu items for which they are substituted (such as low fat biscuits versus standard recipe biscuits, etc.). Such a comparison would provide a control data base, and prevent respondents from identifying the new items as "different", which may bias their future thoughts on the items.

3. In Phase III, it appears that one week of standard menus will be followed by the week of modified menus. (Or, will the seven days of each set be intermingled with daily testing conducted?) It is important to make sure that the same individuals (to the extent possible) participate in both standard and modified menu acceptance testing. With schedule changes for personnel, might there be a different group eating in the garrison the first week versus the second week? If so, it would be necessary to intermingle the modified days with the standard days.

4. Will the graduate student surveys be administered only during the week of modified menus, or will they be administered randomly between both weeks? The presence of the graduate student surveys may affect (at least temporarily) the respondents' food selection patterns, thus it would be important to make sure that questions were asked during both the modified and standard meals.

5. No research design is provided for either part of Phase III. What data are to be collected? How are interfering variables to be controlled? How are the data to be analyzed?

6. The evaluation form provided is not adequate to determine acceptability. Also, why does the score sheet to be given to the troops list a "Code" for the menu item? This could surely be accomplished in another less obvious way.

7. In addition, the acceptability trial is too simplistic. One overall scan for a menu item is not adequate. Questions need to be asked that reflect various aspects of preference, not just a single overall score.

8. Sensory testing results need to be compared with acceptability scores versus actual ingestion. It is important to remember that highly preferred foods (such as desserts) are not necessarily eaten frequently, while moderately preferred foods, for example, bread, are eaten daily.

9. Clear mention and delineation of important principles of menu development including such factors as variety, flavor, color, texture, etc. need to be included in the project objectives and project plans.

10. The issue of ethnic food preferences must be addressed in the project plan.

11. It is suggested that an Advisory Committee of NCO's be established at Fort Polk to advise the project staff. This committee would be analogous to the use of student advisory groups in school feeding programs.

12. As a result of the travel distances and time involved, the use of graduate students to collect data at Fort Polk is very inefficient and may be costly. There may be experienced dietitians, as dependents at Fort Polk or its environs, who would be interested in working on this project. This alternative at least should be explored.

13. The nutrition knowledge and nutrition education components are not relevant to the basic mission of this particular project and should receive lowest priority.

14. The committee is not enthusiastic about ancillary graduate student projects in the area of nutrition education. There is considerable likelihood that such activities will be disruptive and are not likely to yield information of consequence. Unless a much more persuasive argument can be put forth the ancillary graduate student research projects should not be undertaken.

15. The committee does not see enough evidence of the kind of expertise needed for this project in the two curriculum vitae that accompanied the proposals. The lead investigator has good expertise with data bases, but has little experience with large scale food services. The chef is dedicated but also an individual with little experience in very large scale food service with tight cost and ingredient constraints. Someone who had formerly been involved in Army food service would bring more realistic perspectives to the task. As a result, the investigators should lean very heavily on the expertise of personnel involved with Army food service.

Recommendations

- The specific objectives of the project need to be written down so the results of the project can be evaluated, for example, targets need to be delineated for fat, cholesterol, and salt reduction, acceptability level, nutrient composition (including the relationship to the current Military Recommended Dietary Allowances [MRDAs], etc.

- A systematic approach to the menu modification project should be carefully described, for example,

  a) initially a schedule of interactions should be outlined that will take place with Army menu planners to ensure that the project investigators fully understand the menu development process, cost restrictions, etc., and to insure relevance of the planned project to future use in Army menu development;

  b) extensive computer modeling should be planned that is based on interaction as suggested in (a) to identify the best opportunities to modify menus to meet objectives as established in the first recommendation stated above;

c) initial acceptance criteria for modified items need to be clearly developed; and

d) a detailed plan for adequate testing in an adequate military setting must be established.

- Plans for familiarization with actual preparation capability in the Army menu system must be made to obtain a clear understanding of the actual sites and circumstances under which meals are prepared in the Army, more understanding of the preferences of personnel, and more understanding of the Master Menu and other procedures.

- The investigators need a more intimate and comprehensive working relationship with USARIEM.

- Staff on the project should include those who have expertise and credentials in large scale menu planning, and previous experience working with the Army food services.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

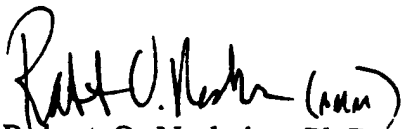
The first two programs in particular address high priority research questions, employ creative yet sound experimental methodologies, and have the potential to yield unique and important insights for the broader question concerning the relationship between diet/nutrition and function, with far reaching applications. However, because these projects may impact not only broad scientific objectives and hypotheses, but also specific future scientific methodology and measurement, it would be important to see the philosophy guiding this research clarified with respect to the scientific issues of acute versus chronic and pharmacologic versus physiologic dietary intervention. Frequent and meaningful communication between the Basic and Clinical neuroscience researchers will greatly benefit both programs. In contrast, the CMNR has serious concerns about not only the staffing but also the adequacy of the approach of the menu modification program.

The physical resources available at the PBRC are adequate to accomplish these three projects. While the overall staffing for the basic and clinical neurosciences projects appears well developed, the CMNR believes the projects require on going advice from nutritional scientists with expertise in dietary factors that alter behavior and neurotransmitter levels. This could be accomplished through the addition, at least as consultants on a regular basis,

of nutrition research scientists who are trained in conducting human dietary studies. The committee has reservations about the relevance and appropriateness of the menu modification project. Without appropriate planning, coordination, and staffing this project cannot make a significant impact on modifying Army menus in keeping with current healthful diet concepts. If improperly executed it would likely be a waste of time and resources.

The CMNR is pleased to provide this review as part of its continuing response to the U.S. Army Medical Research and Development Command.

Sincerely,



Robert O. Nesheim, Ph.D.
Chairman, Committee on Military
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Enclosures

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Brief Report

Pennington Biomedical Research Center
September 1996 Site Visit

Pennington Biomedical Research Center

September 1996 Site Visit

A Brief Report of

The Committee on Military Nutrition Research

Food and Nutrition Board

Institute of Medicine

to

Brig. General Russ Zajtchuk

Commander

U.S. Army Medical Research and Materiel Command

November 21, 1996

Produced under grant number DAMD17-94-J-4046 between the National Academy of Sciences and the U.S. Army Medical Research and Materiel Command

NOTICE: The project that is the subject of this report was approved by the Governing Board of the National Research Council, whose members are drawn from the councils of the National Academy of Sciences, the National Academy of Engineering, and the Institute of Medicine. The members of the committee responsible for the report were chosen for their special competencies and with regard for appropriate balance.

This report has been reviewed by a group other than the authors according to procedures approved by a Report Review Committee consisting of members of the National Academy of Sciences, the National Academy of Engineering, and the Institute of Medicine.

The Institute of Medicine was established in 1970 by the National Academy of Sciences to enlist distinguished members of the appropriate professions in the examination of policy matters pertaining to the health of the public. In this, the Institute acts under both the Academy's 1863 congressional charter responsibility to be an adviser to the federal government and its own initiative in identifying issues of medical care, research, and education. Dr. Kenneth I. Shine is president of the Institute of Medicine.

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INTRODUCTION AND BACKGROUND

This brief report, *Pennington Biomedical Research Center (PBRC): September 1996 Site Visit*, is the latest in a series of reports of the activities of the Committee on Military Nutrition Research (CMNR) of the Food and Nutrition Board (FNB), Institute of Medicine, National Academy of Sciences, provided to the Commander of the U.S. Army Medical Research and Material Command (USAMRMC) concerning issues brought to the committee through the Military Nutrition Division (MND) of the U.S. Army Research Institute of Environmental Medicine (USARIEM) at Natick, Massachusetts. The purpose of this site visit was to assist the MND in its review of the progress by the PBRC on research supported by USAMRMC Grant No. DAMD17-92-V-2009, "Military Nutrition Research: Six Tasks to Address Medical Factors Limiting Soldier Effectiveness," and to review proposed nutrition research activities to be conducted at the PBRC with funds earmarked for military nutrition research activities at the PBRC in the 1997 Department of Defense (DoD) Appropriations Bill.

The PBRC is a member of the Louisiana State University (LSU) system and thus is linked administratively to the LSU Medical Center, LSU Agricultural Center, and LSU's main campus in Baton Rouge. Some of the center's major research funding comes from the state of Louisiana, as well as the federal government, to include DoD funded projects, which total 17 percent of its revenues.

History of the Committee

The CMNR was established in October 1982 following a request by the Assistant Surgeon General of the Army that the Board on Military Supplies of the National Academy of Sciences form a committee to advise the DoD on the need for and conduct of nutrition research and related issues. The committee was transferred to the FNB in 1983. The committee's tasks currently are to identify nutritional factors that may critically influence the physical and mental performance of military personnel under all environmental extremes, to identify deficiencies in the existing database, to recommend research that would remedy these deficiencies as well as approaches for studying the relationship of diet to physical and mental performance, and to review and advise on standards for military feeding systems. The CMNR periodically also has

been requested to review ongoing or proposed research activities funded by USAMRMC, particularly this program at the PBRC. Although the membership of the committee has changed periodically, the disciplines represented consistently have included human nutrition, nutritional biochemistry, performance physiology, food science, dietetics, and psychology. For issues that require broader expertise than exists within the committee, the CMNR has convened workshops or utilized consultants to provide additional state-of-the-art scientific knowledge and informed opinion to aid in deliberations.

Focus of the Report

This report focuses on the research program conducted at the PBRC in support of the military nutrition research program at USARIEM. In the initial appropriation dated July 27, 1988, the U.S. Army awarded Grant No. DAMD17-88Z-8083, entitled "The Effect of Food, Diet, and Nutrition on Military Readiness and Preparedness of Military Personnel and Dependents in a Peace Time Environment," to the PBRC. The total grant award was $3,500,000. In the second appropriation dated March 1992, the PBRC was awarded $13,086,567 over 5 years to conduct "Military Nutrition Research: Six Tasks to Address Medical Factors Limiting Soldier Effectiveness." The grants did not allow for equipment purchase, and the PBRC used other funding sources to provide over $5.4 million in equipment to support these research projects.

The U.S. Army staff at USARIEM, in consultation with the CMNR, periodically reviews and makes recommendations on research projects proposed by the PBRC. In June 1989 the CMNR was first asked to review the research plans of the PBRC funded through the DoD appropriations and had submitted a report with their recommendations to the Army. In September 1991 as the initial 3-year grant to the PBRC was nearing completion, the CMNR was asked to review the progress of the PBRC. This review resulted in a report that was submitted to the Army in May 1992. The CMNR again visited the PBRC in June 1992 to review new research plans as proposed by the PBRC for a renewal of their contract with the Army. The role of the CMNR at the meeting on June 3, 1992 was to assist the Army with identifying research activities that fell within the mandate for the appropriation. The responsibility for all decisions regarding the program remained with the Army. At this particular visit, the CMNR was asked to focus its attention on projects in the areas of neuroscience and menu modification. These reviews were transmitted as reports in May and December 1992 (see Appendix IV).

This current review was conducted at the request of Harris R. Lieberman, Ph.D., Chief, MND, USARIEM, who is the Grant Officer Representative for USAMRMC for Grant No. DAMD17-94-J-4046 to the National Academy of Sciences for support of the CMNR. This site visit is thus the third to the PBRC that the CMNR has performed at the request of USARIEM. The members of the CMNR met at the PBRC in Baton Rouge, Louisiana on September 18–20, 1996. The purpose of this meeting was to review and evaluate the progress on work related to USAMRMC Grant No. DAMD17-92-V-2009, "Military Nutrition Research: Six Tasks to Address Medical Factors Limiting Soldier Effectiveness," and to hear

proposals for research to be initiated under the new appropriation of the Pennington military nutrition research program.

Committee Procedure

Prior to the meeting, the CMNR reviewed: (1) the preproposal requesting funding for the continuation of the agreement between PBRC and USARIEM for 5 years beginning April 1, 1997 for military nutrition studies at the PBRC and background materials; (2) the Pennington Biomedical Research Center Annual Reports for 1991 and 1996 submitted by the principal investigator Donna H. Ryan, M.D., to the Army; and (3) past CMNR reviews of PBRC activities in the form of reports as transmitted to USAMRMC. Copies of the preproposal, the 1996 Annual Reports, and the previous CMNR reports, plus the meeting agenda and list of participants, are appended.

The Committee on Military Nutrition Research's activity during this site visit included (1) hearing presentations by PBRC staff members on the progress of current research efforts and proposals for research to be initiated under the new appropriation to the PBRC military nutrition research program; (2) discussing the progress and proposals in a closed session of CMNR members with the Army sponsor; (3) evaluating the progress and proposed activity in an executive session of committee members; and (4) developing a brief report to the Army stating the committee's conclusions and recommendations.

Subsequent to approval of the final draft by the CMNR, this report was reviewed in confidence by a separate anonymous scientific review group in accordance with National Research Council guidelines. The CMNR reviewed the anonymous comments of this review panel and incorporated their suggestions where appropriate. Staff members then wrote a letter in response to the review coordinator and obtained approval of the final report draft from the review coordinator. This report is thus a thoughtfully developed presentation that incorporates the scientific opinion of the Committee on Military Nutrition Research members and the anonymous National Research Council reviewers.

The following is the Committee on Military Nutrition Research's evaluation of the research program presented to them and to Army personnel at the Pennington Biomedical Research Center during the September 1996 site visit.

GENERAL COMMENTS

The committee continues to be impressed with the excellence of the facility at the PBRC for laboratory and clinical research. Continued progress has been made in the staffing of this large facility. The laboratories are extremely well-equipped for research in the areas of the Army's interests. The equipment for supporting this research program has largely been provided by a USDA grant. Financial support for the research activities has progressed significantly, with funds provided by the U.S. Army, USDA, NIH, and various other sources. George A. Bray, M.D., director of the PBRC, and Donna H. Ryan, M.D., principal investiga-

tor for the military nutrition grant, have effectively recruited a qualified staff to conduct the research outlined in the Army grant. The scientific and administrative direction appear to be solid. It is the committee's judgment that as the PBRC receives new directives from its military sponsor, the center now has the staff and expertise to develop appropriate programs utilizing its individual laboratory units as interactive modules. The PBRC has noted that the center's military nutrition research goals for 1997–2002 are: to increase publications, patents, and technology transfer; to increase collaboration with Army scientists; to increase the integration of specific tasks and laboratories; to conduct a military nutrition symposium every other year; and to invite a "military nutrition visiting professor" six times yearly for consultation and peer review.

SPECIFIC PROJECT REVIEWS[1]

Overview of Project Tasks

As described in the introduction, the PBRC is requesting funding for an additional 5 years to conduct research concerning issues of nutrition relevant to the military. The research as outlined in the preproposal consists of the following:

- measuring energy and water needs of troops in the field,
- providing laboratory support for field studies,
- providing nutrition assessment support in field studies,
- enhancing the nutrition of military menus, and
- developing nutritional strategies for improved military performance under stressful conditions.

A total of nine specific projects were developed by the PBRC to meet the U.S. Army objectives. These specific tasks that the CMNR were asked to review include:

- Clinical Laboratory for Human and Food Samples (Task 1),
- Stable Isotope Laboratory (Task 2),
- Stress, Nutrition, and Mental Performance (Task 3),
- Stress, Sleep Deprivation, and Performance (Task 4),
- Stress, Nutrition, and Work Performance (Task 5),
- Menu Modification/Enhancing Military Diet Project (Task 6),
- Nutrient Database Integration Laboratory (Task 7),
- Stress, Nutrition, and Immune Function Laboratory (Task 8), and
- Metabolic Unit Projects.

[1] Please note that the task numbers correspond to the task numbers assigned in the preproposal (see Appendix II).

Task 1: Clinical Laboratory for Human and Food Samples

Project Summary

This project is headed by Richard T. Tully, Ph.D., with major assistance from Jennifer C. Rood, Ph.D. The primary function of this laboratory under the U.S. Army grant is to provide nutrition laboratory research support to the military nutrition research program at USARIEM. This support includes performing biochemical assessment of nutritional status and performing analyses of nutrient and biochemical substances in foods used in military rations and clinical studies. This laboratory is the central laboratory responsible for analyzing blood, urine, stool, tissue, and food samples. In the future, the laboratory proposes in-field computerized data processing of samples to speed processing time, as well as establishing a database that would be accessible to many Army facilities.

General Comments

This review of the laboratory's operation reveals that Dr. Tully and his staff are well qualified and knowledgeable about the wide range of analytical methods required to support the research program. The procedures used for sample transportation, receipt, storage, preparation, and analysis are clearly defined. Sample analyses are accomplished in a timely manner to meet USARIEM's program needs. The laboratory has a sound quality assurance program and uses standard reference materials to check the adequacy of procedure performance. It is noted that the laboratory follows Good Laboratory Practices regulations and is accredited by the College of American Pathologists. It also is noted that additional method development is underway for tests needed for future research. For the size of its staff, the number of procedures currently being performed by the laboratory is very impressive.

The progress that has been made in rendering this laboratory fully operational is excellent. The laboratory houses state-of-the-art equipment and a well-trained staff. It is evident by the level of clinical laboratory support provided to USARIEM in conducting military nutrition research that this laboratory is vital to the DoD nutrition research program.

Specific Comments, Concerns, and Questions

The staff of the Clinical Research Laboratory has been consulting with the U.S. Department of Agriculture Food Composition Laboratory to obtain expertise in the accomplishment of food analysis. The committee agrees that this relationship is important and that such consultation should continue.

Recommendations

The committee suggests that the laboratory should facilitate additional contacts, such as with the FDA's Regional Laboratory in Atlanta, for nutrient analysis. In the area of clinical methods, it may be useful to establish a relationship with the laboratories at the Centers for Disease Control and Prevention in Atlanta. Participation in interlaboratory collaborative studies to verify new methods under the guidelines of the Association of Official Analytical Chemists is also recommended. These laboratories will provide guidance in the development and implementation of new laboratory tests that will best support the current clinical laboratory projects as well as in the development of the methodology required and proposed for food composition and analysis. As the Menu Modification/Enhancing Military Diets Project develops and broadens in scope, maintaining expertise in methodologic issues will be key.

The committee recommends that the additional expenditure of resources be permitted for the development and implementation of methods necessary for the assessment of immune function (in support of Task 8). The development of these methods will be coordinated by the immunologist who is directing the project on Stress and Immune Function (Task 8).

The committee recommends that efforts associated with expanding the capability of chemical analysis of food composition be restricted to obtaining data that are not available from other reliable sources and which directly support metabolic unit studies.

Finally, the committee recommends continued financial support of the Clinical Research Laboratory, as this laboratory provides services to the Stable Isotope Laboratory, Menu Modification/Enhancing Military Diets Project, and the Nutrient Database Integration Laboratory (see Tasks 1, 2, 6, and 7). Financial support should be maintained at a level consistent with USARIEM needs.

Task 2: Stable Isotope Laboratory

Project Summary

This project is directed by James P. DeLany, Ph.D., and involves the use of the doubly labeled water technique to determine energy expenditure in the field and total body water measurements to monitor changes in body composition. The work of this laboratory represents one of the major service functions of the PBRC for the Army. In the past funding period, the laboratory has been involved in 17 completed studies in collaboration with USARIEM, accomplishing 169 doubly labeled water, 222 water turnover, and 616 total body water determinations. At present, the laboratory is involved in analyses of samples from four additional studies conducted in conjunction with USARIEM.

General Comments

Although no specific new work is proposed in the preproposal presented to the CMNR, it is assumed that this laboratory will continue to be available to the Army on an "as needed" basis to complete similar kinds of measurements in studies yet to be specified.

Dr. DeLany has been funded by the Defense Women's Health Research Program to evaluate total daily energy requirements and activity patterns in servicewomen performing a wide range of tasks.

Dr. DeLany indicated that new equipment had been ordered for the laboratory (and is due for delivery) to allow for an increased volume and variety of analyses to be conducted. This equipment will expand the capability of the laboratory to assist in the conduct of studies on various metabolic pathways, such as protein turnover, muscle protein synthesis, and metabolic fuel use during exercise, which could be done in conjunction with other studies proposed to meet the needs of the military (see Tasks 5 and 8).

Specific Comments and Concerns

The lack of integration between this laboratory and others that was suggested by the preproposal is disturbing to the CMNR. Given the directions proposed by other investigators in that preproposal, there are several opportunities for collaboration. These would include investigations such as the ones proposed under Tasks 5 and 8, to determine protein requirements and turnover as well as fuel use and the optimal type of fuel under conditions of intense physical stress that impact on immune function.

Recommendations

The committee recommends that the Stable Isotope Laboratory continue in its service function to assist the Army in its on-going research program. In addition, Dr. DeLany and coworkers should continue to assist the Army with developing experimental designs that ensure the optimal execution of studies using doubly labeled water as well as other stable isotopes. The committee believes that to date this facility has been underutilized, and ample opportunity exists for greater collaboration within the PBRC. The funding of this laboratory should be consistent with the level required to support the Army's nutrition research program.

Task 3: Stress, Nutrition, and Mental Performance

Project Summary

The basic project, headed by Ruth B. S. Harris, Ph.D., involves studies of the impact of stress on neurochemical and behavioral indices of performance using a rodent model. This

line of investigation has developed from an interest in ascertaining how stress modifies brain chemistry and function, with the goal of designing appropriate nutritional and pharmacologic strategies to ameliorate the adverse effects of stress. A number of behavioral, nutritional, sensory, and neurochemical findings have been documented, some of which are being prepared for publication and many of which suggest productive future avenues to pursue.

Rodent models have been developed to evaluate the impact of nutritional interventions for their potential to moderate or prevent stress-induced neurochemical changes and their subsequent behavioral deficits. Many models have been evaluated, including a model of acute stress; a model of chronic stress induced by sleep deprivation or by restraint; chronic mild stress induced by exposure to a random mild stressor for several weeks; and an alternate model to study stress and retention utilizing a fixed-ratio training schedule in operant chambers. The stress models currently in use by the Nutritional Neuroscience Laboratory include a chronic stress and an acute stress model. The chronic stress model utilizes 4 days of Rapid Eye Movement sleep deprivation (REMd) to induce a state of stress. The acute stress model measures the effect of either 3 hours of restraint in a small cylinder or immobilization restraint for 30 minutes to 3 hours. This apparatus is a cylindrical enclosure attached to one side of an open field. A rat is placed inside the chamber, and the time to leave the chamber, number of reentries, total time in the chamber, and locomotor activity in the open field are recorded.

The studies proposed for the renewal project period continue several of the earlier lines of investigation into the mechanisms and effects of stress, in addition to a new project entitled "Genetic Markers for Stress Susceptibility" to investigate the interaction of stress and genetic background in the rodent model. A variety of strategies will be used to identify potential candidate genes.

General Comments

This laboratory has had changes in leadership since it was first established 6 years ago. Under the current leadership of Ruth B. S. Harris, Ph.D., significant progress has been made toward the aims proposed in the last project submission (see Appendix III).

While some of the laboratory's approaches will no doubt yield interesting outcomes, the committee feels that some focus is needed owing to the complexity of stress as a physiologic phenomenon and to the various techniques that have been used to evaluate the stress response experimentally.

Specific Comments, Concerns, and Questions

Specifically, the committee believes that a re-evaluation is needed of the models of stress being examined, so as to focus on one or two models that have the greatest potential relevance to the military mission. Once identified, these models should be examined behaviorally, neurochemically, pharmacologically, and nutritionally to identify vulnerable brain

functions and potential countermeasures. Unless such an approach is taken, there is the possibility that this important area of investigation will remain unfocused, and thus unlikely to lead to the important insights regarding stress and mental function that are necessary to improve human performance under stressful military conditions.

Recommendations

The committee recommends that the investigators seek expert help in stress, pharmacology, and formulation of research diets to assist in the identification of appropriate pharmacologic and nutritional models and interventions. This may be accomplished in conjunction with the PBRC's 1997–2002 research goals as presented at the site visit, one of which is to bring in visiting professors; it may also be accomplished by holding a 1-day meeting specifically dedicated to examining animal models of stress, that have been used successfully in measuring stress. Speakers would evaluate models of stress with which they are familiar in the context of the stresses experienced in the military.

The committee strongly believes that the PBRC investigators must seek a model which more appropriately fits the human experience of stress; with the use of this model, important insights may be gained regarding the manner by which stress compromises brain function, an issue of particular importance to the military mission. Such insights may ultimately lead to effective nutritional and pharmacological measures to combat the negative effects of stress in a military context. In addition, the committee believes that investigations attempting to identify the genes responsible for controlling the response to stress are premature as experienced investigators and facilities are lacking at this time.

Task 4: Stress, Sleep Deprivation, and Performance

Project Summary

This clinical project, as presented by George A. Bray, M.D.; Richard A. Magill, Ph.D.; and William F. Waters, Ph.D., involves studies in sleep deprived human subjects to ascertain whether chemical and nutritional interventions can minimize the cognitive deficits associated with sleep loss. The investigators have pursued these studies for several years, examining such agents as tyrosine, amphetamine, caffeine, and phentermine. The preliminary presentation of results during the site visit indicates that amphetamine clearly improved several of the cognitive deficits produced by sleep deprivation. Smaller effects were noted for phentermine and caffeine, while unremarkable effects were observed for tyrosine. As presented, the results are consistent with earlier findings for each agent and suggest that weak pharmacologic agents are not particularly potent in improving performance deficits associated with sleep loss.

A proposal has been made to continue this line of investigation and to examine the effect on performance of administering tyrosine alone or in combination with caffeine during

a longer period of sleep deprivation. Another proposed study would compare the effects of tryptophan alone with that of tryptophan in combination with melatonin to evaluate whether these agents can improve sleep efficacy during short naps provided intermittently during the sleep deprivation period.

General Comments

At the time of this review, while a large number of studies were reported to have been completed, the data had not yet been thoroughly analyzed statistically.

Specific Comments, Concerns, and Questions

Overall, it would appear that this project has succeeded in fulfilling its mission: it has shown that caffeine may be useful as a nonprescription agent for enhancing performance during sleep deprivation, but that tyrosine is not.

Based on the data collected and analyzed to date, it appears that additional studies are unlikely to lead to new and potent intervention strategies using these agents. Hence, the need to continue such studies is not evident. The committee is aware of other, well-established neuroscience sleep laboratories within the military system that currently are working on similar projects.

Recommendations

The CMNR feels that it would not be in the Army's best interest to continue to provide support for the conduct of the sleep-deprivation studies within the Sleep Laboratory.

Task 6: Menu Modification/Enhancing Military Diets Project and Task 7: Nutrient Database Integration Laboratory

Project Summary

In its preproposal, the PBRC has divided the original Menu Modification Project into two projects. The newly named project, Enhancing Military Diets Project, was initiated during September–October 1995 and will continue the recipe development activities using testing procedures similar to those developed for the previous project under the direction of Catherine Champagne, Ph.D. Chef Kelly Patrick has developed new recipes and has begun bench-top testing. The PBRC currently is assembling an acceptability panel that will consist of 50 to 100 panelists who fit the profile of U.S. military personnel to evaluate the recipes. Acceptability and consumption studies will follow at the Army Quartermaster Cooks School at Fort Lee, Virginia.

Past activities in the Menu Modification Project focused on the development of recipes to achieve the stated reduction in fat and cholesterol necessary to achieve the desired goals of no more than 30 percent of total calories from fat, and approximately 300 mg/d of cholesterol, for meals served in the Army garrison dining halls (A Rations). Forty-seven recipes have been tested for acceptability by panels at the PBRC and at Louisiana Tech. Similar methods of acceptability testing done at Fort Polk gave results comparable to those at Louisiana Tech, supporting the use of such nonmilitary panels to predict acceptability in an actual Army feeding situation. The lack of such comparable data was a concern expressed at the last CMNR site visit (see Appendix IV).

A 2-week study also was conducted at Fort Polk in which the new recipes were incorporated into dining hall menus during the second week. Data collected on food consumption in the dining hall plus food records for food consumed off base showed a significant reduction in fat intake (from 34.5 percent to 31.8 percent) with the modified menu compared to the regular menu for food eaten in the dining hall. It was noted, however, that approximately 50 percent of the food intake of personnel in Army facilities is from food consumed off base (outside of military dining facilities). The total fat consumed from these meals averaged 35.4 percent during each period of the study.

Based on interviews with Army personnel, PBRC investigators have proposed a list of modified recipes for testing, with an emphasis on ethnic foods and new breakfast items.

The new task proposed for the Nutrient Database Integration Laboratory involves the development and expansion of activities to assist the Army in estimation of the nutrient content of recipes and menus, as well as in the evaluation of dietary intake records from field studies conducted by USARIEM. Since 1989, the PBRC has participated in a support role in a number of studies. The Nutrient Data Systems Section at USARIEM has been involved in active data collection since 1993. In assessing military diets, a modified visual estimation method as well as food records have been utilized. In January 1995, the PBRC was approached by the Army sponsor to facilitate a more efficient form of collecting and disseminating dietary intake data from garrison and field studies. One such collaborative study which has been undertaken is the Savannah study conducted in July and August 1996. The leader of the data collection team for this project was Dr. Champagne, and the co-leader responsible for data entry was Ray Allen, Ph.D. A database system called MiDAS (Military Data Acquisition System) has been developed for use in the assessment of food intake in military settings. The PBRC staff will integrate all Armed Forces recipes, special formulations (Meals, Ready-to-Eat), and other food formulations into one centralized database system as part of this task.

General Comments for Tasks 6 and 7

In the letter report dated May 12, 1992, the CMNR noted several shortcomings in the Menu Modification Project. These included: (1) a lack of sensitivity to the needs of the military garrison feeding program, (2) inadequate evaluation procedures of modified menus,

and (3) lack of interaction between the menu developers and the military menu system (see Appendix IV). The CMNR is pleased to note that PBRC investigators have overcome many of these shortcomings and have established working relationships with Army dietitians at USARIEM and with Army facilities such as Fort Polk and Fort Lee. Future activities with USARIEM include a visit of the PBRC team to the Army Quartermasters Cooks School at Fort Lee, Virginia to observe current training of Army cooks and a meeting with personnel responsible for determining the Army Master menu, as well as meeting with personnel responsible for food purchasing.

The proposed new leader of this project, Alana Cline, Ph.D., has extensive knowledge of military feeding systems and will facilitate further strengthening of the interactions of the PBRC with USARIEM. The past and proposed activities of this project are viewed as highly valuable by the Army and will make possible the accomplishment of tasks they currently are unable to do.

The Nutrient Database Integration Laboratory project will take advantage of the excellent PBRC computer facilities and expertise in dietary intake methodology to provide USARIEM with much-needed assistance in obtaining dietary data faster and more efficiently. Nutrient databases that have been incorporated into the PBRC database laboratory include the Bogalusa Heart Study and USDA Handbook 8 databases, as well as the Army's CAN (Computerized Analysis of Nutrients) database system. The PBRC nutrient database has been validated against the University of Minnesota Nutrition Coding Center database, and also has been utilized in two large, NIH-sponsored multicenter clinical trials.

Specific Comments, Concerns, and Questions for Tasks 6 and 7

Since it was demonstrated that approximately 50 percent of the food intake of personnel in Army facilities represents food consumed outside of military dining halls, it is questionable whether the development of low fat menus alone will achieve the goals of reducing total fat intake. However, any measurable reduction should be considered constructive, and those few individuals selecting all their meals in the military dining halls will benefit substantially.

There is additional concern regarding the micronutrient content of the modified menus. In particular, it is felt that additional attention must be focused on the levels of iron, calcium, zinc, folate, and vitamin B_6 (that is, micronutrients likely to be limiting when the use of ingredients from animal sources is curtailed in favor of lowering fat) provided by these dishes, in addition to the concern about their fat content.

Recommendations for Tasks 6 and 7

The CMNR recommends that estimation of plate waste be included in future field acceptability studies, such as the upcoming study at Fort Bliss, to provide for a more quantitative and qualitative assessment of dietary intakes.

In addition to menu modification, the committee strongly recommends to the PBRC and to the Army the use of nutritional education approaches such as those in the Army's Performance Power nutrition education program in addition to menu modification in order to achieve dietary intakes that meet military dietary goals.

The CMNR believes the Menu Modification/Enhancing Military Diets Project is relevant to the Army mission and provides valuable support to USARIEM. Since there are a number of nutrient databases in existence, it will be important to integrate these to avoid unnecessary duplication and to assure the integrity of this database. The proposed new task to develop the Nutrient Database Integration Laboratory is the next logical step in the development of a unified nutrition program that may facilitate the evaluation and comparison of dietary intake data with comparable data from the civilian sector at a later date.

Task 5: Stress, Nutrition, and Work Performance and Task 8: Stress, Nutrition, and Immune Function Laboratory

Project Summary

As described by Jeffery J. Zachwieja, Ph.D., Task 5, entitled "Stress, Nutrition, and Work Performance," proposes to use outpatient volunteers to develop repeatable and reliable models to assess effects of carbohydrate delivery, amino acids, and caffeine on work performance and tolerance.

In addition, Dr. Zachwieja proposes to conduct studies using a rat model of stress. These rat studies, as described, will be closely integrated with Task 3 (Stress, Nutrition, and Mental Performance) of Ruth B. S. Harris, Ph.D., of the Nutritional Neuroscience Laboratory and with Task 8 of David W. Horohov, Ph.D., of the Stress, Nutrition, and Immune Function Laboratory. Descriptions of Dr. Horohov's task indicate that many, if not all, of his initial studies will be done in a rat model of stress in an attempt to develop a reproducible and predictable rat model for characterizing the mechanics of stress and immune dysfunction.

General Comments for Tasks 5 and 8

The committee believes that the integration of Tasks 5 and 8 is highly desirable but suggests that the objectives of both tasks would best be accomplished in human subjects. The superb PBRC Metabolic Unit would easily allow these performance studies to be conducted so that studies of balance, body composition, protein turnover, energy metabolism (these last three using stable isotopes), and immune function could all be combined and data obtained under closely controlled conditions. The independent variables would be physical activity and nutritional alterations (including modest-to-severe protein/energy deficiency as experienced in Ranger training). The integration of Tasks 5 and 8 will support a key directive from the Military Nutrition Division at USARIEM to test and evaluate nutrients and pharmacologic agents that may enhance performance. Such studies could build upon previous

data obtained in Ranger trainees, as highlighted in previous CMNR publications (IOM, 1992, 1993). These studies documented the effects of demanding physical activity combined with limited food intake and limited sleep, which resulted in a 12 to 15 percent average weight loss over a period of about 9 weeks that was coupled with episodic increases in accidents and infections (IOM, 1993). This situation of low body fat, undernutrition, altered endocrine patterns, and perturbations in immune function suggested the need to develop important and practical nutritional countermeasures. Two important testable issues are whether improving the protein-to-energy ratio, under conditions of modest-to-severe energy deficiency, would improve immune function and physical performance. The carbohydrate delivery studies proposed by Dr. Zachwieja also could be important in the search for effective countermeasures.

Specific Comments, Concerns, and Questions for Tasks 5 and 8

The CMNR feels strongly that the advantages of using the human model for immunological studies far outweigh any advantages of using rat models. As noted earlier in this report, the rat models currently being developed for use in Tasks 3, 5, and 8 leave much to be desired in terms of their direct applicability to military situations involving stress. The use of a human model permits longer-term measurements of antibody response to test antigens as well as delayed dermal hypersensitivity responses. Also, immunological studies of rats can be impaired by lack of species-specific reagents. Both Drs. Zachwieja and Horohov have had extensive experience in conducting studies in human subjects. Combining Tasks 5 and 8 as human studies, and reducing the reliance on rodent studies, may have some economic advantages in the long run.

Recommendations for Tasks 5 and 8

The CMNR believes that the proposed new tasks, once the work being conducted within individual laboratory units becomes integrated and adapted to clinical and metabolic unit studies, will have high degrees of military relevance and should be supported as new areas of research.

Metabolic Unit Projects

Project Summary

An overview of the Metabolic Unit Projects was presented by Donna H. Ryan, M.D.; Jeffery J. Zachwieja, Ph.D.; and Steven R. Smith, M.D. During the past funding period, with input from the Army two metabolic studies were conducted. The first study, "Assessment of Intra- and Inter-Individual Metabolic Variation in Special Operations Forces Soldiers,"

sought to determine the possible need for individually-designed diets to optimize performance in Special Operations Forces soldiers, who are highly trained and severely stressed. The outcome of the study suggests that the inter-individual variation in response to changes in dietary carbohydrate is small and does not warrant individualization of military diets.

The second study, "Effects of Prolonged Inactivity on Musculoskeletal and Cardiovascular Systems with Evaluation of a Potential Countermeasure," reported by Dr. Smith, was done in conjunction with NASA and USARIEM. It sought to establish a rapidly developing model of microgravity that could then be used to test possible interventions for use by the space program. A model has been developed that involves administering low doses of triiodothyronine (T3) in conjunction with bed rest to mimic the effects of inactivity. After 4 weeks, changes in bone resorption markers are similar to those seen in more conventional human models after 4 months.

General Comments

The Metabolic Unit facility, in conjunction with the Stable Isotope Laboratory and Clinical Research Laboratory, provides an outstanding opportunity to control dietary interventions and physical conditions and to perform analyses of related metabolic parameters so that specific questions of interest to the military can be studied effectively. Future plans include testing interventions using testosterone and exercise. The CMNR is concerned with the use of female subjects in further investigations of the musculoskeletal response to microgravity. Until demonstrable countermeasures to maintain bone and muscle mass can be developed in males, female subjects should not be used because of their increased risk related to bone density.

The design of the physical facility and the equipment available for metabolic studies at the Pennington Biomedical Research Center are outstanding, and the available staff are well trained and experienced in the conduct of metabolic studies.

Specific Comments, Concerns, and Questions

No further use of the Metabolic Unit was proposed in the preproposal presented for this funding period. The rationale presented was that use of an animal model would be less expensive than experiments involving the Metabolic Unit. However, in listening to the proposals to evaluate work performance, diet, and immune function, the CMNR believes that much of the proposed work could be conducted better and with more direct application to the needs of the military using human subjects (see Tasks 5 and 8).

Recommendations

The CMNR recommends the use of human subjects in metabolic studies as these studies will be more inclusive and better directed to the needs of the military.

The CMNR recommends seeking the advice of additional experts in the field of bone mineral metabolism in the design and implementation of studies on microgravity, to maximize the efficacy of research while minimizing the risk to subjects. This area of research appears to be of greater importance for NASA than for the Army, and any further development and support should be provided by NASA.

SUMMARY OF THE COMMITTEE'S REVIEW

A summary of the scientific support services and related tasks, linking each task area outlined in the preproposal with its core laboratory support, as well as the recommendations of the CMNR, is provided in Table 1.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

- The committee finds that the Clinical Research Laboratory is vital to the Pennington Biomedical Research Center and to the Military Nutrition Division at USARIEM. The availability of this laboratory to USARIEM has, in large measure, solved a critical need that existed for some time prior to 1990 to obtain timely and accurate analytical support for field studies on the nutritional status of military personnel and for the evaluation of military rations designed to meet their needs.
- Of concern to the committee is the lower than expected rate of publication in the scientific literature of the data produced for USARIEM by the Pennington Biomedical Research Center. The data acquired by this facility on energy requirements under a variety of circumstances (altitude exposure in men and women, extremes of physical training), changes in body composition with dietary and activity manipulations, and optimization of performance with dietary manipulations, would be of significant importance to the military as well as to the general scientific community. While the research may be reported in Army Technical Bulletins, the value of the work done by the PBRC in conjunction with USARIEM would be enhanced by publication in peer reviewed journals.
- The committee recommends continued support for and integration of the Clinical Research Laboratory, Stable Isotope Laboratory, Menu Modification/Enhancing Military Diets Project, and Nutrient Database Integration Laboratory at a level consistent with USARIEM needs. Experimental studies utilizing the technique of doubly labeled water as well as the incorporation of studies within the Metabolic Units Project employing isotopes to evaluate nutrient utilization should receive high priority in developing projects of interest to the Army.

TABLE 1 Summary of Scientific Support Services, Related Tasks, and CMNR Recommendations

Laboratory/Facility	Current Task	Proposed Task	CMNR Recommendation
Clinical Nutrition Reference Laboratory (Task 1)	Clinical lab projects	Clinical lab projects	Continue development and support of clinical lab projects Integrate with Task 2 and Metabolic Unit Projects
Food Chemistry Laboratory	Menu Modification/Enhancing Military Diets Project (Task 6)	Menu Modification/Enhancing Military Diets Project (Task 6)	Promote further development and integration of Tasks 6 and 7
Nutrient Database Integration Laboratory (Task 7)	Nutrient Database Integration Laboratory (Task 7)	Nutrient Database Integration Laboratory (Task 7)	Promote further development and integration of Tasks 6 and 7
Stable Isotope Laboratory (Task 2)	Available as needed	Available as needed	Integrate Task 5 (Stress, Nutrition, and Work Performance, basic and clinical studies) and Task 8 (Stress, Nutrition, and Immune Function, basic and clinical studies) with these and Metabolic Unit Projects
Energy Expenditure/ Exercise Physiology Facilities	Available as needed	Available as needed	Continue stable isotope studies and integrate with Tasks 5 and 8 as well as Metabolic Unit Projects
Body Composition Facilities	Available as needed	Available as needed	Continue stable isotope studies and integrate with Tasks 5 and 8 as well as Metabolic Unit Projects

Continued

TABLE 1 *Continued*

Laboratory/Facility	Current Task	Proposed Task	CMNR Recommendation
Nutritional Neurosciences Laboratory	Stress, Nutrition, and Mental Performance (Task 3, basic studies)	Stress, Nutrition, and Mental Performance (Task 3, basic studies)	Continue Task 3 basic studies with modifications
Sleep Deprivation Laboratory	Stress, Sleep Deprivation, and Performance (Task 4, basic and clinical studies)	Stress, Sleep Deprivation, and Performance (Task 4, basic and clinical studies)	Discontinue clinical studies on sleep deprivation under Task 4
Stress, Nutrition, and Immune Function Laboratory		Stress, Nutrition, and Work Performance (Task 5, basic and clinical studies)	Integrate Tasks 5 and 8 for both basic and clinical studies with Metabolic Unit Projects
		Stress, Nutrition, and Immune Function (Task 8, basic and clinical studies)	

- The committee recommends that additional collaborations be sought for the incorporation of the most current laboratory methodologies for nutrient analysis, with restriction of effort to obtaining data that are not currently available or extrapolative.
- The committee recommends that additional expenditure of resources be permitted for collaboration on and development and testing of various clinical laboratory tests to assess immune function.
- The committee recommends the use of human subjects in metabolic studies that will be more inclusive and better directed to the needs of the military.
- The committee believes that with expert consultation and with the development of an appropriate animal model to evaluate the impact of stress on brain function, the Stress, Nutrition, and Work Performance project can contribute much in the way of basic studies in support of the military mission. On the other hand, the committee does not feel that continuing the development of clinical studies on sleep deprivation in the Sleep Laboratory is of particular value to the Army.
- The committee finds that the Menu Modification/Enhancing Military Diets Project as well as the Nutrient Database Integration Laboratory are valuable to the Army mission and provide needed support to USARIEM. Additional efforts with regard to nutrition education should be incorporated in order to meet Military Dietary Goals.
- The committee recommends integrating the proposed new projects, "Stress, Nutrition, and Work Performance" (Task 5) and "Stress, Nutrition and Immune Function" (Task 8), in both the basic laboratory studies and the clinical studies; they can provide a high degree of military relevance and should be strongly supported. Whenever possible and as appropriate, human subjects should be utilized rather than animal models in these project areas.

The CMNR is pleased to provide this review as part of the committee's continuing response to the U.S. Army Medical Research and Materiel Command. The committee always welcomes comments and suggestions regarding how these reports can better serve the needs of the Army.

ACKNOWLEDGMENTS

The CMNR expresses its appreciation to Donna H. Ryan, M.D., principal investigator for the military nutrition grant, and George A. Bray, M.D., director of the PBRC, for their hospitality during the site visit and the excellent organization of the review procedures and the background material provided prior to the visit. The committee thanks the PBRC scientists who presented summaries of their activities, proposed plans for the future, and patiently answered questions and were very forthcoming on requested information.

The CMNR chair wishes to acknowledge the excellent contribution of the FNB staff, especially Rebecca B. Costello, Ph.D., the project director of the CMNR, and Sydne J. Carlson-Newberry, Ph.D., staff officer of the CMNR, for the organization of the review and handling the many details necessary to prepare the report for publication. The comments by

FNB director Allison A. Yates, Ph.D., provided helpful insights into the development of this final document. The chair also acknowledges the considerable skills of Susan M. Knasiak, research assistant, in assisting in the development of the review document, and Donna F. Allen, senior project assistant, for her work in making arrangements for the site visit. The excellent dedication of the entire FNB staff has made the desired fast reporting on this project review a reality.

Also, the chair continues to be extremely pleased with the dedication, cooperation, and excellence of the critical review that the members of the CMNR bring to this activity in support of the military nutrition research program. It is a real pleasure to work with such a cooperative, hard working, and friendly group.

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Biographical Sketches of Subcommittee Members

JOHN E. VANDERVEEN *(chair)* is the former director of the Food and Drug Administration's (FDA) Office of Plant and Dairy Foods and Beverages in Washington, D.C. His previous position at FDA was as director of the Division of Nutrition at the Center for Food Safety and Applied Nutrition. He also served in various capacities at the U.S. Air Force (USAF) School of Aerospace Medicine at Brooks Air Force Base, Texas. During his time in the Air Force, Dr. Vanderveen participated in the development of USAF body composition standards. He has received numerous accolades for service from both FDA and USAF. Dr. Vanderveen is a member of the American Society for Clinical Nutrition, American Institute of Nutrition, Aerospace Medical Association, American Dairy Science Association, and American Chemical Society. He is a fellow of the Institute of Food Technologists and an honorary member of the American Dietetic Association. He has served as treasurer of the American Society of Clinical Nutrition and as a member of the Institute of Food Technologists' National Academy of Sciences Advisory Committee. Dr. Vanderveen holds a B.S. in agriculture from Rutgers University and a Ph.D. in chemistry from the University of New Hampshire.

DENNIS M. BIER is a professor of pediatrics, director of the U.S. Department of Agriculture (USDA) Children's Nutrition Research Center, and program director of the National Institutes of Health (NIH) General Clinical Research Center at Baylor College of Medicine. Dr. Bier is a member of the Institute of Medicine (IOM) and has served as a member of various scientific committees including the Department of Health and Human Services–USDA Dietary Guidelines Advisory Committee, IOM and Food and Nutrition Board committees, the Food and Drug Administration Food Advisory Committee, the Medical Science Advisory Board of the Juvenile Diabetes Foundation, and the Task Force and Steering Committee of the Pediatric Scientist Development Program. In addition, he has chaired the NIH General Clinical Research Centers Committee and the National Institute of Child Health and Human Development's Expert Panel Five-Year Plan for Nutrition Research and Training. He currently serves as president of the International Pediatric Research Foundation, councilor of the American Pediatric Society, associate editor of the *Annual Review of Nutrition*, and chairman of the USDA Human Studies Review Committee. Dr. Bier received his B.S. in biology from Le Moyne College and his M.D. from the New Jersey College of Medicine. Dr. Bier's research interests include the role of nutrition in human health and in the prevention and treatment of disease, with a particular focus on macronutrients, intermediary metabolism, tracer kinetics, diabetes, obesity, and endocrine disorders.

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JOHN D. FERNSTROM is professor of psychiatry, pharmacology, and behavioral neuroscience at the University of Pittsburgh School of Medicine, research director of the University of Pittsburgh Health System, Weight Management Center, and director of the Basic Neuroendocrinology Program at the Western Psychiatric Institute and Clinic. He received his B.S. in biology and his Ph.D. in nutritional biochemistry from the Massachusetts Institute of Technology (MIT). He was a postdoctoral fellow in neuroendocrinology at the Roche Institute for Molecular Biology in Nutley, New Jersey. Before coming to the University of Pittsburgh, Dr. Fernstrom was assistant and then associate professor in the Department of Nutrition and Food Science at MIT. He has served on numerous government advisory committees. He presently is a member of the National Advisory Council of the Monell Chemical Senses Center. He is also a member of numerous professional societies, including the American Institute of Nutrition, American Society for Clinical Nutrition, American Physiological Society, American Society for Pharmacology and Experimental Therapeutics, American Society for Neurochemistry, Society for Neuroscience, and Endocrine Society. Among other awards, Dr. Fernstrom has received the Mead-Johnson Award of the American Institute of Nutrition, a Research Scientist Award from the National Institute of Mental Health, a Wellcome Visiting Professorship in the Basic Medical Sciences, and an Alfred P. Sloan Fellowship in Neurochemistry. His current major research interest concerns the influence of diet and drugs on the synthesis of neurotransmitters in the central and peripheral nervous systems.

STEVEN B. HEYMSFIELD is professor of medicine at Columbia University College of Physicians and Surgeons in New York. He also currently serves as deputy director of the New York Obesity Research Center and is director of the Human Body Composition Laboratory. Dr. Heymsfield is immediate past president of the American Society of Parenteral and Enteral Nutrition and is an active member of the American Society of Clinical Nutrition and the North American Society for the Study of Obesity. He was recently made an honorary member of the American Dietetic Association. He received his B.A. in chemistry from Hunter College of the City University of New York and his M.D. from Mt. Sinai School of Medicine. Dr. Heymsfield has done extensive research and has clinical experience in the areas of body composition, weight cycling, nutrition, and obesity, especially as they relate to women.

ROBIN B. KANAREK is professor of psychology and adjunct professor of nutrition at Tufts University in Medford, Massachusetts, where she also is the chair of the Department of Psychology. Her prior experience includes research fellow, Division of Endocrinology, University of California, Los Angeles School of Medicine, and research fellow in nutrition at Harvard University. In addition to reviewing for several journals, including *Science*, *Brain Research Bulletin*, *Journal of Nutrition*, *American Journal of Clinical Nutrition*, and *Annals of Internal Medicine*, she is a member of the editorial boards of *Physiology and Behavior, Nutritional Neuroscience,* and the *Tufts Diet and Nutrition Newsletter*, and is a past editor-in-chief of *Nutrition and Behavior*. Dr. Kanarek

has served on ad hoc review committees for the National Science Foundation, National Institutes of Health, and U.S. Department of Agriculture, as well as the Member Program Committee of the Eastern Psychological Association. She is a fellow of the American College of Nutrition, and her other professional memberships include the American Institute of Nutrition, New York Academy of Sciences, Society for the Study of Ingestive Behavior, and Society for Neurosciences. Dr. Kanarek received a B.A. in biology from Antioch College and her M.S. and Ph.D. in psychology from Rutgers University.

MELINDA M. MANORE is currently a professor and chair, Department of Nutrition and Food Management, Oregon State University, and a registered dietitian. Her research interests include the interaction of nutrition and exercise in health, exercise performance, disease prevention, and reduction of chronic disease across the life cycle. Dr. Manore's research also focuses on factors regulating energy balance (i.e., energy expenditure, eating behaviors, body weight and composition), and the role of nutrition, exercise, and energy balance in the reproductive cycle. She is a fellow of the American College of Sports Medicine, and a member of the American Dietetic Association (ADA), American Society of Nutritional Sciences, American Society for Clinical Nutrition, and North American Association for the Study of Obesity. She is currently chair of the ADA Nutrition Research Practice Group and received the ADA's Sports, Cardiovascular and Wellness Nutritionists Excellence in Practice award in 2001. Dr. Manore currently serves as a member of the U.S. Gymnastics National Health Advisory Board, the Gatorade Sport Science Institute Nutrition Board, and the Arizona Osteoporosis Coalition Medical Advisory Board. She is associate editor for *Medicine and Science in Sports and Exercise* and *Woman's Health and Fitness Journal,* and is on the editorial boards of the *Journal of the American Dietetic Association,* and *International Journal of Sport Nutrition and Exercise Metabolism.* Dr. Manore obtained her M.S. in health education and community health from the University of Oregon and her Ph.D. in human nutrition from Oregon State University.

PATRICK M. O'NEIL is professor of psychiatry and behavioral sciences at the Medical University of South Carolina, where he is also director of the Weight Management Center. Dr. O'Neil has been involved in the study of obesity and its management since 1977, including clinical trials, basic research, teaching, and public education. He has been the principal investigator of a number of clinical trials of weight loss agents. He is the author of more than 100 professional publications primarily concerning psychological, behavioral, and other clinical aspects of obesity and its management. Dr. O'Neil has served on the Education Committee of the North American Association for the Study of Obesity (NAASO) since 1994, was program chair of the 1999 NAASO Annual Meeting, was a member of the NAASO Ad Hoc Committee for Development of the Practical Guidelines, and is the NAASO Website editor. He is also immediate past president of the South Carolina Academy of Professional Psychologists, former member and chair of the South Carolina Board of Examiners in Psychology, and former chair of the Obesity and Eating Disorders Special Interest Group of the Association for the Advancement of Behavior Therapy. Dr. O'Neil received his B.S. in economics from Louisiana State University and his M.S. and Ph.D. in clinical psychology from the University of Georgia.