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To assess, maintain or improve a soldier's physical/physiological/psychological capability to function effectively under environmental and emotional stress and to minimize adverse effects of stress on health, safety and performance, the Pennington Biomedical Research Center (PBRC) performed the following six research tasks: 1) The Clinical Laboratory for Human and Food Samples performed laboratory analyses of samples from studies conducted by the U.S. Army Research Institute of Environmental Medicine (USARIEM). 2) The Stable Isotope Laboratory performed analyses to measure the energy expenditure and body composition of soldiers during prolonged field exercise. 3) The Nutritional Neuroscience Laboratory conducted multidisciplinary basic research studies on the effects of diet on biochemical, morphologic and behavioral variables. The lab evaluated the role of diet in sustaining performance under conditions of stress and sleep deprivation. 4) The Nutritional Neuroscience Clinical Studies evaluated nutritional and other strategies to optimize cognitive performance in conditions of operational stress and sleep deprivation. 5) The Menu Modification Project evaluated and improved garrison meals at an actual garrison in Fort Polk, Louisiana. 6) The Metabolic Unit Project allowed for the PBRC's inpatient unit to conduct metabolic studies of military relevance in an inpatient setting.

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NA In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

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Donna H Ryder, MD 6/19/98

PI - Signature Date

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FINAL REPORT
U.S. ARMY GRANT
3/1/92 - 3/31/98

Introduction

On April 1, 1992, Grant #DAMD 17-92-V2009 was awarded to Pennington Biomedical Research Center (PBRC) to address the following **Hypothesis: Medical factors limiting soldier effectiveness can be addressed through nutritional strategies**.

The goal of this research is to assess, maintain, or improve a soldier's physical/physiological/psychological capability to function effectively under environmental and operational stress and to minimize adverse effects of stress on health, safety and performance.

Technical Objective

This research continues the research relationship between the PBRC and USARIEM over a five year period. Those research relationships were established under a prior cooperative agreement, #DAMD 17-88-Z-8023, "The effect of food, diet and nutrition on military readiness and preparedness of military personnel and dependents in a peace time environment."

The project allows for the continuation of the Clinical Laboratory for Human and Food Samples, Stable Isotope Laboratory, Menu Modification Project, and Nutritional Neuroscience Laboratory, all of which were initiated under Grant #DAMD 17-88-Z-8023. The project also expands the scope of research to involve Nutritional Neuroscience Clinical Studies. The project also allows for the utilization of the PBRC's inpatient metabolic unit for a study designed by USARIEM investigators as detailed in the Metabolic Unit Project section.

The six tasks performed under this project are listed and described below.

Task 1: Clinical Laboratory for Human and Food Samples

The Clinical Laboratory performs procedures (assessment of protein, mineral, vitamin and immunologic status) to assess the nutritional status of soldiers participating in military nutrition research studies conducted by USARIEM.

Task 2: Stable Isotope Laboratory

The Stable Isotope Laboratory continues the development and field validation of stable isotope technologies to unobtrusively measure the energy expenditure of soldiers during prolonged (1-4 weeks) field exercise in extreme climates. The technology also measures changes in body composition and body fluid status.

Task 3: Nutritional Neurosciences Laboratory

Continuation of the research in the Nutritional Neuroscience Laboratory includes multi-disciplinary studies in rats on the effect of diet on brain function and structure. The biochemical and morphologic variables are related to changes in behavior measured by arousal, shuttle box

performance, operant chamber performance, food selection and swimming performance. The effect of stress and in particular the stress of rapid eye-movement sleep deprivation are studied using the above noted morphologic and behavioral parameters. Mechanistic hypotheses are explored and ameliorative dietary strategies are tested.

Task 4: Nutritional Neurosciences Clinical Studies

The Nutritional Neuroscience Clinical Studies are designed to evaluate cognitive performance and man-machine interface under conditions of sleep deprivation. Different nutritional intervention strategies to favorable influence mental performance in conditions of rapid eye-movement sleep deprivation are tested.

Task 5: Menu Modification Project

PBRC nutritionists evaluate, through a computer data base, the Moore's Extended Nutrient Database (MENU), and laboratory analyses, the nutritional content of garrison meals. In a project conducted at Fort Polk, Louisiana, menus are modified and tested in an actual garrison setting to meet the improved Army guidelines for nutrition. Follow up of the testing includes further modifications and testing, using the quality improvement approach. PBRC personnel travel to field sites for assistance in ARIEM-directed studies of food intake in the field.

Task 6: Metabolic Unit Project

The PBRC inpatient 14 bed unit is made available for research studies and has accommodated Special Operation Forces volunteers in a research study designed by a collaboration of USARIEM scientists and participation by PBRC personnel.

Military Significance and Relevance to USARIEM Needs

The Stable Isotope and Clinical Laboratory methodologies are critical components of in-house military nutrition research of the U.S. Army Research Institute of Environmental Medicine. These extramural projects provide critical capabilities that do not exist in house, but are needed to fulfill the Army Surgeon General's responsibility to provide nutritional research support to the DOD and Nutrition RDT&E Program.

The Clinical Laboratory also provides support for three projects of Defense Women's Nutritional Health Research.

The Nutritional Neuroscience Laboratory and Clinical Studies Programs expand our knowledge of the effects of stress and sleep deprivation and explores the ameliorative effects and mechanisms of action of dietary-induced alterations in behavior and cognitive function. Advances in this knowledge are the basis for developing safe and effective nutritional strategies to sustain and enhance soldier performance under conditions of environmental or operational stress. The project also provides insight into the roles of corticotrophin releasing factor (CRF) and locus coeruleus (LC) noradrenergic mechanisms in mediating anxiety in rats exposed to restraint stress.

The Menu Modification Project fulfills military needs to promote health, maintain

readiness and sustain soldier performance.

The Metabolic Unit Project fulfills military need for an inpatient site for performance of specialized research utilizing the body composition assessment, energy expenditure assessment, metabolic kitchen services, and clinical laboratory expertise of the PBRC.

This report describes progress during the six years of the grant.

The major administrative and scientific high points are outlined below:

- On June 2-3, 1992, the Pennington Biomedical Research Center was the site of a review by the following members of the Committee on Military Nutrition Research: Robert O. Nesheim, Ph.D., Allison Yates, Ph.D., Johanna Dwyer, D.Sci., R.D., Bernadette Marriott, Ph.D. In addition, special consultants included James G. Penland, Ph.D. and other attendees, Colonel David Schnakenberg, Colonel E. Wayne Askew, Dr. Harris Lieberman, Major Mary Mays, and Major Cecelia Thomas. At this meeting initial plans were laid for development of an inpatient metabolic ward project to be developed jointly with the Pennington Center and staffed at USARIEM.

- In the 9th quarter, the grant officer, Colonel Wayne Askew, was replaced by Dr. Jim Vogel (USARIEM Director for Occupational Health and Performance). The project was overseen by Dr. Harris Lieberman, in charge of the USARIEM Military Nutrition Research Division. Colonel Bob Gifford, Director of Army Systems Hazards Research Program in the Army's RAD III unit, was assigned to oversee the project's grant funding.

On November 6-8, 1995 the Pennington Center hosted the Military Neuroscience Symposium which was attended by 60 scientists in the field. The title of the symposium was "Current Progress in Military Neuroscience Research: Countermeasures for Battlefield Stressors."

- On September 18-20, 1996 the PBRC hosted a site visit for the CMNR. On Wednesday, September 18 the PBRC faculty presented proposed research activities for the site visitors. On September 19-20 the CMNR met at the PBRC Conference Center and pursued their own agenda. Details of the site visit agenda are found in the 18th Quarterly Report. The members who attended the meeting included the following:

  Robert O. Nesheim, Ph.D., Chair
  William R. Beisel, M.D.
  Gail K. Butterfield, Ph.D.
  John D. Fernstrom, Ph.D.
  G. Richard Jansen, Ph.D.
  Robin B. Kanarek, Ph.D.
  Orville Levander, Ph.D.
  John Vanderveen, Ph.D.

  The CMNR staff who attended included:

  Rebecca Costello, Ph.D.
  Sydne Carlson-Newberry
  Susan Knasiak
  Donna F. Allen

- On February 5, 1997 we received correspondence from Judy Pawlus, Chief, Research Data Management, informing us of the Army's intent to downgrade our reports from limited to unlimited, approved for public release, status. This correspondence can be found in the 20th Quarterly Report.

- We received confirmation that the final report for this project is to be submitted in 1998. Correspondence documenting this is found in the appendix of the 20th Quarterly Report.

- On March 18, 1997 we received notification from Wendy A. Cockerham, Procurement Technician, of a modification to our cooperative agreement that allowed us a no cost extension of one year to enable us to complete approved projects. This document is found in the appendix of the 20th Quarterly Report.

- We received notification that five annual reports were reviewed and acceptable as written.

Discussions of individual projects funded under this grant follow.

I. Clinical Research Laboratory

A. Introduction

The Clinical Research Laboratory continued to perform testing for USARIEM for many of its studies. In the past six years the laboratory grew from six to twenty two employees. This growth was necessary to keep up with the increasing demands placed upon the laboratory by our in-house clinical activity as well as Army studies. New equipment was also acquired to handle this increasing workload. This includes the following for the clinical lab: Beckman Array Rate Nephelometer, an Abbott IMx enzyme immunoanalyzers, a Beckman CX7 automated chemistry analyzer, a Beckman CX5 automated chemistry analyzer, an Instrument Laboratories ACL 3000+ coagulation instrument, a Bio Rad ELISA reader and plate washer, a DPC Immulite, and general laboratory equipment, refrigerators, centrifuge, and a laboratory computer system. The Food Analysis Laboratory became operational during this time period. Equipment obtained for the food lab includes the following: a Perkin Elmer nitrogen analyzer, a CEM microwave for moisture determination, a CEM microwave for ash, Soxtek fat extraction system, two GLCs for cholesterol and fatty acids, two HPLCs for vitamins and carbohydrates, a Hewlett Packard capillary electrophoresis system, and general laboratory equipment.

Due to the growth of the laboratory, it was reorganized in mid-1995. Dr. Tulley continued to serve as the laboratory director and director in charge of Personnel, Business, and Continuing Education. Dr. Rood became director of Technical Services. Managing these activities were Joanie Wilson and Ken Smith (Business and Continuing Education), Janaki Vaidyanathan (Technical Services), and Mendy Richard and Deonne Bodin (Research Facilitator). The Research Facilitator coordinates the implementation of lab aspects of research projects (including Army) and manages the Good Laboratory Practices of the laboratory. Dr. Tulley served as coordinator for regular army studies; Dr. Rood was coordinator for women army studies.

Ken Smith took over the duty of Business Manager in 1997 and Deonne Bodin became Research Facilitator, replacing Joanie Wilson and Mendy Richard, respectively.

In the Food Analysis Laboratory, Regina Louviere, one of our Research Associates, resigned and was replaced by Dianne Ratcliff.

B. Body

Early studies included the Ranger 2 study, Ranger 2.5, Pikes Peak study, New Generation Survival Ration Study, Metabolic Variation Study, and the Fort Jackson Women's Study. A total of 57 different tests were run for these studies.

Studies (and tests) in which samples were also analyzed include Hot Weather Field Study (chem 24, ferritin, B12, folate transferrin, RBC folate, prealbumin, 24 hydroxy vitamin D, lactate, NEFA, BHBA, and red cell EAST, ETK, and EGR), Antioxidant Study (vitamins A, E, C, and selenium), Menu Modification (cholesterol, HDL, folate, vitamin E, vitamin A, vitamin C, B12, ferritin, transferrin, and retinol binding protein), Sleep Deprivation (insulin, T3, LH, melatonin, screening tests, and urine creatinine), Navy Seal Caffeine studies (salivary caffeine), Glycemic Index (insulin), Ranger 3 (amino acids), AMS Glycerol Study (bromide), and the SOF study. Samples were also analyzed for the SAFS-5 study, and the Banderet Tyrosine Study (catecholamines, prolactin, lactate, glucose, and insulin).

Representatives from the Clinical Laboratory attended blood draws for four blood draws for the Ranger 3 study, three draws for the Sergeant Major Academy Nutritional Survey, two draws for the SAFS-6 study, the Ranger Regiment Nutritional Survey (one draw), the IDNS study (one draw), and the CASH study (one draw).

Women's studies analyzed included the study "Assessment of the relationship between iron status, dietary intake, physical endurance, and mood state of female army soldiers in a basic population" (IDNS). We received two sets of samples for a total of 112 samples. For both blood draws in San Antonio, Texas, the Clinical Research Lab at PBRC sent representatives to process the blood at the site. Blood samples were analyzed for magnesium, iron, total iron binding capacity, vitamin C, ferritin, haptoglobin, transferrin, serum folate, whole blood folate, vitamin B12, retinol binding protein, copper and zinc.

We completed analyses for the Women's study entitled "The effect of pregnancy on the performance, health, and nutritional status of postpartum soldiers" (Return to Fitness). We continued to receive samples through August 1996. For this study we measured albumin, calcium, phosphorus, iron, transferrin, transferrin saturation, total iron binding capacity, ferritin, transferrin, folate, vitamin B12, vitamin D, and PTH in serum. In urine, we analyzed creatinine, phosphorus, calcium, and deoxypyridinoline.

Work was completed on the study entitled "The prevalence of negative iron nutriture and relationship with folate nutriture, immunocompetence, and fitness level in US Army servicewomen"(WISP). Four sets of samples for a total of 1141 were sent to PBRC. We analyzed the samples for iron, total iron binding capacity, ferritin, haptoglobin, transferrin, transferrin saturation, serum folate, whole blood folate, and vitamin B12. We purchased a hematofluorometer which was transported to the field sites for measurement of erythrocyte protoporphyrin. We completed 10,269 tests for this study.

Results for the majority of the chemistries ordered for the Sergeant Major's Academy study (first, second, and third draws) were completed. Lipids were run on approximately 500 soldiers at three draws and 24 tests on approximately 300 soldiers at three draws for a grant total of approximately 27,600 tests. Additional analyses for the Sergeant Major's Academy Nutritional Survey study were completed. These included C Reactive Protein, vitamin B12, and folate. Reactive Proteins were also requested by Major Bill Karge on the Sergeant Major's Academy Study. These were run but showed very low values.

The analysis of Vitamins A and E were completed for the Sergeant Major's Academy Study and the SAFS-5 study.

In addition, red cell enzyme assays for the CASH study and for the Hot Weather Feeding Study were completed.

Although the majority of tests have been completed, studies which still have one or more tests pending include the Ranger Regiment Nutritional Survey (SAFS-5) (homocysteine), Sergeant Major's Academy (homocysteine), the SOF2 study (RBC Enzymes), and the CASH study (homocysteine, carotenoids, and vitamins A and E). Homocysteine and carotenoid methods are awaiting method development and vitamins A and E will utilize the same sample so have not been analyzed yet. These projects will be completed as part of a subsequent grant.

Data was completed for statistical analysis for the Sleep Deprivation Study.

The Food Analysis Laboratory analyzed total and soluble dietary fiber for thirteen foods for the SAFS-5 study. This was done only following extensive method development .

A new nitrogen analyzer was put out for bid because results using our old analyzer were not consistent. In addition, our analyzer was not supported by its manufacturer due to its age. We decided that a new analyzer should therefore be ordered.

A shipment of samples from two studies was received in January. Major Jeff Kennedy sent samples from the Ranger 4 study and SAFS-6 study. He asked for us to run TSH, T3 free and total, T4 free and total, TBG, GH, IGF-1, liver function tests, cholesterol, HDL, triglycerides, vitamin A, vitamin E, folate, beta carotene, sodium, potassium, calcium, BUN, and glucose on both studies. We are still awaiting word from Major Karge on the status of these samples.

R&D and Method Development

Vitamins A and E. An HPLC method which had previously been used for vitamin A and retinyl palmitate was adapted to analyze vitamin E in the same run. Vitamin A is measured at 1.2 minutes at 320 nm and vitamin E at 2.6 minutes at 292 nm. Mean recoveries were 90%, 88%, and 96% for retinol, retinyl palmitate, and alpha tocopherol, respectively. CV's were less than 5% for vitamins A and E. Studies were conducted to determine stability of vitamins A and E to light. Results showed stability for up to at least 3 hours in normal laboratory lighting.

Selenium. A method for the analysis of selenium by graphite furnace atomic absorption was set up for the army. The non identity of the slope between the standard additions curve and that of aqueous standards makes it necessary to prepare blood based standards.

Blood based standards were prepared by pooling whole blood, spiking different levels, and preparing standard additions curves. Composite standard additions curves were used to determine the endogenous selenium in the pool. Adding this amount to each level spiked gave the total selenium concentration in each blood based standards. Performing standard additions on six different blood samples shows identical slopes. This shows that using a blood based pool is adequate. Precision of analysis is 2-3% for within run and less than 3% for day to day. Recovery of selenium from spiked samples was 104%.

RBC Enzymes. Studies were performed to improve performance of the three red cell enzyme assays. Reproducibility and accuracy were enhanced by optimizing reaction conditions.

Amino Acids. Work was completed on the development of a method of analysis of amino acids by HPLC with pre-column derivatization with OPA. Analyses for Ranger 3 amino acids were completed using this method.

Pyruvate. A method for pyruvate on microtiter plates was developed. The method showed good linearity and precision.

Catecholamines by RIA. A correlation study of a new catecholamine method by RIA proved disappointing, therefore catecholamines were analyzed by our old HPLC method for this study.

Vitamin C. A method for the analysis of vitamin C by capillary electrophoresis was developed. Excellent speed and resolution were achieved. Sensitivity is approximately 4-5 mg/L, limiting this method for low values, however, the method could be used as a discriminator between normal and low nutriture. An attempt to improve sensitivity was made by using Hewlett Packard's new high sensitivity flow cell, however, results have not been completely satisfactory to this point. It is hoped that correlation studies will be conducted between the capillary electrophoresis method and the in-house developed automated method.

Homocysteine. A method for the analysis of homocysteine by capillary electrophoresis was investigated. The first method tried was an indirect method using dimethyl aminobenzoate and alpha cyclodextran. This method was able to resolve homocysteine, however, appears to suffer from lack of sensitivity. We hope to continue to investigate methods for homocysteine by capillary electrophoresis.

Work was done on the development of a method of analysis of homocysteine by HPLC. This method showed great promise. Separation and resolution were very good. We were at the point of performing precision and recovery studies when the column had to be replaced. Results with this new column, however, were disappointing. Even though this column was identical in type, order number, etc., the column packing was of a different batch and did not exhibit the resolution of the first column. We are awaiting a replacement column from the company to continue our development.

3-Methyl Hisitidine. A method for the analysis of 3-methyl histidine using our amino acid method of HPLC showed what appeared to be good separation and recovery but gave disappointing results. Results showed values which were higher than expected physiologically. We believe there

are interfering substances present. This project was put on indefinite hold until more man-power is available for the method development.

Nitrogens. The new nitrogen analyzer was put in use and doing very well for urine nitrogens; however, it is still having problems with digested samples (fecal and food). The problem is apparently due to the catalyst or strong acid used in the digestion. Since this instrument uses a direct needle injection rather than a boat as in the old instrument, there are problems with the sample needle getting quickly blackened. For some reason very low counts are being obtained on these samples. We are still working with the company to solve the problem.

Specimen Integrity Project. We completed a year long study on sample stability at -4oC and -80oC for over fifty analytes. Results of this study will be forthcoming pending data analysis.

C. Conclusions

Tests performed by the Clinical Research Lab for the army since the inception of this grant include the following:

General Chemistry
Chem 26 panel
urine creatinine
albumin
iron
TIBC
glucose
HDL
cholesterol
triglyceride

Metabolites
lactate
free fatty acids
BHBA
amino acids
catecholamines

Minerals/Trace Elements
calcium
phosphorus
selenium
copper
zinc

Hormones
PTH
growth hormone
melatonin
cortisol

insulin
ACTH

Vitamins
25 OH vitamin D
folate
RBC folate
vitamin B12
vitamin A
vitamin E
vitamin C
RBC enzymes

Proteins
transferrin
ferritin
haptoglobin
retinol binding protein
prealbumin

Miscellaneous
CBC
urinalysis
caffeine
bromide
deoxypyridinoline
nitrogen

Services which the laboratory has provided include:
- Help in design & implementation of studies
  - metabolic variation
  - sleep deprivation
  - women's studies
- Consultation on sample processing/storage/shipping
  - all studies
- Help in processing of samples
  - Ranger 2 study
  - Metabolic Variation
  - Sleep Deprivation
  - IDNS
  - Ranger 3 study
  - CASH
  - Sergent Major's Academy study
  - Ranger Regiment Nutritional Assessment Study

- Reporting of results
  - hard copy
  - compiled data in database

- Help in manuscript writing
- Original method development
    Adapted to automation:
        NEFA
        glycerol
        BHBA
        lactate
        ammonia
        amino acids/tyrosine
        RBC enzymes (transketolase, glutathione reductase, aspartate aminotransferase)
        bromide
        vitamins A/E
        salivary caffeine
        retinol binding protein
    Original methods
        urinary caffeine
        vitamin C
        ICP analysis of Na, K, P, Mg, Ca in food, urine
    Methods set up for army
        food & urine nitrogen
        selenium
        salivary melatonin
        ACTH
        25 OH vitamin D
        RBC folate
        vitamin B12
        folate
        catecholamines
        ferritin
        transferrin
        haptoglobin
        prealbumin

Work was performed on a total of 25 studies between 1992-8. The laboratory was expanded in personnel and equipment in order to handle this workload. Our estimation is of successful completion of this task.

D. References

None.

II. Stable Isotope Laboratory

A. Introduction

The research conducted by the Stable Isotope Laboratory is in the area of energy and water requirements, and changes in body water, of soldiers, often under harsh environmental conditions. The method used to determine energy requirements is the doubly labeled water (DLW) technique, which involves oral administration of water labeled with the stable isotopes, ^{2}H and ^{18}O. Saliva and urine samples are then obtained for periods of 4-14 days, longer with redosing. Water intake can be determined using only the ^{2}H labeled water.

The use of doubly labeled water for measurement of energy expenditure was developed as a field technique for use in small animals (1). The method is based on the premise that after a loading dose of $^{2}H_{2}{}^{18}O$, ^{18}O is eliminated as CO_2 and water, while deuterium is eliminated from the body as water. The rate of CO_2 production, and, hence, energy expenditure, is calculated from the difference of the two elimination rates. Doubly labeled water, using the two-point method, is an ideal method for use in free-living subjects because it is noninvasive and nonrestrictive. The only requirement of subjects is to give urine and saliva specimens before and after drinking an initial dose of $^{2}H_{2}{}^{18}O$, and then return in one to two weeks to give a final urine specimen. During the period between the two urine and saliva samplings, subjects are free to carry out their normal activities and are not required to maintain extensive diaries.

The doubly labeled water method has been extensively validated in humans under controlled settings (2), but there are confounding factors that need to be considered in field studies, particularly in Army Field Studies. Among these are change in location or food and water supply immediately preceding, or during an energy expenditure study. These changes may cause a change in baseline isotope abundance and, therefore, interfere with the accuracy of the energy expenditure measurement. This has occurred in a previous field training exercise involving the study of the MRE and RLW rations (3). This is a particular problem with studies such as the Ranger Training Studies (4), in which soldiers are moved to different parts of the country during the study. Therefore, a group not receiving labeled water must be followed to make any corrections in baseline isotope shifts.

Hydration status is another main focus for some Army studies. Using the cheaper and more readily available deuterium tracer, either changes in total body water (5,6) can be followed during a study, or water turnover (intake) (7,8) can be measured during a study.

One advantage of the DLW method is that it uses stable isotopes so there is no radiation exposure. The method uses two heavy isotopes of water, which are naturally occurring in food and water. There are no known side effects of either isotope at the doses given in DLW studies and has been used extensively to study energy expenditure during pregnancy (10,11) lactating women (12), and infants for measurement of energy expenditure and human milk intake (13-15).

The Stable Isotope Lab was involved in many Army research projects during the current grant. These are described below.

B. Body

Pikes Peak 92

Deuterium analyses for the Pikes Peak 92 Study samples for total body water and water turnover measurements were completed. Data were reported in the 3rd Quarterly Report and sent to Tanya Jones for further calculations. This data was included in a paper presented at the Experimental Biology 93 meeting in New Orleans by Tanya Jones.

Ranger 92

Deuterium analyses for the Ranger 92 Study samples for energy expenditure were included in the 3rd Quarterly Report. The ^{18}O data, elimination rate calculations and dilution space measurements, as well as the deuterium elimination rate and energy expenditure data are included in the 4th Quarterly Report. Preliminary energy expenditure calculations for the Rangers 92 Study were prepared for presentation at a meeting. Energy expenditures during this Ranger study were very similar to those observed in the previous Ranger study. As was the problem with the first study, water turnovers were so high during the Fort Benning Phase, that the isotopes washed out quickly, causing inaccuracies in the later part of the Phase.

Energy calculations using isotope data corrected for baseline isotope shifts were calculated for the Ranger 92 Study. Energy expenditures for this study were similar to that observed in the previous Ranger study, with the exception that the energy expenditure during the Mountain phase was considerably lower.

	Fort Benning	Mountain "Classes"	Mountain FTX	Jungle	Desert
Ranger 1	4200	6045	4540	3770	4330
Ranger 2 (92)	4160	5200	3240	4240	4080

IDF Medical Corps

Isotope analyses were completed for two IDF studies. Major Burstein of the IDF Medical Corps, Institute of Military Physiology came to the Pennington Center to discuss the results of the analyses of ^{18}O and ^{2}H for the Summer Study. Isotope results, elimination rates, total body water and preliminary energy expenditures by the two point and regression methods were given in the appendix of the 5th quarterly report. The other study completed was a winter study.

SFAS

The Special Forces Assessment and Selection course is designed to test Special Forces volunteers bodies and minds to determine if they can operate effectively as individuals and team members under prolonged periods of stress. The procedures involve administration of mental, learning and personality tests, physical challenges, and mental challenges in a field environment. The 21 day course consists of two separate phases. One portion of a "Nutritional Assessment of Soldiers During the Special Forces Assessment and Selection Course" study was to assess energy

expenditure. To carry out this assessment, DLW was administered to 14 soldiers. Eight soldiers were studied in each phase, with only two soldiers completing both phases. Detailed energy expenditure data is included in the 8th Quarterly Report. The energy expenditure during each phase was quite similar with a mean of 5270±600 kcal/day during the first phase and 5260±960 during the second phase.

Pikes Peak '93

The dehydration associated with high altitude acclimatization (HAA) was examined in the Pikes Peak study. Total body water measured with deuterium dilution in 10 male lowlanders at sea level, during 16 days of HAA at 4300 m, and after return to sea level. A decrease in TBW was observed within one day at altitude (49.1±50.3 l vs 50.9±5.0 l at sea level) and a further decrease was observed with chronic exposure (47.5±4.2 liters). TBW levels remained below sea level values even after 6 days after return to sea level (49.0±5.1 l).

PERC

The PERC study involved several measurements of total body water. Complete isotope analyses were included in the 7th annual report.

Marine Corps Mountain Warfare Training

A USARIEM study was conducted at the Marine Corps Mountain Warfare Training Center in Bridgeport, CA, from 1 to 13 September 1994. A subset of 18 soldiers was dosed with doubly labeled water for measurement of energy expenditure. An additional 6 subjects not receiving doubly labeled water were also studied to make any necessary corrections for drinking water changes that would affect background isotope abundance. A total of four subjects did not finish the study, 3 labeled and 1 placebo subject. One of the dosed subjects did not drop out until half the study was over, so some useful data will be obtained from this subject. Results from isotope analyses for the 5 placebo subjects demonstrated a significant decrease in deuterium and ^{18}O abundance of 25.0 and 1.97 o/oo of the course of the study. The isotope data from the placebo group were used to correct for the significant decline in isotope abundance observed. The average energy expenditure for this study was 5185±840 kcal/day.

Erythrocyte infusion effects on fluid redistribution at high altitude

Results of a study designed to examine the effects of erythrocyte infusion on changes in total body water, extracellular and intracellular fluid shifts at altitude were reported by Tim Lyons at the Experimental Biology 95 meeting in Atlanta (9).

Emerging Technologies For Nutrition Research

Dr. DeLany was invited to speak at a workshop sponsored by the Committee on Military Nutrition Research titled "Emerging Technologies For Nutrition Research: Potential For Assessing Military Performance Capability." A copy of the agenda appears in the Appendix of the 13th Quarterly Report. Dr. DeLany gave a presentation in the "Advanced Tracer Techniques and Metabolism" session entitled "Doubly-Labeled Water for Energy Expenditure," and prepared a chapter for the CMNR Report that came out of this conference (16).

Chocolate Mountain Study

A USARIEM extreme environment field feeding test was conducted at Chocolate Mountain. It was anticipated that the desert temperatures would reach 100 F during the day, and drop to about 40 F during the nights. The study was 10 to 11 days in length, and dosing began about October 8, 1994. A subset of 20 soldiers were dosed with isotope with an additional 5 serving as placebo subjects. Of the 20 dosed subjects, 10 consumed a high carbohydrate drink, and the other 10 consumed a placebo drink. Stable isotopes (deuterium and ^{18}O) were administered for the assessment of water turnover and energy expenditure. Mean declines in isotope abundance over time for the placebo subjects were reported in the Appendix in the 13th qtr rep. These data were used to correct isotope data in those receiving the heavy water, for calculation of water turnover and energy expenditure.

Energy Expenditure Data for the dosed subjects is given in the table below. The individual data are reported in the Appendix in the 14th quarterly report.

Day	*Average Energy Expenditure*
6	3990±980
10	4200±1570
12	4020±1240

Camp Parks Study

A study titled "The effects of diet composition on food intake, food selection and water balance in a hot environment" was conducted at Camp Parks Reserve Training Area, CA. The primary purpose of this study is to assess whether current military rations meet the nutritional needs of soldiers in hot environments. Water turnover and total body water changes were measured using deuterium labeled water. Ten male and 17 female subjects were dosed. There appeared to be a sample or dosing problem for subject #1969. Even upon reanalysis this subject's TBW was 30.4 kg at the beginning and 43.0 kg at the end of the period. There were no changes due to repeat analyses, so the data presented in the 15th Quarterly Report are still the final TBW and deuterium elimination rates for calculation of water turnover. The raw data are presented in the Appendix in the 16th quarterly report.

USARIEM Altitude Chamber Studies

Two projects were conducted in the USARIEM altitude chambers. These accumulated samples were sent to the Stable Isotope Lab for analyses. The two studies were the Acute Mountain Sickness Glycerol (ACM) 95 study on men and the Women at Altitude 95 study on AMS and the menstrual cycle. These two studies are outlined below:

I. Women at Altitude 95: PI, LTC Rock

A. 160 saliva samples for deuterium analyses

This study included samples from three different phases of the menstrual cycle; early

follicular, late follicular and mid-leuteal. Four samples were taken for each period, a 6 am and 10 am sample at sea level and a 6 am and 10 am sample after 24 hours at altitude. Therefore there were 12 samples for each subject. Isotope analyses for calculation of total body water were completed. A summary Table was included in the 17th Quarterly Report, with raw data presented in the Appendix of that Report.

II. Acute Mountain Sickness 95: PI, CPT Lyons

A. 94 saliva samples for deuterium and bromide analyses

This study includes six samples per subject per trial, with placebo or with experimental glycerol solution, for total body water by deuterium dilution and extracellular water by bromide dilution. Samples were taken at 6 am and 10 am after an overnight fast at sea level, high altitude day 2 and high altitude day 4. Thus there are 12 samples for each subject. Isotope analyses for this study were completed and given in a Table in the 18th Quarterly Report.

Greenland Expedition

The isotope analyses for the Greenland Expedition were completed and presented in the 19th Quarterly report. The energy expenditure values for the two subjects by period are given in the table below. There was a problem with the later samples (13 and 15 day) for the third period for both subjects.

Energy Expenditure, kcal/d

		Subject	
Period	day	R	T
1	7	3250	3380
	13	4700	5350
	15	5250	6170
2	7	3970	3900
	11	3790	3600
	13	3750	3790
3	7	4713	4940

Norwegian Ranger Study

Isotope analyses for the Norwegian Ranger Study were completed and reported in the 20th Quarterly Report. The deuterium and Oxygen-18 baseline isotope shifts for the two placebo groups were calculated to correct the isotope data for the labeled subjects. The energy expenditure values for subjects by period are given in the table below. The raw data for TBW determinations and deuterium and O-18 elimination rates are presented in the Appendix of the 20th Quarterly Report.

	Energy Expenditure	
	06/24/96	06/24/96
Mean	5941	6232
SD	648	665

C. Conclusions

The doubly labeled water method continues to be a valuable tool for examining energy requirements of soldiers in a variety of settings, from garrison to standard field training exercises to exercises under extreme conditions that military personnel undergo for training. The DLW method is the only method to provide truly free living energy expenditure under the conditions of military training. In the studies conducted over the past several years, energy expenditures between 3200 and 6200 kcal/d have been measured, under various environmental and training conditions. Within a single study energy expenditures can vary widely. In the Ranger 92 Study for example, energy expenditure varies between 3200 and 5200 kcal/d, depending on the phase of the study. Very high energy expenditures have been observed during the Mountain Phase of the Ranger study (5200 kcal/d), the SFAS study (5270 kcal/d), the mountain warfare study (5200 kcal/d), the Greenland Expedition study (4000-6000kcal/d) with the highest energy expenditures observed during the Norwegian Ranger Study (5940 and 6230 kcal/d).

The DLW method also provides measures of total body water (TBW) and water turnover. In fact, administration of the cheaper, and easily obtained deuterium alone can provide this information. Several studies were conducted in which deuterium was given alone, and TBW and/or water turnover were studied, particularly as they pertain to adjustments to altitude or hot environments. The Pikes Peak Study, the erythrocyte infusion study, the Camp Parks Study and the two Altitude Chamber studies at USARIEM are examples.

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III. Basic Neuroscience Laboratory

A. Introduction

The objective of this project is to identify nutritional interventions that will minimize, or prevent, negative behavioral responses to stress in animal models. We utilize two different

approaches in our research. The first is to use existing literature to identify potentially beneficial interventions that can be applied to our models of stress-induced behavior. The second is to investigate the mechanisms responsible for the changes in behavior of stressed rodents, with the goal of using this information to identify potential nutritional supplements that would modulate these mechanisms. There have been a number of personnel changes over time on this project but the current group possesses a diverse range of research skills that allow us to determine the molecular, biochemical and neurochemical mechanisms associated with animal behavior.

We have developed a number of reliable rodent models that provide robust and repeatable stress-induced behaviors. Currently we are focusing on four primary areas of research that are described below as ongoing projects.

B. Body

STRESS AND TASTE PREFERENCE

Leigh Anne Howell, Crystal Clarke, Bradley Youngblood, Tim Gilbertson and Ruth Harris

The objective of this project was to elucidate the effects of stress on taste preference in rodents. There is a significant amount of literature indicating that stressed rats lose their preference for sweet solutions (Blass et al., 1986; Plaznik et al., 1989; Mathews et al., 1995) but there is little information on other tastants or other rodents (Kuta et al., 1984). As sweet taste is usually associated with delivery of nutrients and stressed rats have a depressed appetite (Marti et al., 1994; Dess et al., 1989) it is possible that the depression of sweet preference is secondary to a reduced desire for food. Alternatively, the change in taste preference could be independent of satiety. In our experiments taste preference for sweet, sour, bitter and acid solutions was tested in rats and hamsters following either a single, acute 1 hour restraint or repeated restraint. The results demonstrated that the effect of stress on taste preference was not specific to sweet solutions. In rats the response was limited to preferred solutions whereas hamsters gave a stress-related response only when offered an aversive tastant. This suggests that, in hamsters, the response is not simply an end point measure of anhedonia. The results from the experiments with repeated stress indicated that by offering the rats the saccharin solution close to the start of the period of restraint a conditioned aversion was induced with restraint stress acting as the conditioning stimulus. The minimal change in sweet preference when the rats were offered tastant after the end of the restraint period imply that the loss of preference is a weak behavioral effect and is not reliable enough for us to use as a behavioral marker of stress. The results from these experiments are described in a manuscript that has been submitted to Physiology and Behavior.

The Effect Of Chronic Mild Stress On Saccharin Preference and Open Field Activity

Ruth Harris and Jun Zhou

The objective of this study was to explore an alternative model of chronic stress that induces behavioral changes. Chronic mild unpredictable stress (CMS) involves subjecting animals to a variety of stressors in an unpredictable manner, to prevent habituation or adaptation to the stress. This has been reported to result in depression, behavioral changes typical of anxiety and loss of preference for sweet taste (Willner et al., 1992; Mathews et al., 1994). We conducted a series of studies, exposing rats to chronic mild stress, and were unable to induce any significant changes in saccharin preference unless the animals were water deprived for 24 hours prior to

preference testing. We concluded that this was not a useful model for our studies as the end point behavior was dependent upon hydration state of the animal. These results are described in a manuscript published in Physiology and Behavior.

The Effect of Repeated 3 Hour Restraint on Spatial Memory
Bradley Youngblood, Molly Nicodemus, Ruth Harris

We have found that the chronic stress of 96 hours of REM sleep deprivation (REMd) causes a significant impairment of spatial reference memory and Luine et al. (1994) have reported that restraint stress impairs spatial memory in a radial arm maze. In order to determine whether this response was specific to the radial arm maze or was measurable in the Place Learning Set Task in a Morris Water Maze we measured spatial working and reference memory in rats that were subjected to 3 hours of restraint stress for up to 5 consecutive days. This was considered an appropriate model because restraint causes a significant increase serum corticosterone and catecholamines (Kvetnansky et al., 1996) and in norepinephrine turnover in a number of brain regions (Keim and Siggs, 1976). Others have reported that serotonin metabolism is increased during restraint stress (Haleem and Parveen, 1994) and may be responsible for the rebound sleep that is induced by stress (Cespuglio et al., 1995). In the REMd rats we have found no change in hypothalamic or hippocampal norepinephrine concentrations or turnover, however, serotonin metabolism is increased in both brain areas, compared with controls.

The results from the Place Learning Set Task for rats exposed to repeated restraint showed that this model of repeated stress does not induce a substantial impairment of reference or working memory in either Wistar or Sprague Dawley rats. Although restraint stress is reported to cause activation we did not observe any change in swimming speed of the rats, which could be considered an index of activation.

Involvement of Central Corticotrophin-Releasing Factor in Memory Processing in the Rat
Gennady Smagin, Bradley Youngblood, Jun Zhou

Corticotrophin-releasing factor (CRF) is a 41 amino acid polypeptide that stimulates the release of ACTH and beta-endorphin from the anterior pituitary gland. In addition to its neuroendocrine functions, CRF has been shown to produce behavioral activation. I.c.v. injection of CRF enhances locomotion and rearing. Acute administration of CRF also affects learning/memory performance in rats as i.c.v. injection improves acquisition/retention of a visual discrimination task (Koob and Bloom, 1985). Intra-amygdala CRF injection improves memory retention of a passive avoidance task (Liang and Lee, 1988) and intra-hippocampal and intra-locus coeruleus injections of CRF dose-dependently enhance retention performance of passive avoidance learning in rats (Chen et al., 1992).

There is little information available concerning the effect of chronic administration of CRF on memory or learning, however, chronic stress, in which the CRF system is activated, is associated with impairments of cognitive function (Luine et al., 1994). Rats deprived of REM sleep for 4 days experience chronic activation of the hypothalamic pituitary axis and have a significant impairment of reference spatial memory. We performed two experiments to investigate the effects of chronic modulation of CRF on spatial memory of rats. In the first study, male Wistar rats received 5 days infusions of 3 ug or 5 ug CRF/24 hours. These levels of

CRF raised circulating concentrations of corticosterone but did not cause significant changes in food intake or in spatial memory. In the second experiment, sleep deprived rats were infused with a CRF receptor antagonist (50 ug ahCRF/24 hours). The antagonist did not reliably suppress stress-induced corticosterone concentrations and may not have been adequate to influence central CRF concentrations. There was no beneficial effect of the antagonist in relation to the physiological responses or spatial memory of REMd rats.

The Effect of Dietary Vitamin E on Stress-Induced Behaviors

Jun Zhou

It is well established that stress can impair immune function (Keller et al., 1981) and that antioxidants can improve immune function (Bendich et al, 1986) and may also protect against exercise induced oxidative stress (Goldfarb, 1992). In stressed animals corticosterone increases catecholamine, serotonin (5-HT) and 5-hydroxyindolacetic acid (5-HIAA) concentrations in many areas of the brain including the hypothalamus, hippocampus and cortex (Jhanwar-Uniyal et al., 1987; Hastings et al., 1996). Increased metabolism of these neurotransmitters has been associated with oxidative damage and neuronal degeneration (Hastings et al., 1996) and has been implicated in the development of several neuro-degenerative diseases that occur with increased frequency during aging (Coyle and Puttfarcken, 1993). Based on these observations we conducted a study to examine the effect of dietary antioxidant on behavioral responses to a variety of stressors in rats.

Male Wistar rats (300g) were divided into 3 groups matched for average body weight. They were fed ad libitum one of three diets containing different concentrations of the antioxidant, Vitamin E. The diet was a tocopherol-free diet (ICN) supplemented with (+)-a-tocopherol acid succinate (Sigma Chemicals). The lowest level of 30 IU/Kg Vit E met AIN guidelines for rodent diets, the intermediate concentration of 75 IU/Kg met NRC guidelines and the highest dose of 150 IU/Kg doubled this amount. The rats were fed this diet for 5 weeks and were then tested in three different stress-behavior conditions: (1) The effect of acute restraint stress on open field activity and food intake. (2) The effect of 48 hours of REMd on spatial memory. (3) The effect of repeated restraint on food intake and weight gain. The results from this experiment suggested that varying dietary Vit E concentration above or below NRC recommended levels (75 iu/Kg) influenced the behavioral response to chronic stress. There was no effect of diet composition on measures of anxiety when the rats were exposed to a single, acute, three hours restraint. However, when the rats were subjected to the chronic mixed stress of 48 hours of sleep deprivation both the low and high doses of Vit E exaggerated deficits in spatial memory of the rats. Therefore, although antioxidants may protect the brain from oxidative damage associated with increased metabolism of catecholamines and serotonin, excessive levels of anti-oxidant may also have negative effects on cognitive performance.

In this experiment we adapted rats to an experimental diet, supplemented with antioxidants and then tested their response to a series of stressors. We anticipated that this would allow us to evaluate the effects of specific dietary manipulation on various aspects of stress-induced behavioral changes. However, some of the stressors induced prolonged, or irreversible, changes in the rats which had the potential to confound the response to a subsequent stress. Therefore, all future studies involved animals that had not been exposed to any previous stressor.

Stress and Appetitive Behavior

Bradley Youngblood and Ruth Harris

Operant schedule-controlled behavior has been used to evaluate the effects variety of drugs, brain lesions and thermal stressors (Ahlers and Salander, 1993; Ahlers et al., 1992; Gauvin et al., 1994; Cancela et al., 1996). Rats have to be food restricted prior to being trained to lever press to receive food pellets. There is no information available on the effects of acute stress on appetitive operant behavior in rats or on whether stress inhibits food intake of restricted rats, however, it is well documented that stress suppresses food intake of ad libitum fed rats (Michajlovski et al., 1988). The studies described here used a small fixed-ratio (FR15 or FR5) schedule of reinforcement for rats, eliminating post-reinforcement pausing, resulting in a maximal rate of rapid responding with frequent reinforcements. We determined the effect of acute restraint stress, or restraint stress combined with partial water immersion, on performance of the rats in the operant task and on free-feeding behavior to determine whether this would be useful model for investigating the effects of stress on motor performance.

In an initial experiment we found that rats trained to an FR5 schedule and exposed to 15 minutes of restraint plus partial water immersion (RWI) stress significantly suppressed response rate and reinforcers earned in rats but the stress did not affect free feeding food intake. Injection of either saline or 100 mg/kg L-tyrosine 30 minutes before 15 minutes of RWI stress did not prevent the stress-induced suppression of operant behavior and there was no effect of stress or tyrosine on any monoamine or metabolite measured in the striatum. In a second experiment we found that tyrosine injected immediately after RWI did not attenuate the stress induced suppression of operant motor performance. The results from these studies have been published in Physiology and Behavior. We are not pursuing this model as the animals take a number of weeks to train prior to being included in the experiments making this a very work-intensive paradigm.

The Effect of Dietary Interventions on Spatial Memory of Rapid Eye Sleep Deprived (REMd) Rats

Bradley Youngblood, David Elkins, Jun Zhou, Gennady Smagin and Ruth Harris

We have established that 96 hours of REMd, induced by the "flower-pot" technique (Mendelson et al., 1974) results in a substantial impairment of reference spatial memory in rats, measured in a Place Learning Set Task in the Morris Water Maze (Youngblood et al., 1997). In the first dietary manipulation studies that we completed we determined whether changing the macronutrient composition of the diet would have any beneficial effect on the REMd rats. Everson and Wehr (1993) reported that survival of sleep deprived rats was improved by feeding a high energy diet but no behavioral data was reported. Therefore, two experiments were completed to determine whether a high-fat diet or a macronutrient self-selection diet improved spatial memory in REMd rats. In the first experiment rats were offered either a high carbohydrate diet (60 % kcal CHO, 28 % Kcal fat) or a high fat diet (54% kcal fat, 33% kcal CHO). There was no effect of diet composition on spatial memory of the rats although 96 hours of REMd caused a significant impairment of both working and reference spatial memory. In the second experiment the rats were allowed to select their macronutrient intakes from fat, protein and carbohydrate diets offered simultaneously. The stressed rats reduced their intake of fat and increased carbohydrate intake, but there was no beneficial effect of macronutrient selection on

behavioral or physiological responses to REMd.

A consistent finding in our REMd studies is that central serotonin metabolism, measured as the 5-HIAA:5-HT ratio, is increased in several brain areas, including the hippocampus, hypothalamus and brain stem. Changes in central serotonin metabolism in response to stress are well documented. Central serotonin concentrations and metabolism are stimulated by corticosterone (Jhanwar-Uniyal et al., 1987; Luine et al., 1993) and reduced in adrenalectomized rats (Jhanwar-Uniyal et al., 1987). Luine et al. (1993) also reported that the corticosterone-induced change in serotonin metabolism may be associated with impaired spatial memory, measured in a radial arm maze. Recently , Graeff et al., (1996) have proposed that the increased serotonin metabolism is an adaptive response to stress that attenuates stress.

We have conducted several experiments attempting to modulate central serotonin metabolism in REMd rats by altering availability of the serotonin precursor, tryptophan. In the first experiment we tried to reduce tryptophan availability by feeding a diet containing a high concentration of leucine, an amino acid that competes for tryptophan transport at the blood brain barrier. In the second experiment we attempted to increase tryptophan uptake by increasing dietary tryptophan concentration.

Rats were fed control diet or a diet containing 3% leucine for 3 days prior to being exposed to 96 hours of sleep deprivation. In rats fed control diet, there was no effect of treatment on performance in trial 1, representative of reference memory until day 4 when REMd rats had a significantly impaired spatial memory compared with either TC or cage control rats. In trial 2, indicative of working memory, there was no significant effect of treatment on any of the 3 days of testing. In rats fed the l-leucine diet there was a significant effect of treatment on reference memory on all three days. On day 2 both TC and REMd rats performed less well than cage controls but on days 3 and 4 only the REMd rats were significantly impaired compared with cage controls. There was no effect of diet on weight change, fat pad weight, food intake, rectal temperature or thymus weights of the rats although both TC and REMd rats lost considerable amounts of weight, had elevated body temperatures and had reduced thymus weights compared with cage controls. The L-leucine diet caused a significant increase in serum corticosterone in both the TC and REMd rats but not the cage controls. As corticosterone was already elevated in these animals, it suggests that the L-leucine diet exacerbated the stress response. There was no effect of diet on central concentrations of tryptophan or serotonin turnover. In a second experiment we attempted to increase central concentrations of serotonin by supplementing the diet with 3% tryptophan, the precursor for serotonin. The tryptophan diet reduced food intake and suppressed weight gain in control rats. All stressed rats lost similar amounts of weight. Tryptophan also caused a reduction in thymus weight of all treatment groups, suggesting an exaggeration of the response to stress. There were no beneficial effect of tryptophan supplement on working or reference memory of the rats and there was no effect of diet on central concentrations of tryptophan or on serotonin turnover.

We concluded that some of the negative effects of these diets resulted from feeding a high protein diet with an unbalanced amino acid composition. Therefore, we determined the minimal amount of amino acids needed in the control diet that would support normal growth in the rats used in our REMd studies. By reducing the amount of total amino acids present in our baseline diet it would improve the probability of inducing changes with amino acid supplements. We found that a diet containing 60 % of the NRC requirements for young, growing animals is

adequate to support normal growth in adult male rats. Research Diets have formulated a liquid diet that maintains the amino acid : energy ratio of our 60% diet and we now use this liquid diet as the control diet for REMd studies.

We have carried out two REMd studies using the new diet. The first was a second attempt to modulate central concentrations of serotonin by supplementing the diet to 2.4% valine, an amino acid that competes with trytophan for transport across the blood brain barrier. The results of the study demonstrated that supplementation of the diet with valine did result in a small decrease in brain tryptophan concentrations but did not influence serotonin concentrations . The limitation of tryptophan transport did not prevent stress-induced increases of serotonin metabolism, suggesting that the increased presence of 5-HIAA is due to a suppression of 5-HT and 5-HIAA clearance, rather than increased 5-HT synthesis. The valine supplemented diet had no beneficial effects on either spatial memory or the physiological response of the rats exposed to sleep deprivation. Therefore, although we did manage to produce a small modulation of brain tryptophan it is still not possible to determine, whether, or not, the increased rate of serotonin metabolism is responsible for stress-induced changes in spatial memory.

In a previous study, with rats exposed to restraint stress, we found that dietary histidine, the amino acid precursor for histamine, improved spatial memory of control, but not stressed rats. In this study we determined whether supplementing the diet with histidine had any beneficial effects on memory or physiology of rats exposed to REMd.
Rats were adapted to control diet or the same diet supplemented to 4.5%, by weight, histidine for 4 days prior to exposure to 96 hours REMd. When considering the last 3 trials of the Place Learning Set task, supplementing the diet with histidine totally prevented any SD induced impairment of reference memory on day 2 of REMd. On the two subsequent days of sleep deprivation there were no significant effects of stress or diet on memory of the rats. Supplementing the diet with histidine did not prevent the stress-induced loss of body weight, elevation of body temperature, elevation of serum corticosterone or decline in serum leptin concentrations. Histidine also had an independent inhibitory effect on food intake, consistent with reports that central histamine suppresses feeding in rats (Mercer et al., 1990; Ookuma et al, 1987).

We conducted a dose-response study, feeding rats diets of increasing histidine concentration, to determine whether concentrations lower than 4.5% moderated the effect of sleep deprivation on memory of REMd rats. Animals were allocated to one of 8 treatments groups: cage control or REMd fed diets containing 0.24 (control), 1.125, 2.25 or 4.5% histidine by weight. After 4 days adaptation to the diet the REMd rats were placed in sleep deprivation cages for 48 hours. Results from the Place Learning set task, performed after 48 hours of REMd, indicated a significant effect of both diet and stress on reference spatial memory. Histidine had an independent negative effect on memory which was not additive to the stress-induced impairment. The majority of the increased distance traveled by rats on the histidine diets occurred in set 1 of Trial 1 which implied that they were not learning the task and were not adjusting their search strategy successfully, this may represent an impairment of immediate, or egocentric, memory. In Trial 2 there was a significant effect of stress but no significant effects of diet on working memory. The results from this study indicate that supplementing the diet of rats with histidine, the amino acid precursor of histamine, significantly impairs early events in spatial learning of rats. As there was no additive effect of diet and stress on spatial memory, it is possible that similar pathways are involved in the two responses.

The Involvement of CRF Systems in Feeding Behavioral and Behavioral Responses to Stress

Gennady Smagin

Anxiety

Considerable evidence indicates that corticotrophin-releasing factor (CRF), primary stimulator of adrenocorticotrophin secretion (Bloom et al., 1982; Olschowska et al., 1982, Vale et al., 1981) is involved in non-endocrine functions and serves to integrate autonomic and behavioral responses to stress (Berridge & Dunn, 1987; Dunn & Berridge, 1990). It is established that the central noradrenergic systems are activated in stress (Stone, 1975) and in fear or anxiety-like responses (Redmond & Huang, 1976). A number of reports indicate that CRF receptors located in, or close to, the locus coeruleus (LC), the major noradrenergic cell group in the brain stem (Foote et al., 1983), are involved in altering stress-induced responses. Infusion of low doses of CRF into the LC may produce behavioral effects characteristic of stressed animals (Butler et al., 1990). The objective of this project is to determine the role of interactions between CRF and LC NE mechanisms in mediating stress-induced anxiety behavior and identification of the CRF receptors involved in the response.

The stressor used in these studies is 1 hour of restraint stress and the behavioral measures of anxiety are made using the Defensive Withdrawal apparatus. Activity and movement of animals is registered and analyzed automatically (EthoVision, Noldus Information Technology, The Netherlands). One hour of restraint stress significantly increases anxiety type behaviors and we have shown that bilateral infusion of the CRF receptor antagonist, ahCRF, into the region of the LC significantly attenuates these behaviors. The anxiety is associated with release of norepinephrine from the LC, measured by microdialysis in the prefrontal cortex which receives NE input almost exclusively from the LC. Antagonism of LC CRF receptors also blocks the stress-induced release of NE. These studies support the hypothesis that CRF activation of LC noradrenergic release may mediate anxiety-type behaviors induced by stress.

The identification of a new neuropeptide of the CRF family, urocortin (UCN), suggested a potential physiological role for endogenous UCN in activating central CRF receptors. Experiments were performed to identify the neurochemical changes produced by icv infusion of UCN. Administration of 3 ug of UCN icv produced a significant increase in serum corticosterone 1 and 2 hours after the infusion, consistent with previously published data that UCN activates the HPA. UCN infusion also affected central noradrenergic, serotonergic and dopaminergic systems. Microdialysis was used to monitor extracellular concentrations of selected compounds released in response to icv infusion of UCN (3ug in 3 ul). The concentrations of DOPAC, 5-HIAA, HVA and MHPG increased in the microdialysates collected from the medial prefrontal cortex. DOPAC concentration peaked 2 hours after injection, 5-HIAA and HVA concentrations reached their peak by the third hour after UCN injection. There were no significant differences in NE or MHPG concentrations after UCN infusion suggesting that noradrenergic system to the prefrontal cortex is not activated by UCN infusion, implying that the LC is not activated by UCN. These results suggest that UCN, administered centrally, produces neurochemical and neuroendocrine effects similar to those observed after the CRF infusion. However, UCN increased serotonin metabolism whereas other investigators have failed to demonstrate an activation of serotonergic system by icv CRF. The neurochemical

pattern of response to UCN infusion differs from that observed after CRF infusion, as changes are greatly delayed compared to those caused by CRF.

Development of a collaborative agreement with Neurocrine to provide a specific CRF_1 receptor antagonist (NBI 27914) has allowed investigation of these receptors in feeding and stress-induced behaviors. Systemically administered, NBI 27914 is able to cross the brain-blood barrier and has been shown to attenuate swim-stress induced elevation of plasma ACTH (Lorang et al., 1997). The effect of NBI 27914 was evaluated using restraint-induced defensive withdrawal behavior. Pretreatment of animals with NBI 27914 (5 mg/kg) attenuated the effect of stress on anxiety behaviors. In the second part of the experiment, defensive withdrawal was induced by icv injection of CRF or UCN. Pretreatment of animals with NBI 27914 (5 mg/kg) significantly attenuated defensive withdrawal produced by 0.25 and 0.5 μg of CRF but was not effective in attenuating UCN-induced defensive withdrawal behavior, indicating that UCN may not be involved in stress-associated anxiety.

Food Intake

The CRF system is involved in the regulation of food intake and energy metabolism. CRF treatment induces an increase in the activity of SNS concurrently with a reduction in food intake (Brown and Fisher, 1990; Egawa et al., 1990). Food intake is diminished by administration of CRF or urocortin and treatments that increase endogenous hypothalamic CRF production, such as stress, tumor induction or appetite-suppressing drugs (Appel et al., 1991; Krahn et al., 1986; Spina et al., 1996). The objective of these experiments was to evaluate the involvement of CRF receptor subtypes on UCN and CRF mediated inhibition of food intake.

Antisense oligonucleotides have been used to selectively arrest the translation of target mRNA into functional proteins. A pilot study validated the use of CRF antisense oligonucleotide for stress studies. A CRF antisense oligonucleotide (ON) was designed based on the sequence of CRH mRNA (5' AGC CGC ATG TTT AGG GGC 3') that corresponds to the initiation codon of CRF mRNA. The complementary sense ON and aCSF served as controls. Injections of CRF antisense or sense ON into the lateral ventricle (30 mg of ON 3 times at 12 h intervals) significantly attenuated the response of the HPA axis to 1 hour of restraint stress and reduced anxiety-type behaviors in an open field apparatus.

In a study examining the acute effects of CRF on food intake, rats were infused centrally with anti-sense oligonucleotides designed to down-regulate CRF1 or CRF2 receptors. The complementary sense ON and saline served as controls. CRF_1 and CRF_2 antisense and sense oligos were injected 3 times, every 12 hours icv (20 ug per injection, with the last injection 3 hours before the experiment). Animals were food deprived for 24 hours prior to an injection of CRF icv (3 ug) and food intake was measured and blood samples were collected every 30 min during 2.5 hours. After the experiment with CRF, sense and antisense ON treatment was continued for two more days (four injections), experimental groups were reversed, and animals were injected with urocortin (3 ug icv). Animals that received injections of antisense and sense oligo to CRF_1 receptor mRNA significantly decreased their food intake in response to CRF injection. However, the 24 h food intake was not affected. Treatment with antisense and sense ON to CRF_1 receptor mRNA did not affect the HPA response to CRF infusion, all animals injected with CRF display an increase in serum corticosterone concentration which peaked at 90 min after injection. Infusion of CRF in animals treated with saline and sense ON to CRF_2

receptor mRNA caused a decrease in food intake and increased concentrations of corticosterone. Food intake was not different from the control group in animals treated with antisense ON to CRF_2 receptor mRNA and antisense ON significantly attenuated the HPA response to CRF infusion. Urocortin, produced a significant decrease in food intake in control animals and treatment with antisense ON to CRF_2 receptor mRNA significantly attenuated the effect of urocortin on food intake. The results of this study suggests the involvement of CRF_2 receptors in the regulation of food intake and activation of the HPA axis.

To study the involvement of CRF_1 receptors in CRF-induced anorexia, rats were pretreated with a CRF_1 receptor antagonist (NBI 27914 : 5 mg/kg s.c.) prior to icv injection of CRF or vehicle (3 μg in 3 μl, or 3 μl, respectively). The CRF_1 antagonist had no effect on CRF-induced anorexia or CRF-stimulation of serum corticosterone . Further dose-response studies are required to confirm whether, or not, CRF_1 receptors are involved in regulation of food intake and HPA activity in response to exogenous CRF and/or stress.

Measurement of Tissue Urocortin mRNA by Ribonuclease Protection Assay and Characterization of an Anti-Urocortin (UCN) Antibody

Xiaolang Yan, You Zhou and Gennady Smagin

Urocortin (UCN) is a novel neuropeptide related to CRF in mammals. Rat urocortin was originally cloned from a midbrain cDNA library (Vaughan et al., 1995). *In situ* hybridization studies demonstrated that UCN mRNA was present in various brain areas including Edinger-Westphal nucleus, supraoptic nucleus, hypothalamus, cerebellum, hippocampus and pituitary (Wong et al., 1996). The physiological role of UCN is not well understood but available information suggests that it may be involved in appetite control and possibly in stress-mediated behavioral responses (Spina et al., 1996). The localization of urocortin mRNA in peripheral tissues has not been studied, however, UCN-like immunoreactivity was found in duodenal extracts, indicating possible UCN expression in the periphery (Vaughan et al., 1995).

In order to determine the role played by UCN in stress-induced responses it is necessary to develop appropriate assays for UCN mRNA and protein and to understand the dynamic relationship between stress and UCN. We have tested two commercially available antibodies and have determined that the anti-UCN antibody sold by Phoenix Pharm. Inc. (Mountain View, CA) detects UCN (as low as 10 ng) but not CRF in a Western blot. Preliminary studies also suggest that this antibody can be used for immunohistochemistry but the assay requires further development. A second antibody, provided by Affinity Bioreagents, did not detect UCN and cross-reacted with CRF in Western blots.

The distribution of UCN mRNA was determined by RT-PCR which indicated that UCN mRNA was expressed in both the brain and the periphery including adrenal, fat, heart, hippocampus, hypothalamus, midbrain and pituitary. No UCN mRNA expression was detected in liver, spleen or kidney. To verify that the PCR products were in fact UCN, a Southern blotting was conducted and all PCR products of the expected size, strongly hybridized to the UCN probe whereas there was no hybridization for PCR products of different sizes. To measure relative levels of UCN mRNA in different tissues a ribonuclease protection assay was developed. The assay detected protected fragments from heart, midbrain and hypothalamic tissue. UCN mRNA was almost undetectable in midbrain from control rats but increased dramatically after 1h restraint.

The Effect of Repeated Restraint Stress on Energy Balance

Jun Zhou, Xiaolang Yan, Gennady Smagin, Leigh Anne Howell,
Ruth Harris and Bradley Youngblood

There are conflicting reports in the literature on the effect of restraint or immobilization stress on food intake in rats. Badiani et al. (1996) have reported that brief, 20 minute, restraint results in a stimulation of food intake during the subsequent hour. Others have reported that restraint stress inhibits food intake. Grignaschi et al. (1993) found that 1 hour of immobilization stress inhibited food intake during the next hour and reported that the hypophagia may be mediated by serotonin receptors in the paraventricular nucleus of the hypothalamus. Repeated immobilization stress for up to 38 days has also been reported to cause a significant reduction in food intake and a failure to grow in young animals (Michajlovskij et al. 1988) and Marti et al. (1993) reported that repeated immobilization stress for 2 hours daily reduced food intake, caused weight loss and increased adrenal weight in rats. The repeated stress also changed circadian release of corticosterone, growth hormone and thyroid stimulating hormone. In contrast, Haleem and Parveen (1994) reported that a single 2 hour exposure to restraint caused a significant inhibition of food intake and failure to grow, whereas repetition of restraint for 5 days did not cause the weight loss or aphagia, suggesting an adaptation response.

We have noted a sustained suppression of body weight in rats exposed to 3 hours of restraint for three days (repeated restraint), similar to those responses reported by Marti et al, 1993, and by Michajlovskij et al. 1988. We have previously observed a small but significant decrease in food intake and body weight of rats exposed to chronic mild stress or 96 hour REM sleep deprivation stress. These observations suggest that weight loss is a generalized response to stressful conditions. The objective of this project is to determine the physiological and neurochemical mechanisms that causes sustained reductions in body weight of rats that have been exposed to a relatively mild, mixed physical and psychological stress of repeated restraint.

In studies with rats exposed to a single3 hour restraint stress we found that exposing the rats to stress early in the morning caused a greater reduction in intake and loss of weight than that caused by stress in the afternoon. The change in intake was not associated with significant changes in central concentrations of serotonin or catecholamines although there was a significant increase in hypothalamic NPY concentrations, which would have been expected to increase food intake (Rybkin et al, 1997). Subsequent studies with repeated restraint (3 hours/day for 3 days) have produced a more robust response with a drop in food intake that is not corrected for at least 4 days after the end of stress and a reduction in body weight that is maintained for up to 40 days after the stress is ended. These changes in energy balance can be reliably induced in adult rats but not in young growing animals. When adult rats are exposed to a second bout of repeated restraint they lose more weight and maintain an even lower body weight than after the first bout of restraint. The composition of weight loss during the period of stress is exclusively lean body mass but during a 5 day period after stress the composition of loss is adjusted to be a combination of both fat and lean tissue. We have found that these changes are exaggerated in rats fed a high fat diet, possibly because they have a greater amount of body fat.

As the studies with a single exposure to restraint stress did not provide any obvious candidate neurotransmitter as a mediator of the sustained shift in body weight Jun Zhou initiated a project to determine whether shifts in peripheral metabolism provide erroneous feedback

signals to the brain which are responsible for the decline in body weight. Measurements of whole body glucose uptake, in a glucose tolerance test performed 24 hours after the last restraint, indicate that stressed rats have an improved insulin sensitivity, requiring less insulin that control rats for similar rates of glucose clearance. Measurement of glucose utilization by two of the major insulin sensitive peripheral tissues, adipose and skeletal muscle, indicate that adipocytes from stressed rats take up minimal amounts of glucose, compared with control or food restricted rats, although the cells remain insulin responsive. In contrast there is little effect of stress on glucose uptake by muscle tissue. Measurements of fatty acid utilization suggest that the fat cells are preferentially oxidizing fatty acids over glucose and this may account for the post-stress shift in body composition from an exclusive loss of lean tissue to a combination of lean and fat tissue. Future studies will determine whether shifts in liver metabolism are responsible for the increased whole body insulin sensitivity and the promotion of fatty acid utilization during the post-stress period.

Measurements of serum hormones indicate that there is no sustained elevation of corticosterone in rats that have been exposed to repeated restraint, but we have not excluded the possibility of a phase shift in the circadian pattern of corticosterone release. Immediately following stress the restrained rats are mildly hypoglycemic and hypoinsulinemic. Five days after the end of stress the hypoinsulinemia is maintained and accompanied by a drop in serum leptin levels. We intend to determine whether the change in relative concentrations of anabolic and catabolic hormones is involved in the changes in nutrient metabolism and body composition of the restrained rats. As weight loss in trauma patients is associated with release of inflammatory cytokines and a cytokine-induced fever (Chance et al., 1987; Lennie et al, 1996), we measured IL6 concentrations and rectal temperatures of rats, before, during and after exposure to repeated restraint. Exposure to the restraint protocol caused elevations of IL6 in both the restrained and control rats which were still high when food intake of the restrained animals returned to control levels. There was no maintained effect of restraint on body temperature of the rats , excluding fever as a primary cause of hypophagia and weight loss.

In two experiments designed to determine the role of central CRF receptors in the stress-induced changes in body composition, rats received infusions of ahCRF (50 ug) into the lateral ventricle immediately before restraint on each of the three days of restraint. Krahn et al. (1986) had previously reported that this partially prevented an acute stress-induced hypophagia in rats. We found that lateral ventricle infusions of the receptor antagonist were only partially effective in blocking the effect of restraint on body weight and had no effect on corticosterone release during restraint. As the CRF receptors that modulate food intake are located in the hypothalamus, in a second experiment rats were infused with ahCRF into the third ventricle immediately prior to restraint. The antagonist totally blocked restraint-induced weight loss but did not prevent corticosterone release during restraint. Future studies will examine the role played by CRF, UCN and other neurotransmitters in initiating the sustained changes in body weight of repeatedly restrained rats,

Identification of Genetic Markers for Stress Sensitivity and Stress-Induced Behavioral Responsiveness

You Zhou, David Elkins, Alan Cheshire, Leigh Anne Howell

The objective of this project is to identify potential genetic markers for susceptibility to stress-induced changes in behavior. At the start of the project five genes that were reported to be

related to either behavior or to activation of the hypothalamic pituitary axis were selected as initial candidate markers. These were angiotensin converting enzyme (ACE), apolipoprotein E (Apo E), corticotrophin releasing hormone (CRH), transforming growth factor a (TGFa) and prolactin (PRL). Probes for these genes were made by cloning cDNA fragments, obtained by RT-PCR of rat hypothalamic or pituitary tRNA, into plasmid vectors. Dot blot was used to determine the effect of repeated restraint stress (3 hours/day for 3 days) on expression of the candidate genes in various tissues. Repeated restraint caused changes in expression of TGFa in the hypothalamus, PRL in the pituitary gland and ACE and ApoE in the cerebella cortex. Based on recent reports that ApoE phenotype is a risk factor for age associated dementia and Alzheimer's Disease in humans, and that ApoE knock-out mice give abnormal responses to stress, the project has focused on the role of ApoE as a protective factor against stress- and age-associated neurodegeneration.

Although a majority of behavioral studies are carried out using rat models, the availability of molecular and genetic information for rats is still limited compared to that from rapidly developed molecular genetics in mice. In order to take advantage of the transgenic mouse models available, behavioral measures for mice had to be developed. Two behavioral tests were established for mice: open field test for locomotion and anxiety-type behavior and a water maze test for spatial memory and learning ability. We found that we could detect changes in open field activity and in spatial memory of mice exposed to restraint stress. Having established the behavioral tests for mice we examined the behavior of both wild-type and ApoE-deficit mice in response to restraint stress. Restraint stress caused significant reductions in food intake of wild type but not ApoE deficient mice although mice of both phenotypes lost weight in response to stress. Restraint significantly reduced exploratory activity in mice of both genotype, measured in an open field apparatus, and caused a significant disruption of both reference and working memory of wild-type mice. ApoE deficient mice had a severely impaired spatial memory which was little effected by stress. These results indicated that ApoE-deficient mice have an altered susceptibility to stress which was confirmed when we found that ApoE deficient mice showed less adaptation to repeated restraint, based on serum corticosterone concentrations, than their wild type controls

Measurement of ApoE mRNA expression in hypothalamus and hippocampus from mice exposed to acute or repeated restraint was measured to determine whether ApoE was associated with stress-induced changes of HPA activity and learning behavior. ApoE mRNA expression increased in the hypothalamus of mice killed 24 hours after 3 consecutive days of 20 min-restraint and in mice exposed to acute stress immediately before decapitation. A limited ($p=0.057$) increase in ApoE mRNA expression was also observed in the hippocampus of mice exposed to chronic stress.

Detection of apoptotic cells in the brain of chronically stressed mice indicated a substantial increase in the number of damaged cells in tissue from ApoE knockout mice than from their wild type controls. To determine what mechanisms were involved in this apoptotic process and in the abnormal neuroendocrine activity in ApoE-deficient mice, we studied the autoimmune reactivity of the sera from wild type or ApoE deficient mice that had been exposed to repeated bouts of restraint stress. The results from standard ELISA of these sera, using whole brain homogenates of wild-type mice as antigens, demonstrated a higher titer of anti-brain autoantibodies in the sera from ApoE-deficient mice exposed to chronic stress than in sera from wild-type mice exposed to a similar stress. All the sera were further tested on whole brain

homogenates by Western blot analysis and the sera from ApoE-deficient mice detected 2 major bands with the molecular mass of ~72 kDa and ~48 kDa respectively. The ~48 kDa polypeptide was also recognized by sera from some wild-type mice, although a stronger signal was revealed by sera from mice exposed to chronic stress. None of the sera from wild-type mice detected the 72-kDa autoantigen that was recognized by the sera from both control and stressed ApoE-deficient mice. Tissue distribution of the 72kDa antigen was carried out by Western blotting analysis using the sera from ApoE-deficient mice. The ApoE sera appeared to detect a different antigen (~75 kDa) in non-brain tissues. To determine the cellular localization of the major brain antigens recognized by the sera from ApoE-deficient mice, immunofluorescence microscopy was performed on frozen sections of brain from a wild-type mouse, using the serum from a stressed ApoE-deficient mouse. Strong immuno-reactivity of the serum with neuronal cells was observed in different brain areas, including frontal cortex, hippocampus and hypothalamus-cortex regions. The major components detected by the sera from ApoE-deficient mice were localized to the nuclei and the neuronal fiber-like structures. Such reactivity was not detected by the serum from a stressed wild type mouse.

The results from these experiments suggest that ApoE phenotype may be a marker for stress responsiveness. ApoE knockout mice do not adapt to repeated exposure to stress, experience a significant amount of apoptosis in brain tissue and produce an increased titer of autoantibodies. Some of these antibodies are specific for brain antigens and may be responsible for the neurodegeneration observed in these animals. Characterization of the antibodies and their antigens will elucidate the mechanisms responsible for early neurodegeneration in these mice and may provide valuable insight into the mechanisms responsible for Alzheimer's disease.

C. Conclusions

The results described above demonstrate significant progress on a number of projects aimed at elucidating the relationship between stress, behavior and nutrition. Our ultimate goal is to identify nutritional interventions that prevent, or ameliorate, stress-induced impairments of behavior, however, in order to reach this goal it is necessary to identify the physiological and neurological mechanisms responsible for the behavioral response. To this end we are focusing on four rodent models of stress with end-point behaviors relevant to military personnel exposed to stressful environments: repeated stress and weight loss, acute stress and anxiety, chronic stress and memory impairment and genetic markers for stress responsiveness. We have demonstrated that ApoE deficiency is associated with an exaggerated response to stress and neurodegeneration which may be caused by autoantibodies. Future studies will determine whether ApoE phenotype and serum autoantibody titer can be used as markers for stress responsiveness. Repeated restraint stress is associated with a prolonged reduction in body weight and with tissue specific changes in glucose utilization. This response can be blocked by antagonism of hypothalamic CRF receptors and is accompanied by a shift in the relative concentrations of anabolic and catabolic hormones. Sleep deprivation causes an impairment of spatial memory and an increase in central serotonin metabolism. Dietary modulation of brain serotonin suggest that the changes in metabolism as due to inhibition of serotonin breakdown rather than increased rates of synthesis. Finally, studies with antagonists of specific CRF receptors are enabling us to identify specific neurotransmitters and receptor subtypes responsible stress-induced anxiety and aphagia. Taken together these results demonstrate the high rate of productivity and progress in elucidating the relationships between stress, behavior and nutritional status in specific rodent models of stress

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IV. Clinical Neuroscience Laboratory

A. Introduction

The purpose of this project was to evaluate the effect of tyrosine and psychostimulants on cognitive performance under 40 hours sleep deprivation. In order to execute this study, we equipped a polysomnography unit at the Pennington Biomedical Research Center. Pilot subjects were used to test the polysomnography equipment and to adjust software requirements in order to score sleep stage through computer programming. An extensive cognitive performance battery was established and evaluated through pilot testing. In addition, the day to day procedural details for nursing supervision of inpatient subjects, specimen collection and analysis, nutrition and the sequence of screening procedures, polysomnography and multiple sleep latency procedures was determined. We trained research assistants who were primarily graduate students in phlebotomy and specimen collection and preparation.

We applied to the Food and Drug Administration for an investigative new drug license for tyrosine and this was approved. We recruited volunteers for the sleep study. A total of 212 respondents completed telephone interviews.

An initial study was designed to compare the effects of tyrosine, amphetamine, caffeine and phentermine on performance of cognitive tasks following 40 hours of sleep deprivation.

B. Body

In the 18th quarter, we completed this experiment that evaluated tyrosine, phentermine, caffeine and placebo following 40 hours of sleep deprivation in a total of 76 subjects. The purpose of the study was to evaluate nutrient and drug effects on attention and cognitive performance during 40 hours of sleep deprivation. We presented preliminary results from that study at the Committee on Military Nutrition Research. We intended to conduct a second experiment, "Tyrosine plus Caffeine vs. Caffeine Alone." After accruing only three patients, this research project was suspended based upon the comments from the review at the 1996 Committee on Military Nutrition Research site visit.

The effects of tyrosine, phentermine, caffeine and d-amphetamine on sleep drive during 40 hours of sleep deprivation and on subsequent recovery sleep were compared with the effects of placebo and which each other. Subjects were 76 healthy males, age 18-35 years. Sleep deprivation produced highly significant effects on both sleep drive and recovery sleep. Comparison of the first post drug multiple sleep latency tests with the immediate pre drug baseline multiple sleep latency test, showed that d-amphetamine, phentermine and caffeine all reduced sleep drive, producing a significant increase in time to sleep onset, but tyrosine did not; d-amphetamine had the largest effect. A comparison controlling for circadian effects, the first post drug multiple sleep latency test compared with the multiple sleep latency test done at the same time on the previous day, showed that only d-amphetamine reduced sleep drive, thus preventing the decrease in time to sleep onset normally seen in sleep deprivation. However, d-amphetamine also was the only drug that had significant adverse effects on recovery sleep, quantity, sleep depth, sleep continuity and sleep architecture. D-amphetamine also significantly increased multiple sleep latency times on the morning following recovery sleep. Though phentermine impaired only rapid eye movement sleep, tyrosine and caffeine had no adverse effect at all on recovery sleep. In conclusion, d-amphetamine,

caffeine and phentermine all decrease sleep drive, and though d-amphetamine was the most effective, it also caused the greatest damage to recovery sleep. Therefore, caffeine appeared to be the optimal drug for reducing sleep drive during sleep deprivation, as it was effective and had no adverse effects on recovery sleep.

C. Conclusions

The result of the clinical studies in countermeasures useful in sleep deprivation are of some military significance. The studies demonstrate the effectiveness in caffeine in producing alertness and not interfering with recovery sleep. The studies also demonstrate the effectiveness of caffeine on some of the performance measures. Therefore, it may be possible to utilize these results in military policy implementation, i.e., caffeine may be used in conditions where sleep deprivation is inevitable in order to improve alertness and performance without interfering with recovery sleep. Further studies are suggested using a model to look at alertness and performance in a scenario mimicking duty conditions.

D. References

None.

V. Menu Modification Study

A. Introduction

Part I. Recipe Development at PBRC

Since 1985, nutrition initiatives have been introduced into the Armed Forces Recipe Service, the Army Master Menu and the Army Food Service Program to provide soldiers with diets lower in fat, cholesterol and sodium. The Military Nutrition Division of the United States Army Research Institute of Environmental Medicine (USARIEM) conducted several garrison dining facility studies to assess soldiers' nutrient intakes (Carlson et al., 1987; Szeto et al., 1987; Szeto et al., 1989). It was apparent from these studies that in order to achieve Army Nutrition Initiative goals of reducing fat to 30% of the calories and cholesterol to 300 mg/day, extensive revision of Armed Forces Recipes would be required.

The demographic profile of the Army has changed in recent years. As of 1995, 13% of total Army personnel were female. The racial make-up included 62% white, 27% black, 5% Hispanic with the remaining 6% divided among Asian, native Indian, Alaskan Indian and "unknown". Soldiers have increased their demand for the availability of ethnic food choices in dining facilities. This is most likely a reflection of the various backgrounds of individuals coming into the military and gender differences in food preferences, as well as current eating trends in this country. The Army is therefore trying to incorporate additional ethnic-based recipes into the Armed Forces Recipe File.

In 1990, the Military Nutrition Division, USARIEM, began a collaborative effort with Pennington Biomedical Research Center (PBRC) at Louisiana State University to modify Army garrison menus. The purpose of the project was to create healthful, nutritious menu items which moderate soldiers' fat, cholesterol, and sodium intakes. New ethnic-based recipes were

developed to contain decreased fat, cholesterol and sodium levels. The goals for the project were to develop low-fat ethnic and breakfast recipes, standardize them for 100 portions and test the recipes for acceptability in an actual Army garrison.

It has been standard practice for the recipe developers at Natick RD&E to collect food preference and acceptance ratings before new recipes are added to the Armed Forces Recipe file. Natick RD&E uses two types of test panels - "technical" for quality, flavor and texture and "consumer" panels who participate in acceptance and attitude testing. Consumer panelists are asked to rate their acceptance of foods using a 9 point hedonic scale, where 9 equals "like extremely" and 1 equals "dislike extremely". Generally a new food item must receive a mean score of at least 6.0 to be considered acceptable. Because mean scores can be influenced by scores at either end of the spectrum, another criteria which may be used is the percentage of individuals who rate the product with a 6.0 or higher. Ultimately, those products that are found acceptable under laboratory conditions, are tested in actual military dining facility settings to determine whether the new food products are consumed in sufficient amounts to enter the system.

Judgments of the sensory and hedonic properties of food and food preferences are influenced by a variety of factors. Acceptability and consumption of food items depends on a complex interaction between the sensory properties of the food, the consumer expectations for it, its cognitive associations, convenience, and price (Cardello, 1993). Gender and ethnic origin also influence food preferences. In a study of food preferences in military personnel, women had higher preferences for baked potatoes, green salads and fresh fruit while men had a higher preference for grilled meat (Wyant, 1984). Researchers have documented a preference of cultures toward their own culture-specific foods (Axelson, 1986) and examined cross-cultural flavor preferences (Meiselman & Bell, 1991). Recently a group of investigators found that just labeling a food with an ethnic title increased perceived ethnicity and acceptability of the item (Bell, 1994).

Strong relationships between food choice and attitudes have been documented, especially toward foods with a high fat content (Shepherd, 1985 & 1987). Nutritional information has been found to increase consumers' hedonic response to some products (Cheng, 1990). Solheim (1992) studied the effects of information on fat content and sensory differences on like or dislike ratings by consumers of sausage. When sensory quality was similar, false information that the fat content of the 20%-fat sausage was 12% increased the hedonic rating while correct information on fat content decreased the rating.

There are many factors which influence acceptability of a food product and the ideal situation would be to test the acceptability of a food item in the population for which it is intended, but this is not always feasible. It is possible that groups of similar age, gender and ethnic origin would evaluate food items with similar acceptability ratings. The purpose of this project was to determine if acceptability data from a young college age population was similar to acceptability scores obtained from young soldiers in an actual Army garrison setting.

Part II. Participation in USARIEM Field Studies

The United States Army Research Institute for Environmental Medicine (USARIEM) conducts a variety of research studies led by investigators in the Military Nutrition Division

(MND). Focus of projects has been directed towards nutritional status of the soldier and nutritional information related to enhancement of soldier's performance. Since 1993, PBRC Nutrient Data Systems Section has been involved in active data collection associated with some of these studies. Our first participation was Fort Jackson, SC, March 1993 with Catherine Champagne serving as a visual estimator and Kevin Gilley (former chef) as a recipe specialist. This study was entitled "Nutritional Intake of Female Soldiers During the U.S. Army Basic Combat Training" and the Principal Investigator was LTC Nancy King. In October 1994, Kelly Patrick served as a recipe specialist in a study "Nutritional Intakes of Marines in Hot Weather" under Principal Investigator, MAJ Nicol Hotson.

The "Menu Modification Consumption Study" conducted at Fort Polk, LA, in March 1995 involved Catherine Champagne as a visual estimator, Kelly Patrick, Barbara Eberhardt, Nancy Baker, and Baldwin Sanders as recipe specialists working under the direction of Principal Investigator, LTC Alana Cline. It was at this time that the observation was made that improvements of dietary data collection at field studies was needed. In July and August 1995, PBRC personnel participated in another protocol of LTC Alana Cline entitled "Iron Status of Female Soldiers in Advanced Training" at Fort Sam Houston, TX. Catherine Champagne was the Coordinator of Dietary Data Collection and Cheryl Parker, Barbara Eberhardt, and Nancy Baker serving as data collectors. This study was conducted more efficiently than previous field studies, however it emerged that the receipt of finalized nutrient data was still slow, despite the fact that nutrient matches for foods was completed prior to leaving the study.

Consequently, it was determined that timely receipt of dietary data via computerized nutrient analysis of recipes, menus, and dietary intakes of the soldiers is critical to assessment of the soldiers' needs and interrelationships with other aspects of military life. In addition to including within this project the analysis of recipes developed for Army garrison feeding situations, we proposed to diversify our tasks and more fully integrate with tasks of the Military Nutrition Division by providing analysis of dietary intakes taken during military feeding studies. We proposed to assist effectively in dietary collection protocols and analysis of those collections. Our position is to advise the principal investigators on dietary collection and methods to assure a quick turnaround of nutrition information needed for statistical analyses. We work with the current nutrition staff, data programmers, and other key personnel in the development of a more effective database, somewhat patterned after the now defunct Computerized Analysis of Nutrients (CAN) system. Inclusion of the users of the data set is essential in developing and perfecting a more efficient system that can generate finalized data in a more timely fashion than existed previously in the Military Nutrition Division.

We proposed this additional caveat based on the position that we can offer programming expertise, personnel stability, and personnel who can be involved in precise data collection, both in the garrison and field settings. Our plans included state of the art data programming and high technology data transfer and analysis as a part of our proposal. We plan to take advantage of a client-server situation which will involve data collections transferred to a central site (PBRC) for analysis. We eventually plan to integrate all Armed Forces Recipes, special formulations and feedings into one database system that can meet Military Nutrition needs for both now and the distant future, a database that will be ever changing and modifiable to the unique set of circumstances of each study or at USARIEM, in which computerized nutrient analysis of dietary intakes is undertaken. We also proposed that authorship on all future publications include at least one PBRC investigator from this task who has contributed substantially to the conduct of

the study.

B. Body

Part I. Recipe Development at PBRC

The data from the Fort Polk studies was presented at scientific conferences in 1996 (Champagne et al., 1996; Hunt et al., 1996). The information from those presentations represents the summary of relevant data collected during the completion of this project and is contained in the Appendix.

METHODS

The data were collected in three phases; recipe development, acceptability testing in a young population similar to Army personnel, and acceptability testing in an Army garrison. The first phase consisted of recipe development at PBRC by a culinary research associate and nutrient analysis using Moore's Extended Nutrient Database (MENu) (Pennington Biomedical Research Foundation, 1995).

New ethnic-based recipes were developed and divided into eight categories: American, breakfast, Cajun, Caribbean, Chinese, Indonesian, Italian and Mexican. Initial testing for palatability and acceptability was conducted with a consumer panel .

During phase two, each of the new recipes developed at PBRC were prepared as directed to yield 100 portions in the foods laboratory at Louisiana Tech University. Each of the recipes were evaluated for ease of preparation and clarity of method as outlined on the recipe cards during preparation. On 19 selected days, three of the new recipes were prepared and served in a cafeteria-like setting to individuals recruited from the campus. Subjects were able to select the food item they wished to eat. For each modified food item they selected, they were asked to complete a food evaluation questionnaire.

Phase three was conducted in a garrison dining facility at Fort Polk, Louisiana. Over a three-week period the new recipes were incorporated into the regular Army menu. PBRC culinary research associates were on site in the garrison dining facility to train Army personnel in the correct techniques for preparing the new recipes. A total of 47 recipes were prepared and served at breakfast, lunch and dinner. Two food items for each of five menu categories were selected from the existing menu to serve as controls. During the days selected for the study, evaluation questionnaires were distributed at each meal with a new menu item or a control item in the selected dining facility. Every individual who selected a new menu item or control item was asked to complete an evaluation of the product.

The questionnaire contained closed-ended items related to demographic variables, typical use of product, and addition of condiments. In each of the three phases the recipes were evaluated with a single score for overall acceptance. The US military has a 40-year history of measuring like or dislike of food items to predict consumption. Hedonic evaluation of food started in 1950 with Peryam who developed the nine-point hedonic scale (Peryam & Pilgrim, 1957). The hedonic scale consist of nine separate phrases describing degrees of like and dislike. The scale ranges from 1 corresponding to "Dislike Extremely, to 5 "Neither Like or Dislike, and 9 "Like Extremely".

SUBJECTS

Subjects for the first phase were employees of the PBRC who volunteered to participate in routine taste tests of the recipes. They had no training in sensory analysis, but participated routinely in consumer acceptance tests of a wide range of food products. Age ranged from 18 to more than 50. About half were male, 765 were white and 12% black.

Subjects for the second phase were a convenience sample of university students and staff. Individual subjects changed on a daily basis, but the composition remained fairly constant. The majority, 56% were 29 years old or less, and 43% were male. The majority (82%) indicated their ethnic origin was white, 7% black, while the remaining were of other ethnic backgrounds.

Testing for the third phase occurred in an Army dining facility. Subjects were Army personnel who regularly ate their meals in the dining facility. Army personnel were asked to participate only after they had selected a modified food item from the serving line. Therefore individuals changed from day to day, but were similar in composition to the subjects in phase two. The majority of the Army personnel (76%) were 29 years old or less and 90% were male. Most (62%) indicated their ethnic origin as white , 18% black and the remaining 20% were of other ethnic origins.

DATA ANALYSIS

All analyses were performed using SAS statistical package. Descriptive statistics were calculated on demographic data. Mean hedonic responses for each new recipe were analyzed separately for each of the three phases of the study by ethnic and food type. A T-test was used to assess differences in acceptability scores of new recipes between Louisiana Tech and Fort Polk. The percentage of subjects who rated a food product on the upper end of the hedonic scale, six or better, was determined and Chi Square analysis was used to compare the proportion of mean ratings six and above by ethnic and food categories among the three test sites.

In order to investigate differences in food acceptability ratings with respect to test site, a logistic regression model was employed. The binary response variable consisted of the proportion of mean acceptability ratings falling into the categories of "up to six" and "six and above." The analysis was conducted on proportions because they provided more robust comparisons. Test site was used as an explanatory variable. Results were obtained using PROC CATMOD in SAS. In addition, contrasts were written to examine specific comparisons between test sites. Interpretations of the analysis were eased by grouping the recipes into two categories, ethnic foods and food types.

RESULTS

Acceptability data were compared among the test settings, ethnic categories, and food type. When grouped by ethnic categories, the acceptability ratings were more variable than when grouped by food type. We found ratings varied most between the development and validation settings (7.2 vs 6.6, $P<0.05$), and least between the validation and actual Army setting (6.6 vs 6.6, NS).

Although mean ratings varied least between the validation and actual Army setting, when T-tests were done between the two validation sites for 18-29 year olds, there were significant differences in breakfast foods. Four of the eight items included under the breakfast category had significantly higher ratings in the actual Army setting. There were also significant differences among dessert, salad and starch categories. For the majority of food items, the mean scores were higher in the Army garrison setting than they were at Louisiana Tech (see Appendix, Table 2).

The percentages of acceptable ($\geq$6.0) and unacceptable (<6.0) scores for each testing site are presented in Tables 3A-3B (see Appendix). Chi Square analyses revealed variations in ethnic foods and food types were generally due to differences in Pennington compared with either Louisiana Tech or Fort Polk (see Appendix, Table 4A-4B). With respect to comparisons between Louisiana Tech and fort Polk, significant differences were noted only in food type, specifically in pasta, poultry, salad, starches, and vegetables. Variations were noted based on the proportion of mean ratings falling into the two response acceptability categories, "up to six" and "six and above." For those contrasts that are significant, A "+" or "-" indicates the direction of the difference. For example, if the contrast of Louisiana Tech vs. Fort Polk is significant, then A "+" indicates that the proportion of ratings were significantly higher for Louisiana "Tech than for Fort Polk. A "_" indicates that the proportion of ratings were significantly higher for Fort Polk than for Louisiana Tech.

Since acceptability ratings were so similar between validation and Army garrison, we anticipate that future recipe development can continue without additional testing at an actual Army garrison allowing for considerable cost savings and more timely additions to the Armed Forces Recipe File.

The nutrient analysis of the recipes used in the Menu Modification Study are included in Appendix (3 pages)

Part II. Participation in USARIEM Field Studies

Dietary information is collected during field nutrition studies with the software application MiDAS (acronym for Military Dietary Analysis System). MiDAS was developed at PBRC Nutrient Data Systems Laboratory. It was programmed in Visual Basic 5.0 (Microsoft Corporation, 1997) and utilizes a Microsoft Access Version 7.0 database to store collected data (Microsoft Corporation, 1997). Collected data is analyzed using one of two USDA data sets. Some information is derived from USDA database for standard reference, commonly referred to as Standard Release 11 (USDA, 1996). The Standard Release 12 was released in March of 1998 (USDA, 1998) and has been used to update the system. However, the studies conducted this year used the earlier release. Primarily used during field studies employing food records reflecting intakes of non-military rations is the USDA Survey Database for the Continuing Survey of Intakes by Individuals (CSFII). The version used on the studies conducted prior to 1998 was CSFII "94 (USDA, 1995). The CSFII '96 data was released in March of 1998 and has been used to update the current version of MiDAS (USDA, 1998). In addition, several studies contained military rations. When military rations are used, we contact USARIEM and/or Natick Labs for the nutritional information if we are to analyze the dietary intake information. The development of the MiDAS system was presented at the Experimental Biology meeting in San Francisco in April 1998 (Allen et al., 1998).

We processed several studies during this reporting year and revised data from a summer 1996 study which was not cited in the 1997 reporting year since the data had a number of edits to be completed. Therefore, the studies from Summer 1996 through this year will be reported, with data given which has been disseminated to the principal investigator. Military field studies may use one or more means to collect dietary intake data. During our studies, three main methods of collecting dietary intake have been used. The visual estimation method uses trained data collectors to visually estimate the quantity of food before and after the meal by comparing the portion to weighed standard portions of the same food. This method has been reported elsewhere (Rose et al., 1987). Some studies employ dietary records self-reported by the subject. In these cases, the subjects generally undergo training to some extent and the records are reviewed by data collection personnel trained to probe for additional information when reviewing the record. Several studies this past year have combined a self-reported diet record with acceptability data using a 9 point hedonic scale, routinely used by the military. The MiDAS system allows for the collection of both visual estimation data and dietary record data or a combination of more than one type of collection within the same data entry system.

Nutritional Assessment of U.S. Army Rangers in Garrison and During a Field Training Exercise in a Hot Environment. Hunter Army Airfield, GA from 13 July - 5 August, 1996:

Although conducted in 1996, the data from this study has not previously included in an annual report. The data from this study was provided to the Principal Investigator, LTC John Warber, Dr.P.H., R.D., in March 1997. We have revised the data as needed and shipped the raw data forms to LTC Warber in December 1997. Those individuals taking part in this study from PBRC included Catherine Champagne, PhD, RD; Alice Hunt, PhD, RD (under subcontract with Louisiana Tech University, Ruston, LA); Ray Allen, PhD; Mary Baldwin Sanders, MS, RD; Barbara Eberhardt, BS; Anyce Griffon, BS; Philippe Hebert; Stacy Heilman; and Leslie Favie. The protocol included six days of intake in garrison gathered by visual estimation combined with food records for snacks or meals consumed elsewhere, seven days of MRE and pogey bait intake during the field training exercise using special food record forms developed by Natick, and an additional two days of intake recorded by food records designed to determine types of food choices following the hot environment field training.

PBRC processed the garrison intakes and the intakes following the field training. The total number of lines of data entered was 7,531. PBRC was responsible for the data entry for the field exercise portion of the study which involved MRE and pogey bait collection, however the data analysis for that portion was done by Natick Labs. The results from the portion of data collected and processed by PBRC can be found in the Appendix.

Conclusions on the Nutritional Quality of the Ranger Diet in Garrison

- Reported mean food energy intakes were slightly below recommended energy intakes for moderately active adults (3000-3200 kcal).
- Mean intakes of most vitamin and minerals met the MRDA recommended values for gender.
  mean intake of B6 was low, 2.4mg (2.8mg)
  mean intake of magnesium was low, 325mg (350-400mg).
- In general, Rangers subsisting in garrison dining facility were following the principles of the 1995 Dietary Guidelines for Americans.
  mean percentage of total energy from fat was 30.7%, recommended 30%
  mean percentage of total energy from saturated fat was 10.7%, recommended 10%

mean intake of dietary cholesterol was 349mg, recommended less than 300mg

Data from the Savannah Rangers Study was presented as a poster session at the 16th International Congress of Nutrition in Montreal, Canada in July 1997 (Champagne et al., 1997)

Nutritional Assessment and Dietary Education of U.S. Army Sergeants Major Academy Students in Garrison. A Three Phase Study conducted in September, 1996; December, 1996, and March 1997. Biggs Army Airfield, Fort Bliss, El Paso, TX.

This study was initiated during the 1996-97 reporting year, however has not previously been referred to in the annual report. The data was provided to the Principal Investigator, MAJ William H. Karge, III, Ph.D., in July 1997. We have revised the data as needed and shipped the raw data forms to MAJ Karge in December 1997. Those individuals taking part in this study from PBRC included Catherine Champagne, PhD, RD; Ray Allen, PhD; Barbara Eberhardt, BS, RD; Anyce Griffon, BS; April Hebert, BS, RD; Philippe Hebert; Fatemeh Ramezanzadeh, MS; Regina Louviere, BS; and dietetic interns from Louisiana Tech University, Ruston, LA and North Oaks Hospital, Hammond, LA.

The protocol included three days of intake recorded by the subject and reviewed by the data collector. Intakes were Sunday, Monday, and Tuesday intakes with interviews with the soldiers scheduled on Monday, Tuesday and Wednesday to review the previous day's intake.

PBRC processed the dietary intake data. The total number of lines of data entered was 12,498.

Objectives:

- Evaluate the nutritional adequacy of diets of career NCOs
- Assess changes in diet after health promotion courses
- Assess changes in cholesterol levels in entire class

Study Design

- PBRC and USARIEM investigators used dietary analysis to assist in measuring the effectiveness of dietary education in the most recent class
- PURPOSE
  To decrease serum cholesterol levels and coronary heart disease risk in soldiers
- SAMPLE SIZE - 106 Soldiers
- STUDY DESIGN
  Blood draw and diet records pre, after 10 weeks, after 20 weeks
  Dietary education at beginning of program
- DEPENDENT MEASURES
  Serum lipids: Total Cholesterol, LDL, HDL, Triglycerides
  Body composition: weight, height, body fat
  Dietary analysis

Data from the El Paso Study was presented at the Experimental Biology meeting in San Francisco in April 1998 (Champagne et al., 1998).

Assessment of Nutritional Status and Energy Expenditure and Determination of Gender Differences in Dietary Intakes of Combat Service Support Personnel Subsisting on Meal-Focused Versions of the Meal, Ready to Eat, Camp Mackall, North Carolina. 29 April 1997 to 13 May 1997.

This study involved collection of MRE intake data. Data entry was on site and involved the largest number of people to date to input data into the MiDAS system. PBRC was responsible for the data entry only, and the data files were given to the Principal Investigator, Cory Baker-Fulco, before departure from Camp Mackall. The following individuals from PBRC assisted in this study: Catherine Champagne, PhD, RD; Ray Allen, PhD; Bill Glover, PhD; Barbara Eberhardt, BS, RD; April Hebert, BS; Troy Fontenot, BS; Eric LeBlanc, BS; Kelly Patrick, BS; Fatemeh Ramezanzadeh, MS; Regina Louviere, BS; and Alan Pesch, BS. The following information was derived from both the proposal and some of the data collection on acceptability, and was presented at the PBRC Work In Progress seminar series in May 1997, just following our return from the study.

Objectives:

- To determine gender specific differences in MRE component selection and preferences.
- To assess nutrient intakes of Army combat service support personnel subsisting on two different MRE rations (version XVII and a meal focused, carbohydrate-enhanced version.
- To estimate average energy expenditures of male and female combat service support personnel during a 14-day field training exercise.
- To ascertain the knowledge and attitudes of male and female soldiers toward MREs.
- To ascertain the dietary habits of male and female combat service support personnel when in garrison.

Data Collection:

- Food and fluid intake
  - MRE cards for lunch only for 5 days plus pogey bait
  - MRE cards for 3 meals plus pogey bait for 7 days
  - Fluids measured in canteens, canteen cups, and beverage cups
- Food preference and acceptance using 9-point hedonic scale
- Typical practices and nutrition knowledge
  - Diet Habit Survey (Conner, Oregon)
  - Nutrition Knowledge and Attitudes
- Blood analyses
- Body height, weight, and anthropometric data
- Questionnaires
  - Demographic
  - Final Ration Opinion Questionnaire
- Energy expenditure, total body water and water turnover on a subsample of 36 volunteers to include 23 women
- Activity monitors on a subset of the DLW volunteers
- Foot contact monitors to estimate the metabolic cost of walking and running based on body weight and the time during each stride that a single foot contacts the ground

Subjects and Data Collection

- There was data collected on 263 total subjects
  169 males (64.3%)
  94 females (35.7%)
- A total of 4061 meal cards were processed
  MRE consumption, rating, reason not finished
  beverage consumption (bulk)
  pogey bait
- A total of 53,131 lines of data was entered

The remainder of the slides presented at the Work In Progress Seminar Series are included in the Appendix.

Impact of Creatine Intake on Physical Performance, Fort Bragg, North Carolina. 28 August 1997 through 28 September 1997.

In this study data was collected during three phases during August and September 1997. The data was provided to the Principal Investigator, LTC John Warber, Dr.P.H., R.D., in December 1997. Those individuals taking part in this study from PBRC included Catherine Champagne, PhD, RD; Ray Allen, PhD; Barbara Eberhardt, BS, RD; and April Hebert, BS, RD.

Twenty-seven Rangers participated in the study and three-day dietary intake records were processed at three different times during the study: baseline, following one week of placebo, and following one week treatment with creatine. The intake portion of the study began on September 5 and was completed on September 24, 1997. The protocol included three days of intake recorded by the subject and reviewed by the data collector during each of the three phases of the study. Intakes were Thursday, Friday, and Saturday intakes with interviews with the soldiers scheduled on either Monday or Tuesday, as their schedule permitted, to review the data.

PBRC processed the dietary intake data. The total number of lines of data entered was 3,857. Some preliminary data was released to LTC Warber, therefore we will include final data in our next annual report.

The Effects of Carbohydrate Supplementation on the Performance of Combat Relevant Activities. Fort Lewis, WA, February 8-13, 1998.

The following personnel from PBRC assisted in the USARIEM study on the ERGO carbohydrate beverage which was completed under the direction of MAJ Stephen Slade, the British Exchange Officer stationed at Natick.: Catherine Champagne, PhD, RD; Ray Allen, PhD; Eric LeBlanc, BS; Barbara Eberhardt, BS, RD; Bradley Prather, BS, RD; and Alana Cline, PhD, RD. This protocol involved a field test to identify whether the Ergo Drink improves physical performance in typical military scenarios. The study was designed to identify whether the consumption of the ERGO (Energy Rich Glucose Optimised) Drink developed by Natick RD&E Center can help to improve "performance" while performing typical, physically arduous military tasks in the field.

PBRC processed the dietary intake data. The total number of lines of data entered was 5,080. Preliminary data from the study was presented to MAJ Slade on March 6, 1998, during a

visit to USARIEM. A more finalized data set for this study will be released in June 1998.

C. Conclusions

Part I. Recipe Development at PBRC

With the completion of the Menu Modification initial phase, we have geared up for additional work and have brought on LTC Alana Cline (retired). With the successful completion of the Fort Polk study and her interest in furthering these projects, we will bring a new and revised look to this project.

Part II. Participation in USARIEM Field Studies

During this project, several studies have come to complete closure in terms of dissemination of nutritional intake information, namely the Savannah and El Paso studies. The Camp Mackall study indicated our ability to enter dietary data on an extensive number of subjects and provided delivery of that data on the final day of the study. For the Camp Mackall study, we had been advised that of a planned 300+ subjects, data was to be entered on about half of the subjects. This did not turn out to be the case, however, and all data on all subjects was entered for an almost unimaginable 50,000+ lines of data (we had approximately 5 data entry people working 8-12 hours each day).

We believe that we are currently carrying out the mission of this part of the task, efficiently and effectively working to deliver nutritional intake information from USARIEM's field studies that we are involved in. We are working to further streamline delivery of dietary intake information and setting up a version of the MiDAS system which can be used from the USARIEM end.

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VI. Metabolic Unit Project

A. Introduction

Two projects will be discussed. One experiment was executed in 1993.

B. Body

During the second year of this grant, activity in this project consisted of carrying out an experiment on two cohorts of Special Operation Forces volunteers. That project was, **"Assessment of Intra- and Inter- Individual Metabolic Variation in Special Operations Forces Soldiers."** The Principal Investigator for the project is Ms. T. E. Jones affiliated with the Military Nutrition Division at USARIEM. Co-Investigators are C. Gabaree, Lt. Col. T. C. Murphy, Donna Ryan, M.D., E. Brooks, R.N., M.N.

The purpose of the study was to evaluate a group of Special Operations Forces volunteers to determine the metabolic variation during rest, exercise and post-exercise recovery of the individual soldiers. The complete amended protocol can be found in Appendix VI of the Fifth Quarterly Report. On June 11 ten SOF soldiers arrived to serve as the first cohort for testing. Army personnel at the PBRC included Tanya Jones, Principal Investigator, 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, 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 ten members of the Special Operations Forces from the 10th SFG at Fort Devens, Massachusetts participated in the study. All procedures were carried out safely and satisfactorily.

In the last quarter of the third year of the project we discussed the publication process with Dr. Harris Lieberman. PBRC scientists agreed to assist USARIEM in the completion of the draft manuscript begun by Ms. Tanya Jones.

Also in the 12th quarter, planning began on a new metabolic unit project, "Effects of Prolonged Inactivity on Musculoskeletal and Cardiovascular Systems with Evaluation of a Potential Countermeasure." At the January 26, 1995 site visit, Drs. Vogel and Lieberman and Col. Gifford agreed to support the development of a protocol to evaluate the use of a pharmacologic countermeasure to the physical and functional effects of prolonged inactivity. However, based upon review by the CMNR (see appendix of 19th and 20th Quarterly Reports), we decided not to proceed with this project.

C. Conclusions

The Metabolic Unit Project demonstrated that carbohydrate loading produced increments in physical performance in Special Operations soldiers. However, the variation between individual soldiers was not great enough to support developing individualized carbohydrate supplements. As a result of this work, the Special Operations Forces did not pursue a plan to develop individualized soldier supplements for Special Operations. 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 Special Operations Forces soldiers.

D. References

None.

APPENDIX
MENU MODIFICATION PROJECT

Table 1. Mean Daily Intake of Selected Nutrients During Predeployment, Field Training, and Recovery

	7/16/96		7/17/96		7/18/96		7/19/96		7/20/96		7/21/96		6 Day Ave.		7/31/96		8/1/96		2 Day Ave.	
	Average	Std. Dev.	Average	Std. Dev.	Average	Std. Dev.	Average	Std. Dev.	Average	Std. Dev.	Average	Std. Dev.	Average	Std. Dev.	Average	Std. Dev.	Average	Std. Dev.	Average	Std. Dev.
N	81		84		86		78		56		53		73	14.6	41		40		40.5	0.7
Energy (kCal)	3328	1466	2959	1451	2780	1393	2892	1828	2839	1839	1841	1300	2773.2	495.9	2735	1505	3196	1521	2965.5	326.0
Protein (g)	126	55	102	56	109	58	98.3	51.8	81.6	42.5	69.6	40.2	97.8	20.0	95.5	48.3	101	49	98.3	3.9
Total Fat (g)	124	60	107	66	97.1	61.0	90.5	55.3	76.0	51.0	75.8	72.5	95.1	18.7	97.1	54.5	98.2	59.2	97.7	0.8
MUFA (g)	47.3	24.0	42.0	25.6	35.4	21.5	34.2	20.1	29.9	21.4	28.6	30.2	36.2	7.2	41.5	24.1	41.3	26.7	41.4	0.1
PUFA (g)	17.7	12.0	16.8	11.4	15.7	13.3	15.0	15.5	13.5	13.2	14.8	17.3	15.6	1.5	11.6	7.7	13.3	7.9	12.5	1.2
Sat. Fatty Acid (g)	45	22	37.3	24.0	34.8	23.3	31.9	19.5	25.2	16.9	24.5	20.7	33.1	7.7	36.5	22.3	35.6	23.4	36.1	0.6
Carbohydrate (g)	411	201	388	197	356	187	348	211	324	203	218	152	340.8	67.5	291	168	385	198	338.0	66.5
Alcohol (g)	13.9	44.9	10.6	43.6	10.4	31.9	45.8	111	80.4	136	4.2	22.1	27.6	29.8	46.9	105.0	56.2	96.6	51.6	6.6
TDF (g)	18.1	10.6	18.3	11.0	17.0	10.1	15.0	10.7	14.2	9.4	11.0	11.4	15.6	2.8	14.0	8.9	16.6	8.6	15.3	1.8
Calcium (mg)	1455	848	1273	1033	1360	1251	1176	825	811	574	700	508	1129.2	305.9	977	743	1118	703	1047.5	99.7
Iron (mg)	20.6	12.8	20.0	16.5	18.2	12.0	17.0	12.6	14.0	8.4	10.9	7.3	16.8	3.7	15.1	8.3	15.3	7.8	15.2	0.1
Magnesium (mg)	385	207	360	212	345	228	326	189	318	208	218	192	325.3	57.8	288	193	339	166	313.5	36.1
Phosphorous (mg)	2011	960	1791	1098	1795	1200	1675	917	1374	727	1072	723	1619.7	339.6	1477	844	1666	764	1571.5	133.6
Potassium (mg)	4528	1920	4314	2399	3737	2141	3365	1718	3062	1568	2242	1420	3541.3	843.6	2559	1417	3183	1347	2871.0	441.2
Sodium (mg)	4370	2160	4096	2419	3695	1811	3482	1710	2998	1993	2831	2314	3578.7	602.1	3066	1574	3271	1794	3168.5	145.0
Zinc (mg)	19.3	11.5	15.1	12.4	15.5	10.1	13.4	8.9	11.5	7.1	9.3	6.0	14.0	3.5	13.7	7.9	12.6	6.4	13.2	0.8
Copper (mg)	2.00	1.09	1.77	1.38	1.69	1.03	1.60	0.98	1.36	0.83	1.10	0.83	1.6	0.3	1.31	0.75	1.44	0.68	1.4	0.1
Vitamin A (IU)	6296	5656	8610	9282	7112	7337	5885	6575	4032	5955	6016	14797	6325.2	1509.2	2715	2891	3385	3328	3050.0	473.8
Vitamin A (RE)	1169	1089	1320	1251	1187	1006	972	941	617	659	828	1510	1015.5	261.4	454	477	554	428	504.0	70.7
Carotenes (mg)	346	410	419	681	458	676	376	587	292	605	411	1438	383.7	59.0	181	222	233	306	207.0	36.8
Vitamin C (mg)	218	231	241	208	200	208	145	180	101	97	77.7	86.9	163.8	66.2	83.5	119.2	119	132	101.3	25.1
Vitamin E (mg)	17.5	47.6	13.1	20.4	11.1	15.0	11.0	12.5	7.0	6.8	6.7	6.0	11.1	4.0	8.5	8.2	7.9	5.3	8.2	0.4
Vitamin B1 (mg)	2.83	3.64	2.62	3.57	2.71	3.40	1.92	1.26	1.69	0.92	1.29	0.90	2.2	0.6	1.68	1.05	1.81	1.02	1.7	0.1
Vitamin B2 (mg)	3.64	3.39	3.29	3.86	3.36	3.88	2.79	1.76	2.29	1.32	1.72	1.14	2.8	0.7	2.09	1.29	2.44	1.08	2.3	0.2
Vitamin B3 (mg)	33.0	20.0	28.5	19.8	28.9	20.8	29.3	18.6	25.4	16.6	18.6	13.4	27.3	4.9	28.3	15.7	29.4	15.5	28.9	0.8
Vitamin B6 (mg)	3.14	3.13	2.63	3.61	2.72	3.59	2.31	1.61	2.28	1.54	1.28	0.78	2.4	0.6	1.91	1.51	2.18	1.24	2.0	0.2
Folacin (mg)	404	246	430	251	398	326	347	253	307	223	192	155	346.3	87.6	268	235	343	223	305.5	53.0
Vitamin B12 (mcg)	8.61	5.58	6.44	6.53	6.66	6.87	5.36	3.98	4.43	3.05	4.15	3.11	5.9	1.7	5.04	3.38	4.98	3.29	5.0	0.0
Cholesterol (mg)	466	265	398	278	398	278	324	224	246	183	263	265	349.2	86.2	285	226	354	301	319.5	48.8

Table 2. Mean Daily Intake of Selected Nutrients for 6 Days

	7/16/96		7/17/96		7/18/96		7/19/96		7/20/96		7/21/96		6 Day Ave.	
	Average	Std. Dev.	Average	Std. Dev.	Average	Std. Dev.	Average	Std. Dev.	Average	Std. Dev.	Average	Std. Dev.	Average	Std. Dev.
N	81		84		86		78		56		53		73	14.6
Energy (kCal)	3328	1466	2959	1451	2780	1393	2892	1828	2839	1839	1841	1300	2773.2	495.9
Protein (g)	126	55	102	56	109	58	98.3	51.8	81.6	42.5	69.6	40.2	97.8	20.0
Total Fat (g)	124	60	107	66	97.1	61.0	90.5	55.3	76.0	51.0	75.8	72.5	95.1	18.7
MUFA (g)	47.3	24.0	42.0	25.6	35.4	21.5	34.2	20.1	29.9	21.4	28.6	30.2	36.2	7.2
PUFA (g)	17.7	12.0	16.8	11.4	15.7	13.3	15.0	15.5	13.5	13.2	14.8	17.3	15.6	1.5
Sat. Fatty Acid (g)	45	22	37.3	24.0	34.8	23.3	31.9	19.5	25.2	16.9	24.5	20.7	33.1	7.7
Carbohydrate (g)	411	201	388	197	356	187	348	211	324	203	218	152	340.8	67.5
Alcohol (g)	13.9	44.9	10.6	43.6	10.4	31.9	45.8	111	80.4	136	4.2	22.1	27.6	29.8
TDF (g)	18.1	10.6	18.3	11.0	17.0	10.1	15.0	10.7	14.2	9.4	11.0	11.4	15.6	2.8
Calcium (mg)	1455	848	1273	1033	1360	1251	1176	825	811	574	700	508	1129.2	305.9
Iron (mg)	20.6	12.8	20.0	16.5	18.2	12.0	17.0	12.6	14.0	8.4	10.9	7.3	16.8	3.7
Magnesium (mg)	385	207	360	212	345	228	326	189	318	208	218	192	325.3	57.8
Phosphorous (mg)	2011	960	1791	1098	1795	1200	1675	917	1374	727	1072	723	1619.7	339.6
Potassium (mg)	4528	1920	4314	2399	3737	2141	3365	1718	3062	1568	2242	1420	3541.3	843.6
Sodium (mg)	4370	2160	4096	2419	3695	1811	3482	1710	2998	1993	2831	2314	3578.7	602.1
Zinc (mg)	19.3	11.5	15.1	12.4	15.5	10.1	13.4	8.9	11.5	7.1	9.3	6.0	14.0	3.5
Copper (mg)	2.00	1.09	1.77	1.38	1.69	1.03	1.60	0.98	1.36	0.83	1.10	0.83	1.6	0.3
Vitamin A (IU)	6296	5656	8610	9282	7112	7337	5885	6575	4032	5955	6016	14797	6325.2	1509.2
Vitamin A (RE)	1169	1089	1320	1251	1187	1006	972	941	617	659	828	1510	1015.5	261.4
Carotenes (mg)	346	410	419	681	458	676	376	587	292	605	411	1438	383.7	59.0
Vitamin C (mg)	218	231	241	208	200	208	145	180	101	97	77.7	86.9	163.8	66.2
Vitamin E (mg)	17.5	47.6	13.1	20.4	11.1	15.0	11.0	12.5	7.0	6.8	6.7	6.0	11.1	4.0
Vitamin B1 (mg)	2.83	3.64	2.62	3.57	2.71	3.40	1.92	1.26	1.69	0.92	1.29	0.90	2.2	0.6
Vitamin B2 (mg)	3.64	3.39	3.29	3.86	3.36	3.88	2.79	1.76	2.29	1.32	1.72	1.14	2.8	0.7
Vitamin B3 (mg)	33.0	20.0	28.5	19.8	28.9	20.8	29.3	18.6	25.4	16.6	18.6	13.4	27.3	4.9
Vitamin B6 (mg)	3.14	3.13	2.63	3.61	2.72	3.59	2.31	1.61	2.28	1.54	1.28	0.78	2.4	0.6
Folacin (mg)	404	246	430	251	398	326	347	253	307	223	192	155	346.3	87.6
Vitamin B12 (mcg)	8.61	5.58	6.44	6.53	6.66	6.87	5.36	3.98	4.43	3.05	4.15	3.11	5.9	1.7
Cholesterol (mg)	466	265	398	278	398	278	324	224	246	183	263	265	349.2	86.2

Table 3. Mean Percentage of Total Energy for Macronutrients

Date	% Protein	% Fat	% CHO	% Alcohol	% SFA	% MUFA	% PUFA
7/16/96	15.1	33.6	49.4	2.9	12.2	12.8	4.8
7/17/96	13.8	32.6	52.4	2.5	11.4	12.8	5.1
7/18/96	13.6	28.2	48.2	11.0	9.9	10.6	4.7
7/19/96	13.6	28.2	48.2	11.0	9.9	10.6	4.7
7/20/96	11.5	24.2	45.7	19.6	8.0	9.5	4.3
7/21/96	15.1	37.1	47.3	1.6	12.0	14.0	7.2
7/31/96	14.0	32.0	42.6	12.0	12.0	13.7	3.8
8/1/96	12.8	27.7	48.2	12.2	10.1	11.7	3.7
Total	13.7	30.3	48.4	8.6	10.7	11.8	4.8

Table 4. Average Caloric Intake by Meal, by Day and Number of Subjects

		07/16/96	07/17/96	7/18/96	7/19/96	7/20/96	7/21/96	
Breakfast	N	62	72	64	46	44	33	
	Mean	948	958	986	886	993	1139	Mean = 985
	SD	369	466	376	365	392	455	SD = 85
Lunch	N	57	47	56	60	5	2	
	Mean	1122	1089	986	1180	1107	992	Mean = 1079
	SD	423	427	376	419	932	459	SD = 76
Dinner	N	71	62	70	40	38	42	
	Mean	1171	1190	1099	1076	1262	1042	Mean = 1140
	SD	369	387	460	442	465	365	SD = 82
Snack	N	58	51	39	35	27	11	
	Mean	1098	1069	1060	2060	2343	1294	Mean = 1487
	SD	918	718	804	1944	1571	1428	SD = 567
	Day Totals	4339	4307	4131	5202	5705	4467	Average= 4692

Table 5. Distribution of Calcium Intake Among U.S. Army Rangers

	N	%
Above 1200 mg	31	33.7
800 - 1200 mg	24	26.1
700 - 799 mg	16	17.4
600 - 699 mg	7	7.6
500 - 599 mg	4	4.3
Below 500 mg	10	10.9

Table 6. Mean Percent Contributions to Mean Energy Intake for Six Days

		Protein	Fat	CHO	Kcal	Alcohol	SFA	MUFA	PUFA
7/16/96 Total	Breakfast	2394.7	2098.4	9354.9	64897.9	0	791.4	763.9	271.5
7/17/96 Total		2448.9	2417.1	9586.1	69004.3	0	847.5	906.8	325.7
7/18/96 Total		2312.6	2255.3	8362.2	62314.6	0	806.9	803.1	299.7
7/19/96 Total		1587.9	1296.3	5798.4	40737.6	0	497.0	475.6	165.0
7/20/96 Total		1636.0	1495.1	6029.0	43682.0	0	485.8	576.5	267.7
7/21/96 Total		1484.4	1651.8	4289.4	37594.2	0	567.2	597.6	276.7
Total		11864.5	11214.0	43420.0	318230.6	0	3995.9	4123.6	1606.4
% Contribution		14.9	31.7	54.6		0	11.3	11.7	4.5
7/16/96 Total	Lunch	2782.2	2521.3	6050.4	57841.3	0.0	932.9	906.4	366.5
7/17/96 Total		2194.7	2038.9	5969.9	50609.8	0.0	687.4	801.6	331.4
7/18/96 Total		2310.9	2048.1	8226.8	60090.9	0.0	668.9	717.8	382.6
7/19/96 Total		3021.7	2794.1	8530.9	70819.1	0.0	918.1	1003.7	561.5
7/20/96 Total		217.0	122.3	891.6	5532.5	2.1	49.1	48.7	14.7
7/21/96 Total		135.0	90.6	154.3	1984.4	0.0	30.6	37.7	13.5
Total		10661.6	9615.2	29824.0	246878.0	2.1	3287.0	3515.9	1670.1
% Contribution		17.3	35.1	48.3		0.0	12.0	12.8	6.1
7/16/96 Total	Dinner	3298.8	3559.4	9221.2	83112.8	190.1	1239.2	1387.5	508.4
7/17/96 Total		2687.7	2941.7	9386.8	73805.4	0.0	1004.3	1199.3	493.2
7/18/96 Total		3672.8	2870.8	9193.1	76936.3	0.0	1063.7	1089.4	471.4
7/19/96 Total		1672.0	1590.3	5524.9	43049.8	34.6	534.9	614.0	278.6
7/20/96 Total		1894.7	1811.7	5720.1	47974.2	229.0	613.1	698.6	310.5
7/21/96 Total		1716.9	1610.5	5661.5	43753.9	0.5	531.8	598.5	321.6
Total		14942.9	14384.5	44707.6	368632.5	454.1	4986.9	5587.4	2383.7
% Contribution		16.2	35.1	48.5		0.9	12.2	13.6	5.8
7/16/96 Total	Snack	1703.1	1895.3	8633.8	63705.2	938.0	698.9	772.8	287.0
7/17/96 Total		1267.2	1583.2	7655.1	55100.4	886.7	592.5	621.1	260.4
7/18/96 Total		1210.2	1249.7	5112.8	42126.1	896.3	482.6	460.9	218.3
7/19/96 Total		1427.8	1414.3	7464.2	72105.9	3540.3	547.5	584.8	180.8
7/20/96 Total		858.5	892.7	5695.6	63271.5	4270.5	279.7	373.7	176.1
7/21/96 Total		350.4	665.1	1441.9	14237.5	222.7	168.9	280.9	173.7
Total		6817.2	7700.2	36003.4	310546.6	10754.6	2770.1	3094.2	1296.4
% Contribution		8.8	22.3	46.4		24.2	8.0	9.0	3.8
Total for 6 days		44286.2	42913.9	153955.0	1244287.7	11210.7	15039.8	16321.1	6956.5
% Contribution		14.23664	31.03988	49.49178		6.306815	10.87839	11.80512	5.031694

Table 7. Milk Consumption in an Average Day as Observed in U.S. Army Rangers

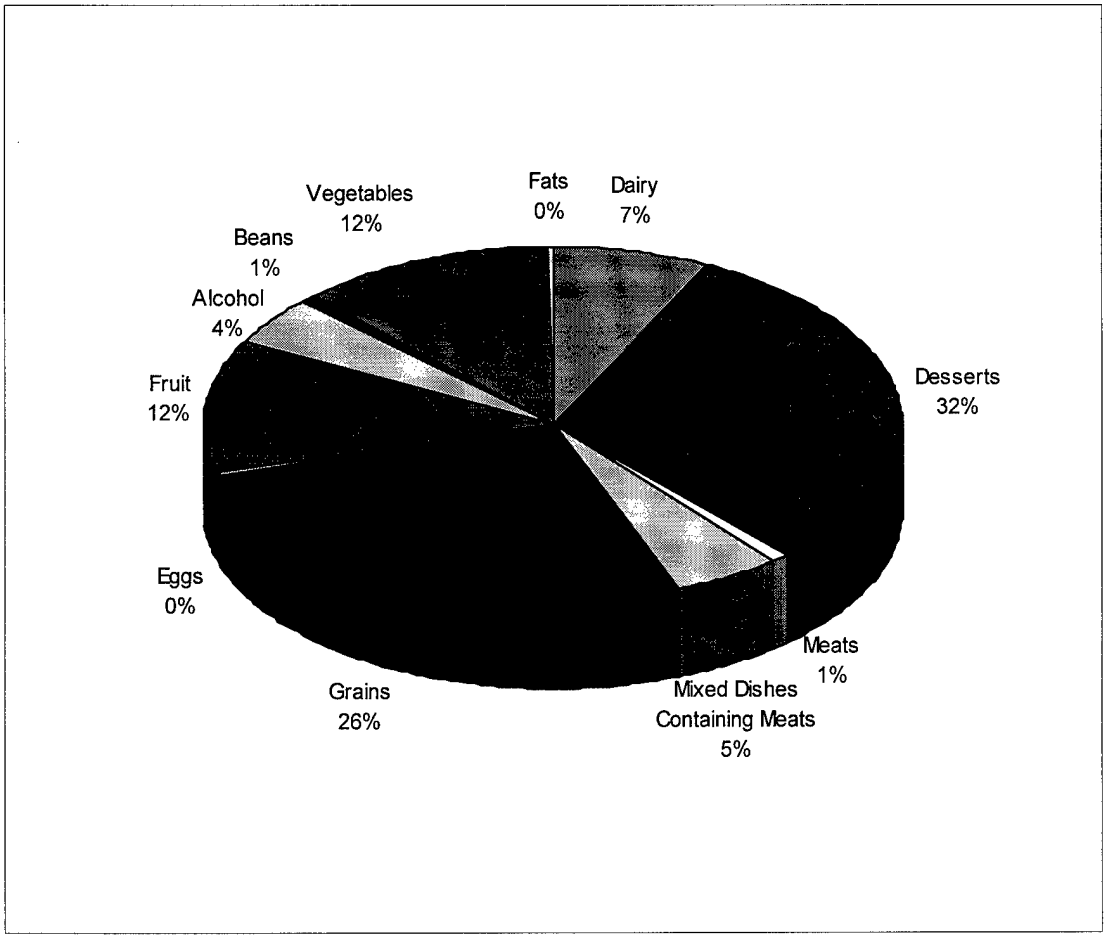
Glasses per Day													
Day	0		0 - 0.5		1		1.5		2		>2		
	N	%	N	%	N	%	N	%	N	%	N	%	Total N
1	36	44	4	4.9	11	13.6	1	1.2	9	11.1	20	24.7	81
2	39	46	3	3.6	11	13.1	1	1.2	13	15.5	17	20.2	84
3	44	51	3	3.4	7	8.0	2	2.3	7	8.0	23	26.4	87
4	45	57	2	2.5	4	5.1	4	5.1	14	17.7	10	12.7	79
5	35	61	2	3.5	8	14.0	0	0.0	8	14.0	4	7.0	57
6	32	60	2	3.8	5	9.4	0	0.0	10	18.9	4	7.5	53

Table 8. Number of Visible Eggs Eaten per Day

		number of eggs consumed							
Day	**Egg Type**	**0**	**0 - 1**	**2**	**3**	**4**	**5**	**6**	**7 +**
1	Whole	47	4	25	3	2	0	0	0
	White	62	0	3	7	8	1	0	0
2	Whole	51	4	23	2	3	0	1	0
	White	65	0	4	3	9	1	1	1
3	Whole	52	5	27	1	2	0	0	0
	White	70	1	4	4	6	1	1	0
4	Whole	59	1	18	0	1	0	0	0
	White	66	0	2	3	6	0	1	1
5	Whole	50	1	5	0	1	0	0	0
	White	47	0	2	2	3	1	2	0
6	Whole	41	5	5	1	0	1	0	0
	White	46	1	2	1	1	0	2	0

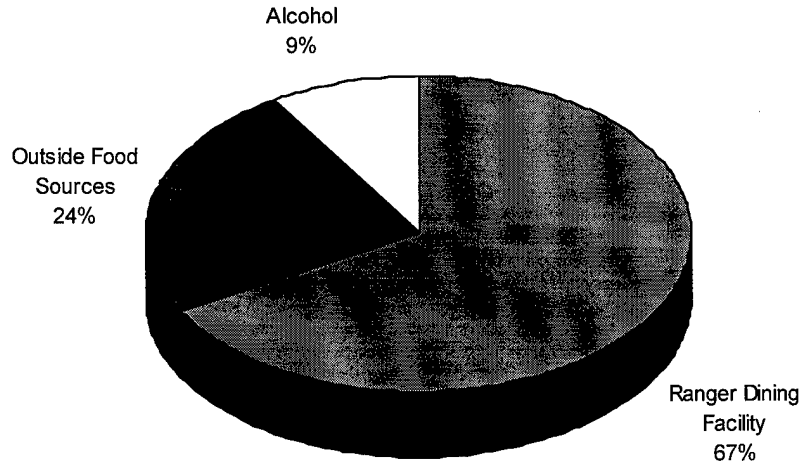
Percent Contributions of Major Food Groups to Total CHO Intake

	% CHO	Protein	Fat	CHO	Kcal	Alcohol
Dairy	7.2	7776.508	5711.72	11090.06	126331.2	0
Desserts	31.1	1549.629	2991.988	47675.25	217734.4	1.227065
Meats	0.9	9277.548	7863.303	1389.08	115720.2	0
Mixed Dishes Containing Meats	5.0	8838.136	7269.134	7740.339	133105.5	0
Grains	26.0	8245.998	6890.766	39888.49	255772.6	0
Eggs	0.3	2748.293	1500.348	404.9673	27014.28	0
Fruit	12.3	920.2777	235.9753	18835.87	76867.88	0
Alcohol	4.5	593.4684	38.9022	6878.264	107411.8	11207.88
Beans	0.7	872.4809	1536.111	1104.327	20433.62	0
Vegetables	11.7	3215.427	4626.143	17985.65	121633.2	0
Fats	0.3	47.42547	4239.54	472.7321	39350.03	0
Total	100.0	44085.19	42903.93	153465	1241375	11209.11



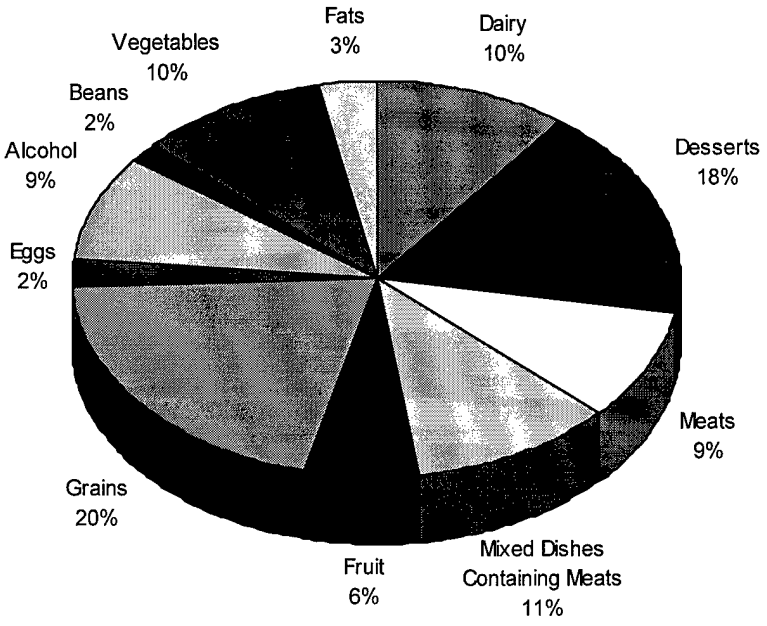
Percent Contribution of Total Calories from Dining Facility versus Meals and Snacks from Other Sources

		7/16/96	7/17/96	7/18/96	7/19/96	7/20/96	7/21/96	%Total
Ranger Dining Facility	N	81	82	86	76	54	48	
	Average	2377.7	2112.5	2026.9	1909.0	1433.0	1556.0	
	SD	951.5	1099.6	949.8	909.1	595.2	794.0	
	%	71.4	69.7	72.2	64.0	48.2	76.5	67.3
	Total	192595.4	173227.3	174311.2	145083.8	77379.7	74688.0	
Outside Food Sources	N	57	53	44	34	28	12	
	Average	1147.049	1273.53	1325.253	1395.65	1430.746	1740.629	
	SD	951.9141	817.3001	962.6721	1240.258	1034.15	1367.055	
	%	24.25525	27.15963	24.14862	20.93051	24.96624	21.40776	24.07727
	Total	65381.77	67497.09	58311.14	47452.09	40060.89	20887.55	
Alcohol	N	13	9	11	22	23	2	
	Average	890.7692	866.1778	804.134	1553.483	1870.423	997.2	
	SD	898.5378	704.1606	430.5227	1376.384	1302.678	300.379	
	%	4.295935	3.13681	3.66321	15.07488	26.8102	2.044072	8.632395
	Total	11580	7795.6	8845.474	34176.63	43019.72	1994.4	



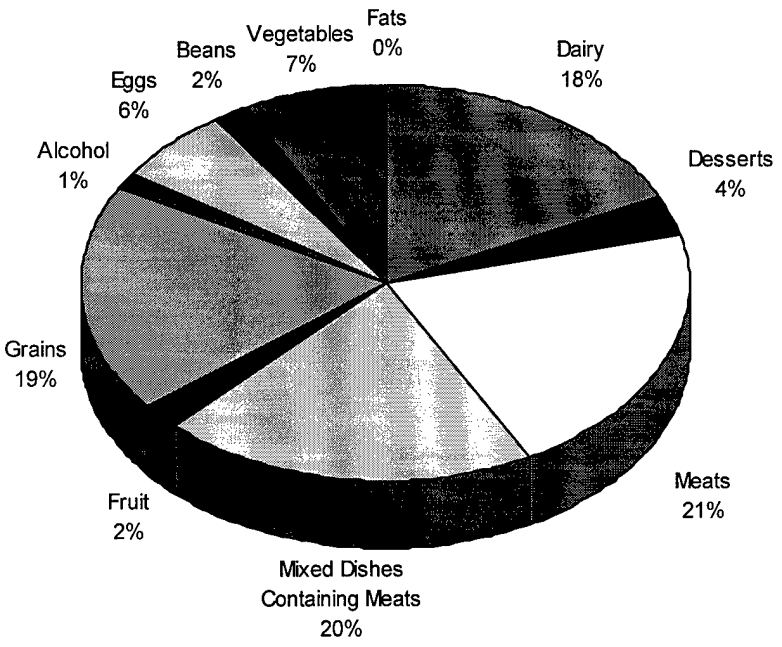
Percent Contributions of Major Food Groups to Total Energy Intake

	% Kcal	Protein	Fat	CHO	Kcal	Alcohol
Dairy	10.2	7776.508	5711.72	11090.06	126331.2	0
Desserts	17.5	1549.629	2991.988	47675.25	217734.4	1.227065
Meats	9.3	9277.548	7863.303	1389.08	115720.2	0
Mixed Dishes Containing Meats	10.7	8838.136	7269.134	7740.339	133105.5	0
Fruit	6.2	920.2777	235.9753	18835.87	76867.88	0
Grains	20.6	8245.998	6890.766	39888.49	255772.6	0
Eggs	2.2	2748.293	1500.348	404.9673	27014.28	0
Alcohol	8.7	593.4684	38.9022	6878.264	107411.8	11207.88
Beans	1.6	872.4809	1536.111	1104.327	20433.62	0
Vegetables	9.8	3215.427	4626.143	17985.65	121633.2	0
Fats	3.2	47.42547	4239.54	472.7321	39350.03	0
Total	100.0	44085.19	42903.93	153465	1241375	11209.11

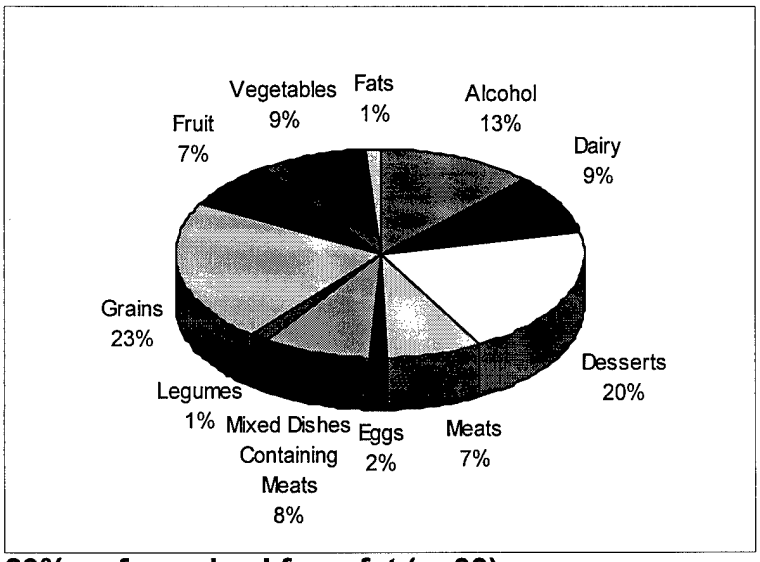


Percent Contributions of Major Food Groups to Total Protein Intake

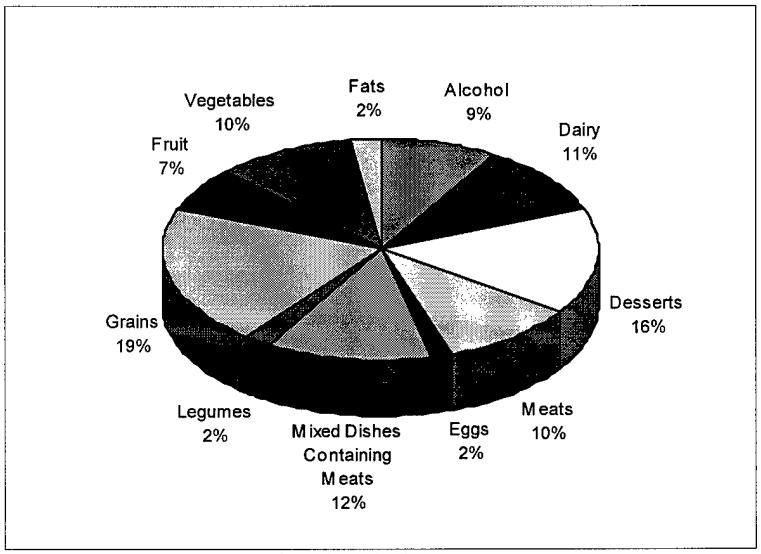
	% Protein	Protein	Fat	CHO	Kcal	Alcohol
Dairy	17.6	7776.508	5711.72	11090.06	126331.2	0
Desserts	3.5	1549.629	2991.988	47675.25	217734.4	1.227065
Meats	21.0	9277.548	7863.303	1389.08	115720.2	0
Mixed Dishes Containing Meats	20.0	8838.136	7269.134	7740.339	133105.5	0
Fruit	2.1	920.2777	235.9753	18835.87	76867.88	0
Grains	18.7	8245.998	6890.766	39888.49	255772.6	0
Alcohol	1.3	593.4684	38.9022	6878.264	107411.8	11207.88
Eggs	6.2	2748.293	1500.348	404.9673	27014.28	0
Beans	2.0	872.4809	1536.111	1104.327	20433.62	0
Vegetables	7.3	3215.427	4626.143	17985.65	121633.2	0
Fats	0.1	47.42547	4239.54	472.7321	39350.03	0
Total	100.0	44085.19	42903.93	153465	1241375	11209.11



Percent Contribution of Major Food Groups at Different Fat Intake Levels of U.S. Army Rangers

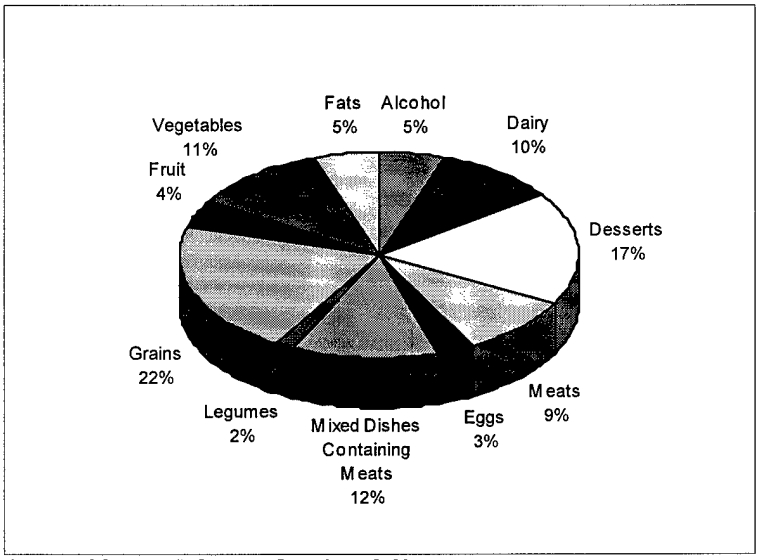


29% or fewer kcal from fat (n=33)

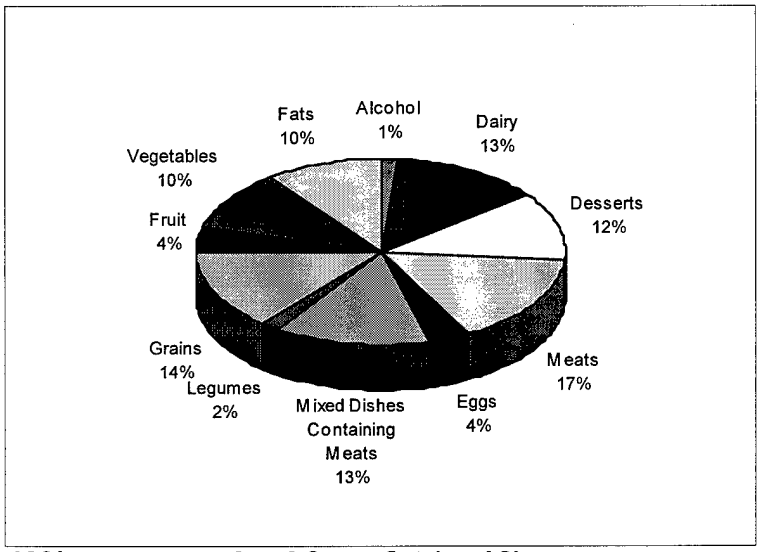


30-34% kcal from fat (n=26)

Percent Contribution of Major Food Groups at Different Fat Intake Levels of U.S. Army Rangers (continued)



35-39% kcal from fat (n=21)



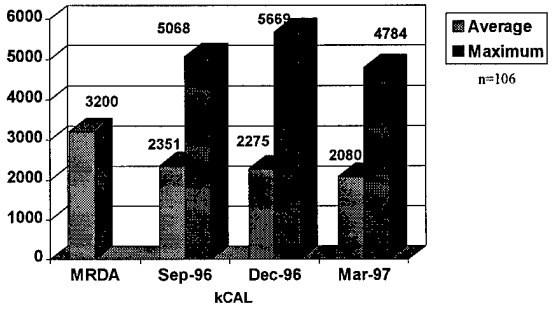
40% or greater kcal from fat (n=12)

Distribution of Dietary Cholesterol Intakes at Breakfast, Lunch, Dinner, and Snacks

		16-Jul	17-Jul	18-Jul	19-Jul	20-Jul	21-Jul	31-Jul	1-Aug	
Breakfast	N	62	72	64	46	44	33	14	23	
	Mean	224.9	230.6	262.1	229.7	162.6	242.0	250.0	285.4	Mean = 235.9
	SD	225.7	261.1	233.5	244.8	199.4	241.3	193.1	293.9	SD = 35.7
Lunch	N	57	47	56	60	5	2	21	27	
	Mean	153.3	128.4	87.2	136.3	68.8	169.0	95.1	98.3	Mean = 117.0
	SD	80.7	83.6	59.0	55.9	76.3	13.6	61.4	49.8	SD = 35.0
Dinner	N	71	62	70	40	38	42	28	27	
	Mean	150.6	120.1	136.5	97.0	144.6	107.8	136.4	134.6	Mean = 128.4
	SD	92.0	89.2	83.9	66.1	79.8	82.2	111.8	94.8	SD = 18.5
Snack	N	58	51	39	35	27	11	26	28	
	Mean	76.0	60.6	82.2	78.1	45.4	97.1	91.8	54.7	Mean = 73.2
	SD	105.2	87.8	170.5	130.4	59.3	175.2	123.9	97.2	SD = 18.2
	Day Totals	604.8	539.6	568.1	541.1	421.3	615.9	573.3	573.0	Avg= 554.6

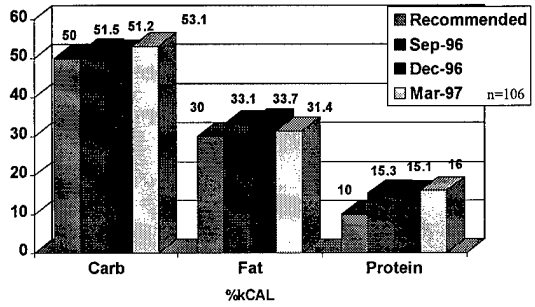
RESULTS

Total Energy Intake



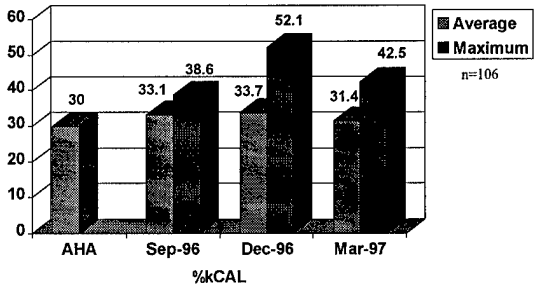
RESULTS

Energy Intake Distribution



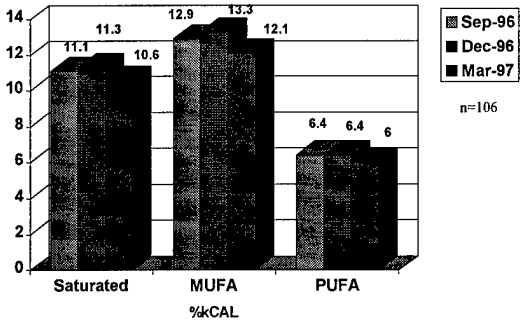
RESULTS

Dietary Total Fat Intake



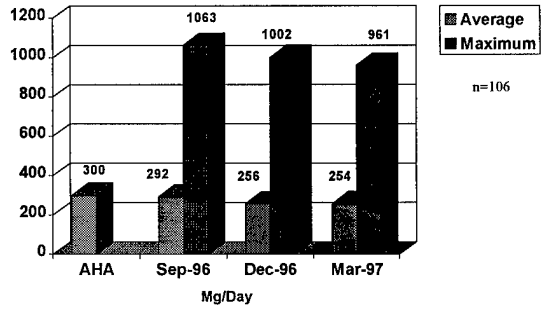
RESULTS

Dietary Fat Intake



RESULTS

Dietary Cholesterol Intake



CONCLUSIONS

- The average diet for USASMA students approaches dietary recommendations.
- There was no significant change in the average diet over time.
- Serum lipid levels increased over time.

Table 1

ETHNIC INCLUDES THE FOLLOWING RECIPES:

AMERICAN

- Apple Betty
- Garlic Cheese Potatoes
- German Potatoes
- Horseradish Potatoes
- Roasted Vegetable Salad
- Tomato Salad
- Turkey Chili
- Turkey Stroganoff
- Turnips and Greens

BREAKFAST

- Biscuits
- Breakfast Burrito
- Breakfast Potatoes
- Eggs Florentine
- Jalapeno Cheese Grits
- Oatmeal Raisin Bar
- Three Berry Muffins
- Turkey Sausage

CAJUN

- Bread Pudding
- Cajun Meatloaf
- Chicken Jambalaya
- Chicken Sauce Piquant
- Eggplant Tomato Salad
- Fish Piquant
- Red Beans with Turkey Sausage
- Summer Squash

CARIBBEAN

- Caribbean Jerk Chicken
- Caribbean Pot Roast
- Jamaican Rum Chicken
- Okra Melange

CHINESE

- Beef with Broccoli
- Chicken with Orange Glaze
- Cucumber Salad
- Oriental Chicken Salad
- Rolled Fish
- Vegetable Rice

INDONESIAN

- Fish and Mushrooms
- Thai Beef Salad

ITALIAN

- Italian Potatoes
- Pasta Primavera
- Pasta Provencal
- Pasta Putanesca

MEXICAN

- Chicken Fajitas
- Mexican Black Beans
- Mexican Cornbread
- Seven Bean Salad
- Southwestern Rice
- Vegetarian Burrito

TABLE 2. COMPARISON OF MEAN ACCEPTABILITY SCORES AND STANDARD ERRORS OF THE MEAN (SE) BETWEEN LOUISIANA TECH UNIVERSITY AND FORT POLK

	LOUISIANA TECH			FORT POLK			T-TEST	
RECIPE	N	MEAN	SE	N	MEAN	SE	P VALUE	DIFF
APPLE BETTY	74	6.77	.22	42	5.76	.32	0.0050 **	-
BEEF AND BROCCOLI	102	7.44	.16	58	7.07	.21	0.0906	-
BISCUITS	97	6.28	.22	63	6.46	.24	0.5468	+
BREAKFAST BURRITO	75	6.68	.20	64	6.52	.24	0.5311	-
BREAKFAST POTATOES	82	7.02	.14	20	7.05	.52	0.1929	+
BREAD PUDDING	89	7.48	.19	40	7.60	.17	0.4448	+
CAJUN MEATLOAF	85	7.42	.11	38	6.28	.33	0.0029 **	-
CARIBBEAN JERK CHICKEN	97	7.22	.14	66	7.41	.17	0.2606	+
CARIBBEAN POT ROAST	62	7.18	.24	39	7.38	.22	0.6038	+
CHICKEN FAJITA SALAD	88	7.81	.15	69	6.88	.25	0.0037 **	-
CHICKEN JAMBALAYA	88	6.95	.15	50	7.14	.24	0.3103	+
CHICKEN SAUCE PIQUANT	61	7.51	.21	60	7.03	.22	0.1738	-
CHICKEN WITH ORANGE GLAZE	91	6.85	.24	50	6.44	.26	0.2425	-
CUCUMBER SALAD	88	6.23	.22	8	7.13	.23	0.2495	+
EGGPLANT TOMATO SALAD	62	3.81	.28	14	5.71	.61	0.0120 *	+
EGGS FLORENTINE	81	5.47	.25	29	5.93	.40	0.4467	+
FISH AND MUSHROOMS	68	6.81	.17	50	6.84	.29	0.5462	+
FISH PIQUANT	86	7.37	.14	47	6.36	.27	0.0028 **	-
GARLIC CHEESE POTATOES	94	7.13	.26	16	6.31	.59	0.1812	-
GERMAN POTATOES	88	6.25	.24	32	5.88	.38	0.3274	-
HORSERADISH POTATOES	81	5.48	.34	39	4.28	.44	0.0187 *	-
ITALIAN POTATOES	100	7.39	.15	31	6.42	.30	0.0036 **	-
JALAPENO CHEESE GRITS	70	5.62	.31	9	4.11	.81	0.0768	-
JAMAICAN RUM CHICKEN	70	7.63	.13	24	6.58	.36	0.0019 **	-
MEXICAN BLACK BEANS	66	6.27	.30	23	7.52	.24	0.0014 **	+
MEXICAN CORN BREAD	84	6.52	.25	70	6.50	.21	0.6567	-
OATMEAL RAISIN BAR	102	6.65	.20	24	6.70	.29	0.5237	+
OKRA MELANGE	60	5.85	.31	25	6.56	.40	0.1625	+
ORIENTAL CHICKEN SALAD	100	6.23	.21	25	6.40	.37	0.4925	+
PASTA PRIMAVERA	70	6.16	.26	96	7.54	.12	0.0001 ***	+
PASTA PROVENCAL	96	6.97	.24	56	6.55	.28	0.5822	+
PASTA PUTANESCA	60	6.62	.19	18	7.33	.40	0.0804	-
RED BEANS WITH TURKEY SAUSAGE	83	7.45	.13	109	7.34	.14	0.6405	-
ROASTED VEGETABLE SALAD	83	4.22	.37	21	6.00	.36	0.0079 **	+
ROLLED FISH	96	5.51	.31	50	6.62	.29	0.0103 *	+
SEVEN BEAN SALAD	116	5.58	.27	24	5.88	.44	0.7058	+
SOUTHWESTERN RICE	60	7.40	.20	64	6.42	.25	0.0087 **	-
SUMMER SQUASH	93	6.60	.25	18	6.72	.47	0.9870	+
THAI BEEF SALAD	81	6.02	.20	84	6.44	.23	0.0833	+
THREE BERRY MUFFIN	70	6.77	.24	40	5.82	.33	0.0069 **	-
TOMATO SALAD	88	6.03	.24	18	6.77	.54	0.0782	+
TURKEY CHILI	100	7.41	.19	33	6.70	.22	0.0014 **	-
TURKEY SAUSAGE	80	7.10	.17	36	5.25	.40	0.0001 ***	-
TURKEY STROGANOFF	81	6.23	.25	83	6.40	.22	0.3700	+
TURNIPS AND GREENS	66	5.03	.30	33	7.09	.37	0.0001 ***	+
VEGETABLE RICE	89	7.48	.16	82	6.60	.22	0.0032 **	-
VEGETARIAN BURRITO	95	7.56	.13	50	6.46	.33	0.0015 **	-

Table 3A PERCENTAGE OF ACCEPTABLE (≥ 6.0) AND UNACCEPTABLE (< 6.0) RECIPE ITEMS BY FACILITY: PENNINGTON BIOMEDICAL RESEARCH CENTER

PENNINGTON	N	< 6.0		≥ 6.0	
ETHNIC		N	%	N	%
AMERICAN	382	90	23.6	292	76.4
BREAKFAST	273	72	26.4	201	73.6
CAJUN	357	70	19.6	287	80.4
CARIBBEAN	167	27	16.2	140	83.8
CHINESE	258	51	19.8	207	80.2
INDONESIAN	93	10	10.8	83	89.2
ITALIAN	160	40	25.0	120	75.0
MEXICAN	240	51	21.3	189	78.7
FOOD TYPE					
BEEF	169	17	10.1	152	89.9
BREAD	104	38	36.5	66	63.5
DESSERT	92	14	15.2	78	84.8
FISH	137	12	8.8	125	91.2
PASTA	119	33	27.7	86	72.3
POULTRY	335	60	17.9	275	82.1
SALAD	346	87	25.1	259	74.9
STARCH	429	108	25.2	321	74.8
VEGETABLE	120	26	21.7	94	78.3

Table 3B Percentage of Acceptable (≥ 6.0) and Unacceptable (< 6.0) Recipe Items by Facility: Louisiana Tech University

La Tech	N	< 6.0		≥ 6.0	
ETHNIC		N	%	N	%
American	755	342	45.3	413	54.7
Breakfast	657	252	38.4	405	61.6
Cajun	647	184	28.4	463	71.6
Caribbean	289	69	23.9	220	76.1
Chinese	566	196	34.6	370	65.4
Indonesian	149	59	39.6	90	60.4
Italian	326	94	28.8	232	71.2
Mexican	509	164	32.2	345	67.8
FOOD TYPE					
Beef	330	79	23.9	251	76.1
Bread	251	97	38.6	154	61.4
Dessert	163	43	26.4	120	73.6
Fish	250	89	35.6	161	64.4
Pasta	226	74	32.7	152	67.3
Poultry	668	162	24.3	506	75.7
Salad	706	361	51.1	345	48.9
Starch	844	240	28.4	604	71.6
Vegetable	219	111	50.7	108	49.3

Table 4A Chi Square Analysis Results for Ethnic Comparisons between Centers

	Chi-Square	P-value	Direction
American:			
Overall	52.37	0.0000	
Pennington vs Ft. Polk	33.41	0.0000	+
Pennington vs La. Tech	49.13	0.0000	+
La. Tech vs Ft. Polk	0.06	0.8058	
Breakfast:			
Overall	18.61	0.0001	
Pennington vs Ft. Polk	17.68	0.0000	+
Pennington vs La. Tech	12.05	0.0005	+
La. Tech vs Ft. Polk	2.20	0.1383	
Cajun:			
Overall	9.44	0.0089	
Pennington vs Ft. Polk	3.05	0.0806	
Pennington vs La. Tech	9.40	0.0022	+
La. Tech vs Ft. Polk	1.42	0.2335	
Caribbean:			
Overall	4.23	0.1206	
Pennington vs Ft. Polk	3.07	0.0799	
Pennington vs La. Tech	3.74	0.0531	
La. Tech vs Ft. Polk	0.00	0.9718	
Chinese:			
Overall	18.72	0.0001	
Pennington vs Ft. Polk	11.65	0.0006	+
Pennington vs La. Tech	18.17	0.0000	+
La. Tech vs Ft. Polk	0.23	0.6342	
Indonesian:			
Overall	20.59	0.0000	
Pennington vs Ft. Polk	14.14	0.0002	+
Pennington vs La. Tech	20.48	0.0000	+
La. Tech vs Ft. Polk	1.10	0.2951	
Italian:			
Overall	1.55	0.4614	
Pennington vs Ft. Polk	0.02	0.8917	
Pennington vs La. Tech	0.79	0.3744	
La. Tech vs Ft. Polk	1.25	0.2643	
Mexican:			
Overall	10.80	0.0045	
Pennington vs Ft. Polk	8.59	0.0034	+
Pennington vs La. Tech	9.46	0.0021	+
La. Tech vs Ft. Polk	0.02	0.8957	

Table 4B CHI SQUARE ANALYSIS RESULTS FOR FOOD TYPE COMPARISONS BETWEEN CENTERS

	CHI-SQUARE	P-VALUE	DIRECTION
BEEF:			
OVERALL	18.38	0.0001	
PENNINGTON VS FT. POLK	18.11	0.0000	+
PENNINGTON VS LA. TECH	13.05	0.0003	+
LA. TECH VS FT. POLK	1.32	0.2514	
BREAD:			
OVERALL	1.82	0.4022	
PENNINGTON VS FT. POLK	1.46	0.2266	
PENNINGTON VS LA. TECH	0.14	0.7098	
LA. TECH VS FT. POLK	1.18	0.2767	
DESSERT:			
OVERALL	8.16	0.0169	
PENNINGTON VS FT. POLK	8.13	0.0044	+
PENNINGTON VS LA. TECH	4.13	0.0422	+
LA. TECH VS FT. POLK	1.59	0.2072	
FISH:			
OVERALL	29.16	0.0000	
PENNINGTON VS FT. POLK	23.22	0.0000	+
PENNINGTON VS LA. TECH	28.17	0.0000	+
LA. TECH VS FT. POLK	0.10	0.7490	
PASTA:			
OVERALL	6.40	0.0408	
PENNINGTON VS FT. POLK	1.65	0.1994	
PENNINGTON VS LA. TECH	0.91	0.3392	
LA. TECH VS FT. POLK	6.39	0.0115	-
POULTRY:			
OVERALL	23.32	0.0000	
PENNINGTON VS FT. POLK	21.83	0.0000	+
PENNINGTON VS LA. TECH	5.17	0.0230	+
LA. TECH VS FT. POLK	10.27	0.0013	+
SALAD:			
OVERALL	68.15	0.0000	
PENNINGTON VS FT. POLK	6.42	0.0113	+
PENNINGTON VS LA. TECH	61.41	0.0000	+
LA. TECH VS FT. POLK	20.70	0.0000	-
STARCHES:			
OVERALL	8.36	0.0153	
PENNINGTON VS FT. POLK	7.90	0.0049	+
PENNINGTON VS LA. TECH	1.52	0.2174	
LA. TECH VS FT. POLK	4.04	0.0445	+
VEGETABLES:			
OVERALL	29.77	0.0000	
PENNINGTON VS FT. POLK	1.33	0.2495	
PENNINGTON VS LA. TECH	25.57	0.0000	+
LA. TECH VS FT. POLK	10.41	0.0013	-

NUTRIENT ANALYSIS OF RECIPES: ARMY MENU MODIFICATION PROJECT, MARCH 1995

RECIPE	KCAL	PROT, g	CARB, g	FAT, g	*% fat kcal*	SAT FAT, g	*% sat fat kcal*	CHOLES, mg	SODIUM, mg
APPLE BETTY	133	0.8	27.5	3.1	*20.8*	0.6	*4.1*	0	75
BREAD PUDDING WITH HARD SAUCE	280	5.3	47.3	8.2	*26.3*	1.8	*5.9*	69	242
BREAKFAST BURRITOS	288	19.0	28.5	12.0	*37.4*	5.0	*15.6*	156	418
BROILED WHITE FISH WITH MUSHROOMS	208	28.2	4.1	8.5	*36.6*	1.3	*5.6*	84	210
BUTTERMILK BISCUITS	238	7.3	44.6	3.1	*11.9*	0.7	*2.5*	2	528
CAJUN MEATLOAF	210	23.2	10.2	8.1	*34.8*	2.9	*12.5*	59	277
CARIBBEAN JERK CHICKEN	160	27.1	3.4	3.6	*20.5*	1.0	*5.6*	73	188
CARIBBEAN POT ROAST	220	32.0	7.2	6.6	*27.0*	2.3	*9.2*	78	313
CATFISH SAUCE PIQUANT	162	16.8	8.7	6.9	*38.3*	1.5	*8.5*	53	411
CHICKEN BREASTS WITH ORANGE GLAZE	197	26.2	6.5	6.9	*31.6*	1.9	*8.7*	72	231
CHICKEN FAJITA SALAD	186	14.1	20.7	5.3	*25.4*	0.9	*4.4*	27	330
CHICKEN JAMBALAYA	226	21.7	22.2	5.2	*20.7*	1.3	*5.2*	57	624
CHICKEN SAUCE PIQUANT	203	33.8	6.5	4.0	*17.9*	1.1	*4.9*	90	313
EGGS FLORENTINE	135	13.3	6.8	6.2	*41.4*	2.0	*13.7*	232	562
FRESH TOMATO SALAD	44	1.5	7.5	1.5	*31.7*	0.2	*4.1*	0	36
GARLIC CHEESE POTATOES	89	3.7	14.5	1.9	*19.8*	1.0	*10.1*	4	220
GERMAN POTATOES	93	2.2	19.6	1.0	*9.4*	0.2	*2.1*	16	138
ITALIAN ROASTED POTATOES	114	3.0	25.6	0.3	*2.7*	0.1	*0.6*	0	96
JALAPENO CHEESE GRITS	89	3.3	16.5	1.1	*10.7*	0.5	*5.3*	3	151
JAMAICAN RUM CHICKEN	153	20.5	6.2	4.5	*26.3*	1.3	*7.4*	61	209
MEXICAN BLACK BEANS	137	8.7	24.0	1.1	*7.1*	0.3	*2.1*	1	234

NUTRIENT ANALYSIS OF RECIPES: ARMY MENU MODIFICATION PROJECT, MARCH 1995

RECIPE	KCAL	PROT, g	CARB, g	FAT, g	*% fat kcal*	SAT FAT, g	*% sat fat kcal*	CHOLES, mg	SODIUM, mg
OATMEAL RAISIN BREAKFAST BARS	120	2.5	22.9	2.6	*19.3*	0.5	*3.6*	8	107
OKRA MELANGE	39	1.7	8.8	0.3	*6.8*	0.1	*1.5*	0	237
ORIENTAL CHICKEN SALAD	80	9.4	6.3	2.2	*24.8*	0.4	*5.0*	18	407
PASTA PRIMAVERA	225	10.2	39.3	3.2	*12.9*	0.7	*2.9*	43	289
PASTA PROVINCIAL	195	9.4	36.0	1.7	*7.7*	0.3	*1.4*	37	342
PASTA PUTANESCA	251	8.7	48.8	2.3	*8.2*	0.5	*1.9*	1	370
RED BEANS AND RICE WITH TURKEY SAUSAGE	248	17.2	36.6	4.2	*15.1*	1.3	*4.6*	21	433
ROASTED BREAKFAST POTATOES	208	7.0	41.8	2.0	*8.7*	0.9	*3.7*	5	84
ROASTED VEGETABLE SALAD	77	2.1	16.3	1.3	*15.2*	0.2	*2.2*	0	249
ROLLED FISH IN RED PEPPER GLAZE	153	16.6	6.2	6.8	*39.7*	1.5	*8.8*	50	331
RUSSIAN TURKEY STEW	326	28.9	37.2	6.6	*18.2*	1.8	*4.9*	81	306
SEVEN BEAN SALAD IN CHILI VINAIGRETTE	138	5.7	20.7	4.3	*28.3*	0.6	*4.2*	0	400
SMOTHERED YELLOW SQUASH	36	2.0	7.5	0.4	*10.6*	0.1	*2.0*	0	247
SOUTHWESTERN RICE	163	7.0	26.9	2.9	*16.2*	1.5	*8.5*	10	157
SPICY EGGPLANT AND TOMATO SALAD	38	1.4	8.6	0.4	*9.4*	0.1	*1.5*	0	80
STIR FRIED BEEF WITH BROCCOLI	162	18.4	10.5	5.7	*31.6*	1.8	*9.9*	41	696
SWEET AND SOUR CUCUMBERS	32	0.7	7.1	0.4	*11.1*	0.1	*2.0*	0	96
THAI BEEF SALAD	205	18.6	16.1	7.2	*31.6*	2.6	*11.3*	48	435
THREE BERRY MUFFINS	153	3.2	24.5	4.9	*28.5*	0.8	*4.7*	1	202

NUTRIENT ANALYSIS OF RECIPES: ARMY MENU MODIFICATION PROJECT, MARCH 1995

FOOD ITEM	KCAL	PROT, g	CARB, g	FAT, g	*% fat kcal*	SAT FAT, g	*% sat fat kcal*	CHOLES, mg	SODIUM, mg
TURKEY CHILI	361	36.7	54.4	11.0	*27.3*	3.8	*9.4*	79	391
TURKEY SAUSAGE	73	9.1	3.2	2.4	*29.8*	0.6	*7.3*	21	226
TURNIPS AND GREENS	25	2.1	4.8	0.3	*9.9*	0.1	*2.1*	0	337
VEGETARIAN BURRITOS	523	27.1	83.0	12.1	*20.8*	4.5	*7.8*	19	756
VEGETABLE STIR FRIED RICE	139	3.6	28.2	1.5	*9.9*	0.3	*1.6*	0	707
ADDITIONAL RECIPES TESTED-WILL VERIFY USE:									
Horseradish Potatoes	*140*	*4.0*	*30.6*	*0.5*	*3.3*	*0.3*	*1.6*	*1*	*335*
Mexican Cornbread	*218*	*6.0*	*36.7*	*5.3*	*21.9*	*1.2*	*5.0*	*27*	*623*
RECIPE YOU RECEIVED WHICH WAS NOT USED:									
Curried Carrot and Bean Pasta									

APPENDIX
BASIC NEUROSCIENCE LABORATORY

Publications

Papers

1. Farooqui, S. J Brock, J Zhou. Changes in the monamines and their concentrations in REM sleep deprived rat forebrain nuclei.. Pharmacology Biochemistry and Behavior. 1996

2. Rapid eye movement sleep deprivation (REMd) impairs spatial reference memory in rats. B. Youngblood, J. Zhou, G. Smagin, D. Ryan and R. Harris. Physiol. Behav. 61: 249-256, 1997

3. Smagin, G.N., R.B.S. Harris and D.H. Ryan The role of the locus coeruleus CRF-norepinephrine interaction in the behavioral response to stress. Pennington Nutrition Series: Current Progress in Military Neuroscience Research (In Press)

4. Harris, R.B.S., B.D. Youngblood, J. Zhou, G. N. Smagin and D.H. Ryan REM sleep deprived rats demonstrate a stress-induced impairment of spatial memory. Pennington Nutrition Series: Current Progress in Military Neuroscience Research (in Press)

5. Smagin, G.N., R.B.S. Harris and D.H. Ryan. 1996. Corticotrophin-releasing factor receptor agonist infused into the locus coeruleus attenuates immobilization stress-induced defensive withdrawal. Neuroscience Letters 220: 167-170, 1997

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12. Smagin, G.N., L.A. Howell, D.H. Ryan, E.B. DeSouza, R.B.S. Harris. Corticotrophin releasing factor (CRF)- and urocortin (UCN)-induced anorexia in rats: the role of CRF_2 receptors. Neuroreports (In Press)

13. Howell, L.A., Harris, R.B.S., C. Clarke, B.D. Youngblood, D.H. Ryan and T.G. Gilbertson. 1996. The effects of acute or repeated restraint stress on taste preferences in rats. Physiol. Behav. (submitted).

14. Harris, R.B.S., J. Zhou, B.D. Youngblood, I.I. Rybkin, G.N. Smagin, D.H. Ryan. The effect of repeated restraint on body weight and composition of rats fed low and high fat diets. Am. J. Physiol. (submitted)

15. Yan, X., Y. Zhou, I.I. Rybkin, D.H. Ryan and R.B.S. Harris. Central and peripheral expression of Urocortin mRNA in Rat. Molecular Brain Research (submitted)

16. Zhou, J., X. Yan, D.H. Ryan, R.B.S. Harris. Sustained effects of repeated restraint stress on muscle and adipocyte metabolism in high fat fed rats. Am. J. Physiol (submitted)

17. Zhou, Y., A. Cheshire, L.A. Howell, D.H. Ryan, R.B.S. Harris. Neuroautoantibody immunoreactivity in relation to aging and stress in apolipoprotein E-deficient mice. J. Neuroimmunology (submitted)

Abstracts

1. Youngblood, B.D., J. Zhou, G. Smagin and R. Harris REM sleep deprivation induces a spatial reference memory deficit and increases brain 5-HT metabolism in rats. Neuroscience 1995

2. Smagin, G.N. Corticotrophin-releasing factor receptor antagonist infused into the locus coeruleus attenuates immobilization-stress induced defensive withdrawal in rats. Neuroscience 1995

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8. Zhou, J., R.B.S. Harris, S.A. Moore, G.N. Smagin, B.D. Youngblood and D.H. Ryan. The effects of restraint stress on rats fed a high-fat diet. Exp. Biol. 1997.

9. Youngblood, B.D., R.B.S. Harris, C. Clarke, D.H. Ryan and T.A. Gilbertson. The effect of restraint stress on consumption of preferred and aversive solutions in rats. Exp. Biol 1997.

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16. Howell, L., R.B.S. Harris, and D.H. Ryan. The effect of naltrexone on food intake and saccharin preference in stressed rats. Neuroscience 1997.

17. Zhou, J., X.Yan, D.H. Ryan, and R.B.S. Harris. The effect of repeated restraint on adipocyte and muscle glucose transport. Exp. Biol. 1998.

18. Bamlett, S.B., R.B.S. Harris, J. Zhou and J.J. Zachwieja. Beta-3 receptor antagonism and lipid metabolism in exercising rats. Exp. Biol. 1998.