

**NUTRITIONAL AND IMMUNOLOGICAL ASSESSMENT
OF
RANGER STUDENTS WITH INCREASED CALORIC INTAKE**

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**U S ARMY RESEARCH INSTITUTE
OF
ENVIRONMENTAL MEDICINE
Natick, Massachusetts**

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SUMMARY

During 1991, two separate research studies on soldiers completing the U.S. Army Ranger Training Course were performed by research teams from the Walter Reed Army Institute of Research (WRAIR), Washington, D.C., and the U.S. Army Research Institute of Environmental Medicine (USARIEM), Natick, MA. The combined results from these studies documented the severe physiological and immunological effects of the stressors placed on Ranger students. The USARIEM study (RGR-I) documented that soldiers lost approximately 15% of the initial body weight and expended 90% of their body fat stores. More important was the documentation by both studies of clinically significant suppression of cellular immune function.

Based on these findings, a second study (RGR-II) was performed by a combined team from USARIEM, WRAIR, Pennington Biomedical Research Laboratories, Baton Rouge, LA, and the USDA Human Nutrition Laboratories, Beltsville, MD. The primary goal of the second study was to determine whether a modest increase in caloric intake over the previous study would decrease weight loss, changes in body composition, and extent of immune suppression. The second study also included additional assessments to define the impact of Ranger training on cognitive performance.

Soldiers from training class 11-92 (August to October 1992) volunteered to participate in the study (n=175). The four phases of this Ranger class were: Phase 1, temperate forest (Ft Benning, GA), Phase 2, desert (Ft Bliss, TX), Phase 3, mountain (Camp Merrill, GA), and Phase 4, jungle (Camp Rudder, FL).

A comprehensive battery of physiological, physical performance, psychological, biochemical, and immunological measurements were made at the beginning and end of every training phase. Each two week phase was divided into approximately 7 days of training geared to the particular geographical environment and then 7 days of a practical application of the training during a non-stop field training exercise (FTX). During the first week of each phase, the soldiers were given a combination of Meal-Ready-to-Eat (MRE) Field Rations and Class A rations prepared in the food dining facility. During the FTX portions of the training, the soldiers were only given Army field rations. Caloric intake was increased by supplementing the MRE with bread and a carbohydrate-electrolyte beverage

during the FTX portions of the first two phases, and by giving the new, Long Range Patrol Ration (LRP) during the FTX portions of the last two phases. Blood samples and measurements were taken at the beginning of the training, at the end of Phase 2, the middle of Phase 3, the end of Phase 3, and at the end of Phase 4.

ATTRITION

Of the original 175 volunteers, 51 were available at the end of Phase 4 for the test measurements. Of the 51 volunteers that completed RGR-II, 40 had achieved the necessary requirements to graduate.

MEDICAL STATUS

Infection rates for RGR-I for the desert, mountain and jungle phases of training were 8%, 25% and 24%, respectively. An overall improvement was shown in the caloric intervention of RGR-II. Infection rates for the same order of phases were found to be 12%, 8%, and 2%.

ENERGY BALANCE

Energy expenditure was estimated on a subset of 8 volunteers using the doubly labeled water technique. Energy intake was calculated with assumptions based on the visual estimation of food intake in the dining facility during the first week of Phase 3. Energy intake for RGR-II was estimated to be 16% higher than RGR-I. Average daily energy balance was negative and calculated to be -1203 and -847 kcal for RGR-I and RGR-II, respectively.

BODY COMPOSITION, ENDOCRINE STATUS, AND STRENGTH PERFORMANCE

The mean body weight loss of 12% during the RGR-II study was an improvement compared to the mean loss of 15% of the RGR-I study. While the volunteers in RGR-I showed a change in mean body fat from 14% to 5%, volunteers in RGR-II shifted from a mean of 14% down to 8.4%. There was approximately a 6% loss of fat-free mass in both RGR-I and RGR-II. There were significant and similar decrements in strength performance in both studies. The decreases in anthropometric measurements from baseline to the end of the training paralleled the more sophisticated measurements of body composition.

However, the anthropometric measurements detected the sharp increases in body composition caused by the relatively short "re-feeding" during the first week of Phase 3. Consistent decline in serum concentrations of selected hormones over the course of the training during RGR-II were similar to patterns shown in RGR-I. However, the change in some of the hormones appeared to be attenuated in the caloric intervention study, and some also showed favorable response to the "re-feeding" period during the first week of Phase 3.

PATTERNS OF SLEEP

Data collected with the use of wrist activity monitors issued to a subset of 30 volunteers showed no change in the amount or pattern of average estimated daily sleep between the two studies. Mean hours of daily sleep for Phases 1, 2, 3, and 4, for RGR-I were 3.78, 3.27, 3.12, and 4.12, respectively. For the same order of phases in RGR-II, amounts of mean daily sleep were 3.45, 3.41, 3.25, and 4.33 indicating the high degree and chronic nature of sleep loss which characterized the course.

COGNITIVE PERFORMANCE

Volunteers who completed RGR-II were highly motivated to perform well on cognitive function tasks. All the volunteers showed substantial impairment of cognitive function. Whenever possible, volunteers traded speed for accuracy in an attempt to maintain baseline levels of accuracy. While the volunteers showed a brief period of recovery during the dining hall feeding portion of Phase 3, deficits appeared to be cumulative over the course.

CLINICAL CHEMISTRIES AND MARKERS OF NUTRITION

While expected changes in markers of energy substrate metabolism were found in RGR-II, the data did not indicate any clinically significant deficiencies of protein, vitamins, or minerals. These findings are in agreement with the findings of RGR-I. A favorable trend due to the caloric intervention was found in the serum concentrations of the metabolic markers, β -hydroxybutyrate, lactate, and non-esterified fatty acids when compared to concentrations found during RGR-I.

HOST DEFENSE MECHANISMS

Although there was a marked improvement in cellular immune proliferation in RGR-II when compared to RGR-I, this proliferation was still suppressed to clinically significant levels. The test of non-immune function performed in RGR-II suggests impairment of gut function leading to enhanced permeation of gut bacteria into the peripheral blood.

LONG RANGE PATROL RATION (LRP)

Analyses of nutritional status taken at the end of a 10-day period during Phase 4 when the volunteers subsisted on only one LRP/day demonstrated that the ration was capable of sustaining acceptable vitamin and mineral intakes during intense field operations. Data indicate that the ration will be fully sustaining if fed at the intended rate of twice per day.

CONCLUSIONS

The 16% increase in caloric intake in RGR-II produced favorable changes in overall physiological condition of the volunteers completing it compared to RGR-I. These conclusions are supported by various physiological, biochemical, and immunological parameters that were measured. Based on the preliminary findings of the RGR-II study, the Ranger Training Brigade has changed its policy of offering one MRE/day during the FTX portions of training to offering three MREs every two days. Based on the favorable results of the LRP, the Brigade plans on switching to the LRP when these rations become available through normal supply channels.

A challenge still exists for Army medical researchers. Despite the improvement in immunological function with the increased caloric intake, a clinically significant suppression persisted. The problem is to find a way to enhance immunological function while still using food restriction as a training stressor. Answers to this problem may have applications beyond the controlled food restriction scenario of Ranger training. Numerous studies performed by the USARIEM Military Nutrition Division over the past 10 years have shown that soldiers subsisting on operational rations for prolonged periods of time fail to maintain body weight. Furthermore, a recent study of the physically demanding Special Forces Assessment Course (SFAS) has shown a clinically significant suppression of the immune

response (manuscript in press). This suppression occurred despite the fact that the soldiers were offered three MREs/day. Successful amelioration of immunological suppression in multi-stressor environments will have important implications for lowering infection rates and disease, and to sustaining fighting strength during both training and combat.

CHAPTER 1

BACKGROUND & OBJECTIVES

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US Army Research Institute of Environmental Medicine

BACKGROUND

The purpose of the U.S. Army Ranger Training Course is (SH 21-75, U.S. Army Infantry School, 1989):

The Ranger Course develops the leadership skills of selected male officer and enlisted personnel by requiring them to perform effectively as small unit leaders in a realistic, tactical environment under mental and physical stress approaching that found in combat. It provides the student with practical experience in the application of tactics and techniques of Ranger operations in wooded, mountainous, lowland swamp and desert environments. Emphasis is placed on development of individual leadership abilities through the application of the principles of leadership while further developing military skills in the planning and conduct of dismounted infantry, airborne, air assault and amphibious squad and platoon-size combat operations.

The current approach to training individuals instead of units in the Ranger course was initiated in 1951. Although the course content has changed over time, this program has always been a proving ground for male junior leaders who voluntarily submit to severe mental, emotional and physical stress. The intended product of this selection and training is a highly confident, tough and capable small unit leader.

The Ranger course is 65 days long, divided across four different geographical locations. These four phases expose the soldier to different types of terrain and environmental stressors including temperate forest at Fort Benning, mountainous terrain in the Blue Ridge mountains in Northern Georgia, coastal swamp along a tributary of the Yellow

River in the Florida panhandle, and in the Chihuahuan desert near El Paso, Texas. The desert phase was added to the end of the other three phases of instruction in 1985 by shortening the individual phases and lengthening the entire course by 5 days. This was the sequence of the phases for the Ranger studies conducted in 1991. Starting in 1992, the course was reordered, with the desert phase inserted into second place. This was a previously planned instructional change designed to progressively increment the level of environmental challenge. The flat desert terrain with unimpeded travel was considered easier terrain to learn patrolling techniques than later phases which required more skill in maintaining patrol command and control, and which offered more individual hazards with rough terrain or constant immersion.

The primary deliberate stressors of this course include repeated periodic food restriction, sleep deprivation, geographical/environmental challenges, prolonged low intensity physical work, and an element of anxiety produced by harassing opposition forces and constant performance evaluation.

Training at each phase is briefly summarized as follows: In the first phase at Fort Benning (16 days), soldiers are evaluated in a 4-day Ranger Assessment Phase (RAP) for physical fitness (Army Physical Fitness Test, chin-ups, 5 mile run, 8 mile foot march) and military skills (day/night land navigation and basic soldier tasks). This is followed by skill and confidence training and then 10 days at Camp Darby where soldiers review the fundamentals of patrolling, including squad recon and ambush. In the second phase at Fort Bliss (16 days), soldiers are instructed in desert operations and platoon-sized operations and drills; this is followed by an 8-day field training exercise including recon, ambush and raid tactics. The third phase occurs at Dahlonaga, Georgia (16 days), with training in mountaineering, operational techniques, and then an 8-day field training exercise which includes foot marching and airborne/air assault operations. The fourth phase takes place at Eglin Air Force Base, Florida (16 days), and includes jungle/swamp techniques (rope bridges, stream crossings, small boat operations) and a 12-day field training exercise including small boat operations and movements, and a night-time airborne operation.

The health hazards faced by soldiers participating in Ranger training have become part of the tradition for those who succeed despite these and other potential pitfalls during the course (Martinez-Lopez et al., 1993). However, medical risks are not an intended

stressor for this or any other Army training. Ranger training was restructured following several tragedies in a winter class in 1976. Similar crises have motivated a heightened awareness of heat injury. The Ranger cadre have addressed this issue by implementing a one week pre-Ranger heat-acclimation phase, performing a thorough screening for prior heat injuries, and strictly enforcing hydration during summer classes.

Attrition rates are normally between 50-60%, with approximately one third of this rate attributed to illness and injury. During the 1991 training year, the school experienced a higher than normal number of cases of pneumococcal pneumonia. Observations by Riedo et al. (1991) indicated that Ranger trainees exhibited a higher *Streptococcus pneumoniae* carriage rate than the general population.

These observations prompted the Ranger Training Brigade to request additional studies of the physiological and psychological effects of Ranger Training. The Ranger Training Brigade Commander, COL Maher, expressed his concern that the degree of sleep and nutritional restriction that had been used for many years might be excessive considering the additional requirements which have been added in recent years. The addition of a desert phase to the training exposed the student to a distinctly different type of climatic challenge. Although COL Maher wanted to proceed as rapidly as possible with a study which could demonstrate appropriate interventions, there was no current physiological and biochemical baseline data available to support intervention regimens. During the planning between COL Maher and research teams from the U.S. Army Medical Research and Development Command (USAMRDC), it was decided to first define the "natural history" of Ranger training; then, on the basis of empirical data, recommend interventions to be tested in follow-up studies.

Although there had been previous studies of Ranger training (Consolazio et al., 1966; Johnson et al., 1976), these focused on caloric intake assessment, body weight maintenance, and metabolic assessments. None of these studies involved assessment of host defense mechanisms.

Because of the logistical constraints and the desire not to overload one class with the experimental testing requirements, two separate studies were performed by research teams from WRAIR and USARIEM (Moore et al, 1992.) The study conducted on Ranger training

class 11-91 by the USARIEM research team assessed changes in body composition, attrition, energy expenditure, nutrient status and cellular immune function. The energy assessment observations showed a net energy deficit of 31%. The students lost an average of 15% of their body weight. With respect to body composition, approximately 90% of the fat stores were lost. Immune function was suppressed throughout the course of the study with the greatest suppression shown during the phase involving the greatest energy deficit. Although the number of students identified as carriers of Group A beta-hemolytic *Streptococcus* remained low throughout the course, *Streptococcus pneumonia* carriage rates increased to 12% of the volunteers by the last phase.

The research group from WRAIR studied endocrine and immune responses of students in Ranger training class 12-91. During the first 6 weeks of training, there was a significant drop in delayed-type hypersensitivity skin test response and the ability to produce antibodies after immunization. Assessment of sleep duration was performed using wrist activity monitors. This method estimated an average of 3.6 hours of sleep/night during the course.

The results of these studies were surprising because they defined more extreme physiological effects than anyone at the Ranger Training Brigade or the research teams had anticipated. The expectation was that some soldiers might lose as much as 10% of their body weight. Instead, 53 of 55 finishers in the RGR-I study lost more than 10% of their initial body weight (Moore et al., 1992). When the preliminary results were presented to the Ranger Training Brigade, COL Maher immediately ordered an increase in rations to all Ranger students.

One of the most surprising results from the RGR-I study was the fact that no evidence of vitamin or mineral deficiencies was found. There was, however, a marked suppression of immune function. This was demonstrated by laboratory tests performed by the USARIEM research team using whole blood cell culture techniques.

The results of these studies were reviewed by a panel of civilian nutrition experts convened by the National Academy of Sciences, Committee on Military Nutrition Research (CMNR). The committee endorsed the findings and recommended that these results be pursued with follow-up studies, as COL Maher had originally proposed. The primary

intervention, first proposed by COL E. Wayne Askew, Chief, Military Nutrition Division, USARIEM, was to increase the intake of Ranger students by 250-400 kcal/day. Overall intake would conform to the cadre's desire for some deliberate challenge of food restriction while preserving more body weight and possibly reducing the adverse effects on immune function and susceptibility to infection. COL Askew suggested that this could be easily incorporated into Ranger training by substituting the new Long Range Patrol (LRP) ration for the Meal-Ready-to-Eat (MRE). The LRP contains approximately 400 more kcal than the MRE. This approach was endorsed by the CMNR and by USAMRDC.

At the urging of COL Schnakenberg, the Director of the Army Systems Hazards Research Program, the present study was conducted as a collaborative effort between two USAMRDC Institutes, a USDA nutritional immunology researcher, Dr. Tim Kramer, and an analytical chemistry laboratory located at Pennington Biomedical Research Center, Baton Rouge, LA.

The purpose of the study was to repeat the measurements made in RGR-I in a class with 250-400 kcal/day more energy intake to determine whether or not this intervention would cause a significant improvement in terms of medical risk. The intent of this follow-up study was not to eliminate the stress of Ranger training, but rather to reduce the risk of a student being eliminated from the course due to illness. Additionally, the follow-up study offered the unique opportunity to further characterize the complex interaction of sleep restriction, negative energy balances, and exhaustive exercise on host defense mechanisms of the human body, as well as cognitive performance.

OBJECTIVES

1. Determine if a small increase in energy intake would attenuate weight loss, strength reductions, in vitro immune function deficits, and other physiological decrements observed in RGR-I.
2. Assess the acceptability and nutritional adequacy of the new Long Range Patrol Ration (LRP) in the high stress realistic combat simulation of Ranger training.

3. Describe in further detail the nature of nutritional, cognitive and immune function deficits which occur during summer Ranger training.
4. Examine the short-term recovery from energy deficit following refeeding within one phase of the course and following completion of the course.

CHAPTER 2

STUDY DESIGN

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TEST VOLUNTEERS

This study was conducted using research volunteers who were trainees in class 11-92, Ranger Training Program, Fort Benning, GA (RGR-II). Only male volunteers were used because Ranger training is not open to female soldiers. This class began in August 1992 and ended in October 1992. Table 2.1 presents the dates and location of each phase of training.

Table 2.1 Training Schedule Ranger Class 11-92

Phase	Description	Location	Dates
I	Benning, General Subjects	Camp Darby, GA	3 Aug-19 Aug
II	Desert Training	Camp McGregor, TX	19 Aug-8 Sept
III	Mountain Training	Camp Merrill, GA	9 Sept-27 Sept
IV	Jungle Training	Camp Rudder, FL	28 Sept-13 Oct

Volunteers were briefed on the purpose of the study and the risks and benefits involved. Before entry into the study, volunteers completed a Volunteer Agreement Affidavit

Volunteers were briefed on the purpose of the study and the risks and benefits involved. Before entry into the study, volunteers completed a Volunteer Agreement Affidavit (Appendix A) and a Background Questionnaire (Appendix B). They were instructed that they could withdraw from this study and remain in the course or withdraw from the Ranger course at any time and return to their sending unit.

Trainees entering the course generally had one of four origins: students who attended a one week pre-ranger training ("zero week") just before the start of the course (n=70), students who started the course in the previous class but who were repeating from the start ("recycle") and who had been in a holding program at the Ranger training Brigade (n=16), personnel assigned to a Ranger Regiment and an Air Force special operations units who participated in a joint pre-Ranger training course (n=89), and students who arrived from their unit to sign in on the first day of training. A total of 175 soldiers, airmen, and marines volunteered.

DATA COLLECTION SCHEDULE

Table 2.2 shows the data collection schedule. Baseline measurements were made at the beginning of the first week of training. This period is referred to as the Ranger Assessment Phase (RAP). Comparison measurements were taken at the end of the Benning and desert phase, in the middle of the mountain phase, end of the mountain phase and the end of the jungle phase (see Table 2.2). Climatic data are shown in Appendix C.

FEEDING PLAN

There is no standard ration cycle during the course, but there is a feeding plan. Trainees are fed a mixture of MRE's, B/T-rations and A-rations throughout the course. Periods of caloric restriction are integral parts of the course during field training exercises (FTX). During the FTX portion of each phase, the students are normally given 1 MRE daily.

Pursuant to the objectives of this study, the feeding plan was altered to achieve a 16% increase in caloric intake over the 8 weeks of training. The original intent was to replace the MRE with the LRP throughout the 8 week course. The LRP contains approximately 1570

Table 2.2 Data Acquisition Schedule Training Phase

	Benning		Desert	Mountain		Jungle
Measurement Date	Start 5 Aug 0	End 19 Sep 17	End 10 Sep 35	Mid 18 Sep 43	End 27 Sep 53	End 13 Oct 64
Training Day						
BODY COMPOSITION						
Body Weight	X		X	X	X	X
DEXA ¹	X					X
Circumference	X		X	X	X	X
Skinfold	X		X	X	X	X
BLOOD DRAW						
Nutritional Assessment	X		X	X	X	X
Immune Function	X		X	X	X	X
Endocrine Assessment	X		X	X	X	X
MRE/LRP Acceptance	X		X		X	X
ENERGY BALANCE						
Food Intake Estimation				X		
Energy Expenditure		X	X		X	X
Cognitive Performance		X	X	X	X	X
Physical Performance	X					X
Activity Monitoring		X	X	X	X	X

Note: Thirty monitors were worn through the training period

¹Dual Energy X-Ray Absorptiometry

kcal/meal as opposed to 1350 kcal for the MRE. However, due to logistical restraints, this was not feasible. As a compromise, the MRE, supplemented with bananas, carbohydrate beverage, or pouch bread, was given during the first two phases of the training. The LRP was given during the last two phases of training.

BLOOD ANALYSES

Clinical Chemistry

Hematology measurements were performed on location using a Coulter JT Blood Analyzer (Coulter, P.O. Box 2145, Hialeah, Florida 33012). This analyzer determined hemoglobin, mean packed volume, mean corpuscular volume, white cell count, red cell count, platelet count, %lymphocytes, %monocytes and %granulocytes.

Biochemical analyses for nutritional assessment were performed by the Clinical Chemistry Laboratory at the Pennington Biomedical Research Center, Baton Rouge, LA. Specific tests and methodologies are presented in Chapter 8.

Immune Function

A heparinized 7 ml fasting blood sample was collected at the time periods indicated in Table 2.2. The blood was collected and transported to the USDA Vitamin and Mineral Nutrition Laboratory, Beltsville Agriculture Research Center, Beltsville, MD. Preparation of whole-blood cultures for lymphocyte blastogenesis and cytokine secretion was conducted (Kramer et al., 1990). Measurement of T-lymphocyte soluble IL-2 receptors, T-lymphocyte and monocyte secreted IL-2 and IL-6 and concentrations of plasma IL-2 and IL-6 were performed using commercially prepared and standardized ELISA kits. Details of the methodology are given in Chapter 9.

A heparinized 7 ml fasting blood sample was collected at the time periods indicated in Table 2.2. Four milliliter aliquots of whole blood were transferred to 3 tubes containing 0.4 ml of Hanks Buffered Saline with either nothing, 100ng/ml bacterial LPS, or LPS +2 ug/ml of indomethacin. Tubes were incubated for 4 hours at 37 degrees. Tubes were centrifuged

and supernatants frozen for IL-1, TNF, IL-6, and PGE2 determination.

The remaining blood was used to test leukocyte oxidative burst activity. The blood was sedimented by addition of Dextran to a final 1% concentration. After 10 minutes sedimentation, the serum/leukocyte phase was removed for analysis. This solution was divided into duplicate wells on 3 microtiter plates for determination of superoxide and hydrogen peroxide production and nitro-tetrazolium blue (NBT) reduction using a microplate reader, as described by Pick (1986). The methodology for these analyses are described in detail in Chapter 10.

Endocrine Analyses

Endocrine markers were determined by the Occupational Health and Performance biochemical laboratory at USARIEM using radioligand assay procedures. Testosterone, estradiol-17b, cortisol, thyroxine, triiodothyronine, thyroid binding globulin, thyroid stimulating hormone, growth hormone, sex hormone binding globulin and immunoreactive LH were measured by direct radioimmunoassay or immunoradiometric assays. Insulin-like growth factor-1 (IGF-1) was measured by radioimmunoassay after extraction procedures. All of these measurements were performed using 5 mls of serum. The details of the methodology are outlined in Chapter 5.

Lymphocyte Phenotyping

White blood cells were stained with appropriate fluorescent labeled monoclonal antibodies specific for Natural Killer cells, T-cells, T-helper cells, T-suppressor cells, Monocytes and B-cells. The cells were fixed and analyzed by flowcytometry as described in Chapter 9.

Trace Element Analysis

In addition to the measurements outlined above, an aliquot of plasma was taken from an acid washed, trace element free vacuum tube (Becton Dickinson, Rutherford, NJ). This aliquot was analyzed for copper and zinc by atomic absorption spectrophotometry according

to the procedures described in Chapter 8.

CHAPTER 3

INJURIES, ILLNESSES & CAUSES OF ATTRITION

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INTRODUCTION

In the RGR-I study, medical causes accounted for 21% of the soldiers who did not graduate in the studied class and 28% of the total number of soldiers who did not graduate following recycling (these proportions are slightly smaller if they are computed only for the number of soldiers who successfully completed the initial 4-day Ranger Assessment Phase, RAP). These medical problems indicate significant cause for concern. They reflect only the most severe injuries and illnesses, with many other problems remaining undetected, successfully treated, or rehabilitated within 30 days (in time to join the next class as a "recycled" Ranger). It was also suggested that the problems may be worse than reflected in this one class. Ranger Training Brigade statistics indicated that as much as half of the normal attrition is due to medical problems (Maher, 1993) and the possibility existed that more intensive medical attention was focused on this class as an artifact of the study attention.

The present study was an attempt to substantiate the findings of the previous summer (Martinez-Lopez et al. 1993). It was hypothesized that even with a small increase in caloric intake, injury, and illness rates might be reduced through better performance and disease resistance.

METHODS

All data on soldiers in the study were collected by periodic reconciliations of the roster of study volunteers and updated rosters from the cadre. Official causes for a dropout were recorded from these lists. Medical problems were followed up individually for confirmation from the medical records. Additional screening of every medical record completed on students who were seen and treated by a medic or physician's assistant provided a complete summary of all reported medical problems.

Five weeks after the end of the course, ten of the soldiers who graduated from this class were brought to USARIEM for follow-up testing including medical examination by a physician.

RESULTS

Of the original 175 volunteers, 3 withdrew from the study before completing all of the initial physical training but remained in the course and successfully graduated; all cited personal reasons for withdrawing. Of the remaining 172 volunteers, 51 (29%) were still present for the final data collection period and 40 had completed the requirements necessary to graduate. Academic failures accounted for the largest reason for attrition (50%). Medical reasons accounted for 13% of the attrition.

Detailed reasons for attrition from the class are shown in Table 3.1. This included more than one fourth of the group eliminated within the first week of the class in the RAP. During the RAP, academic withdrawals included failures on the Army Physical Fitness Test (6), Combat Water Survival Test (2), 5-mile run test (5), 8-mile run test (6), and day/night navigation (4). Administrative reasons for withdrawal from the class included: pre-existing medical problems, such as record of previous heat injury (9), incomplete medical records (5), leave under emergency conditions (3), and other (2). Only four soldiers attrited for infections, including two cases of knee cellulitis. There was only one reported heat strain attrition, and this occurred during the RAP.

Table 3.1 Reasons for Medical Attrition in RGR-II

Category	Phase						
	RAP	I	II	III	IV	Total	%
Medical	8	2	3	4	1	18	13
--orthopedic injuries	(5)	(2)		(2)	(1)	(10)	
--cellulitis/conjunctivitis			(2)	(2)		(4)	
--other	(3)		(1)			(4)	
RAP Testing	23					23	18
Academic ¹		2	31	28	11	66	50
Special Obs Report (SOR)			1		1	2	2
Lack of Motivation (LOM)			1			1	1
Administrative	19					19	17
Total Attrited	50	4	36	32	13	135	
Total Starting Phase	175	125	121	85	53	*40	
% attrited within phase	28	3	30	38	25		

¹Academic reasons include patrolling evaluations, spot reports, & peer evaluations; *grads

This attrition from the class studied accounted for 77% of the original study group (135/175), but many of these soldiers (39%) successfully repeated a phase of training and graduated with the next class, bringing the course success rate to 53% of our original 175 soldiers. A smaller number of soldiers are likely to have been "recycled" twice (or more) and graduated with subsequent classes (not monitored for this study), slightly improving this rate.

Many medical problems occurring during training are treated in the field and are never reported in the medical records (e.g., blisters, rucksack abrasion, and insect bites and stings). Table 3.2 shows a breakdown of the infections that were serious enough to prompt the soldier to seek treatment from a medic or other health care provider. Table 3.2 also shows a comparison of infection rates between RGR-I and RGR-II.

Table 3.2 Infections Documented in Medical Records

Type of Infection	Desert	Mountain	Swamp
Cellulitis	9	3	1
Conjunctivitis	2		
Acute gastroenteritis	1	1	
Otitis	2	1	
Upper respiratory tract infection		1	
Sore throat		1	
Infections/number of soldiers in phase	14/121 (12%)	7/85 (8%)	1/58 (2%)

Infections/number of soldiers in phase (RGR-I study)	9/109 (8%)	19/75 (25%)	14/58 (24%)
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CONCLUSION

Medical problems in this class accounted for 18/135 soldiers who were recycled from the class or dropped from the course. This was substantially lower than the rate in the previous study of a class measured at the same time of year. There are several explanations for the difference. In RGR-I, there was a high rate of heat strain attrition at the start of the course because of unexpectedly hot humid conditions during the RAP, despite appropriate preventive measures by the Rangers (Martinez-Lopez et al., 1993). Students in RGR-II were more rigorously screened and eliminated at the start of the course for prior heat injury. The nine soldiers from the RGR-II study who were dropped in the first week of the course for prior heat injury would have been at greater than average risk for a recurrence of heat injuries. This screening step may have prevented a higher incidence of heat injuries. There was also a reduction in injuries, from 17/190 (RGR-I) to 10/175 (RGR-II) in the current study. Medical care for Ranger students involved aggressive treatment of all injuries and illnesses, as in the previous study.

The incidence of attrition due to infection was unchanged (five cases in RGR-I vs. four cases in RGR-II), but the incidence of soldiers treated for infections while in the course was markedly reduced, from one fourth of the students in each of the last two phases of training to between 2-8% (Table 3.2). Although this cannot be directly attributed to the feeding intervention in this study, the results suggest a beneficial response to the increased caloric intake. Improved disease resistance in the better fed students is also consistent with the findings on cellular immune function reported in Chapter 9. Cellulitis has been typically associated with the jungle phase of training when soldiers are exposed to pathogens during prolonged immersion in stream and river crossings. In RGR-I, the highest incidence of cellulitis occurred in this phase (third phase in the previous plan of instruction). In RGR-II, there was only one case of cellulitis documented in the jungle phase, even though this was now the last phase in the course, and the time when soldiers would be expected to demonstrate greatest susceptibility due to the cumulative stress effects of the course.

The post-training medical sequelae were consistent with the findings of RGR-I, documenting that medical problems tended to be transient and soldiers were in good health by five weeks after the end of Ranger training. The complaint of toe numbness is a problem commonly encountered in basic trainees. In basic trainees, it has been attributed to an adaptation phase associated with new boots and marching with heavy loads . In Ranger students, the problem could be more complex, including overtraining or overuse injury, foot injury, infection from immersion and abrasion, and extreme loss of fat including the protective subcutaneous layer on the foot.

In summary, medical problems and medical causes of attrition were lower than in RGR-I. While this cannot be directly attributed to the feeding regimen, there are several reasons to suggest that it may not be a purely coincidental finding, including the improvement in immune function reported in Chapter 9.

CHAPTER 4

ENERGY BALANCE

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GEO-CENTERS, INC

INTRODUCTION

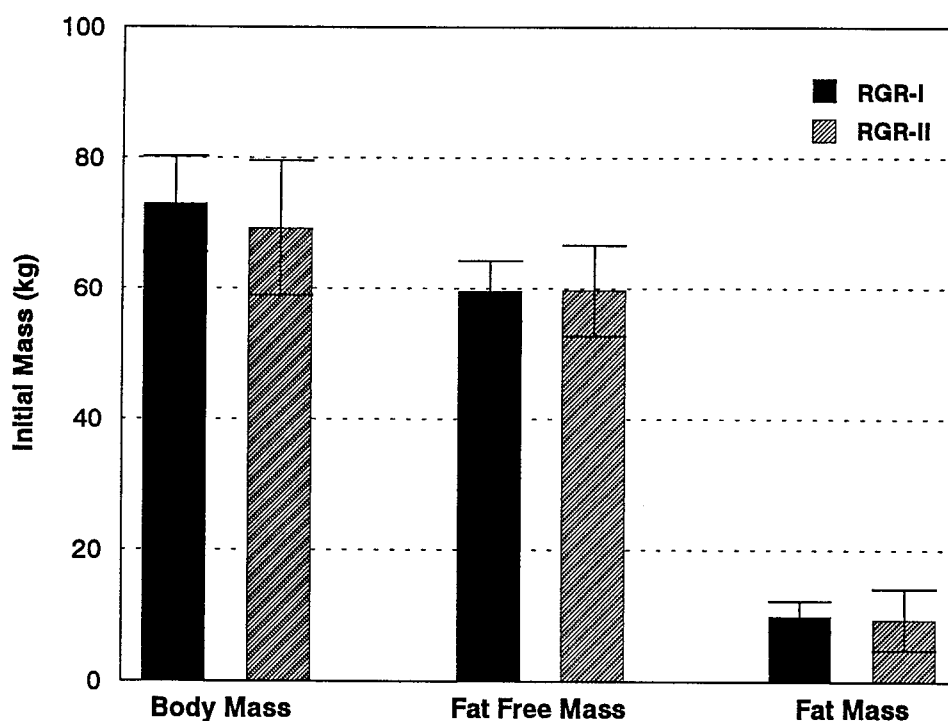
The DLW ($^2\text{H}_2^{18}\text{O}$) method of measuring total daily energy expenditure (TDEE) (Schoeller, 1988) is based on the assumption that after an initial oral dose of stable $^2\text{H}_2^{18}\text{O}$, deuterium (^2H) is eliminated from the body as water, whereas ^{18}O leaves as both water and exhaled carbon dioxide (CO_2). The rate of CO_2 production can be calculated from the difference in elimination rates of the two isotopes. Energy expenditure is calculated from CO_2 production using a metabolic fuel quotient and conventional indirect calorimetric relationships. Changes in baseline isotopic enrichment were simultaneously monitored in similar groups of volunteers who did not receive $^2\text{H}_2^{18}\text{O}$. Total body water, isotopic elimination rates, carbon dioxide production, metabolic fuel respiratory quotient, and energy expenditure were calculated using standard methods (Schoeller et al., 1986) as previously described (Delany et al., 1989; Hoyt et al., 1991). This method has been validated in soldiers in the field (Delany et al., 1989; Hoyt et al., 1991, 1994). The average daily estimated energy expenditure during RGR-I was 4010 kcal/day (Moore et al., 1992). Energy deficits were estimated to be 37%, 33%, and 15% for the mountain, jungle, and desert phases, respectively. This chapter will describe information concerning the energy balance of the RGR-II

trainees.

METHODS

Because of the expense and limited availability of doubly labeled water (DLW), a limited subset of six subjects was studied. Previous work has shown that the DLW method provides accurate estimates of individual rates of energy expenditure (Hoyt et al., 1994). Due to attrition for the various reasons described in Chapter 3, not all of the original six subjects were used during the four dosing periods of the study. The physical characteristics of the DLW subjects that were used in both RGR-I and RGR-II were similar (Figure 4.1) and representative of the main experimental group (Figures 5.5 and 5.6).

Figure 4.1 Body Composition of DLW Volunteers



Intake balance energy expenditure was estimated using standard methods. The estimation of food intake is described below. The change in body energy stores was estimated from DEXA body composition measurements (see Chapter 5), using the equation: $(\Delta ES) = (\text{Fat Mass} \times 9.5 \text{ kcal/g}) + (\text{FFM} \times 0.27 \times 4.4 \text{ kcal/g})$ where FFM is corrected to dry weight assuming a 73% water content.

Mean TDEE during the three periods between DLW measurements was estimated as $[(\text{total intake balance energy expenditure} - \text{total DLW energy expenditure}) / \text{number of days when TDEE was not measured by DLW}]$.

Energy Intake

Food was provided to the Ranger trainees in a controlled and regimented fashion. Over 90% of the food consisted of either packaged U.S. military field rations, or servings of hot food prepared in a garrison dining facility (A rations). Various other meals, such as box lunches and a survival meal, provided less than 10% of the food energy consumed. These miscellaneous meals included a survival meal consisting of known quantities of uncooked vegetables and chicken (total caloric content of 1850 kcal), box lunches, and two other miscellaneous meals where the total caloric content was calculated by factoring the quantities of the various individual food items with their known caloric values (Pennington, 1989). Consumption of unauthorized food or beverages was forbidden and constituted grounds for dismissal from the course.

Over half the meals provided to the Ranger trainees consisted of packaged rations. These rations provided either 1350 kcal/meal (MRE), or 1570 kcal/meal (LRP). It was assumed that packaged rations issued to Ranger trainees were eaten in their entirety.

A variation of the visual estimation method (Rose and Carlson, 1986) was used to estimate representative mean caloric intakes at the garrison dining facility meals. Mean estimates of food intake in garrison were obtained with a minimum of interference with the Ranger training schedule. Energy consumption assessed at the Camp Merrill (Phase III) garrison dining facility was assumed to be similar to that at

the other garrison dining facilities. Food intakes were measured during three breakfast and four dinner meals at the Camp Merrill garrison dining facility. At each meal, 30 Ranger trainees were randomly selected. A data collector recorded the food items and number of portions provided to each subject.

The serving of food was extremely regimented. Each serving of a particular food was considered to be one typical serving. A typical serving of each food was weighed prior to each observed meal using an electronic balance (Sartorius, Brinkman Instruments, Inc., Westbury, N.Y.). Second servings were not allowed at Camp Merrill. However, trading of food and unlimited beverage consumption was allowed. When finished eating, each volunteer returned their tray to a data collector. The data collector recorded uneaten food and asked each subject if he had drunk additional beverages or received or given away any food. The weight of any food returned was subtracted from the food provided. The energy content of the food consumed was calculated using nutrient data from A-ration recipes and the standard food tables (U.S. Department of Agriculture Nutrient Data Base for Standard Reference 8 and Nutrient Data Base for Individual Food Intake Surveys, 1989).

RESULTS

The changes in body mass, fat-free mass (FFM), and fat mass (FM) of individuals used for the data described in this chapter are shown in Figure 4.2. The changes during RGR-II are less severe than during RGR-I due to the caloric intervention. The total daily energy expenditures (TDEE) for RGR-I and RGR-II are shown in Figure 4.3. After attrition, the number of DLW subjects for phases I through IV was 4, 4, 5, and 6, respectively. The overall pattern and intensity of TDEE in RGR-I and RGR-II were similar, with high rates of TDEE in the mountain phase in RGR-I (phase 2) and in RGR-II (phase 3).

Figure 4.2 Change in Body Composition of DLW Volunteers

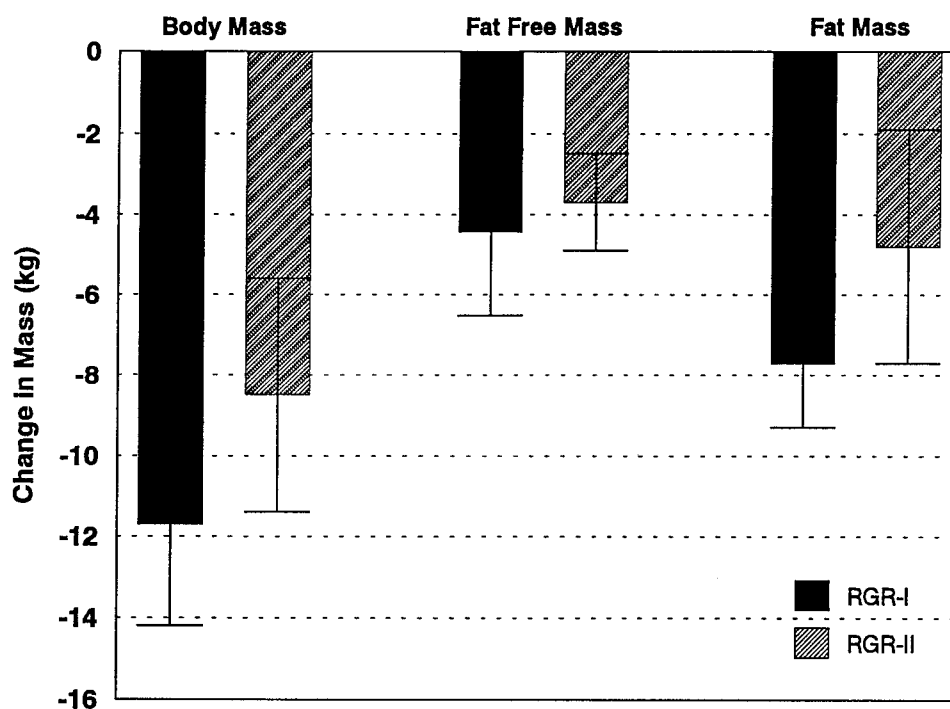
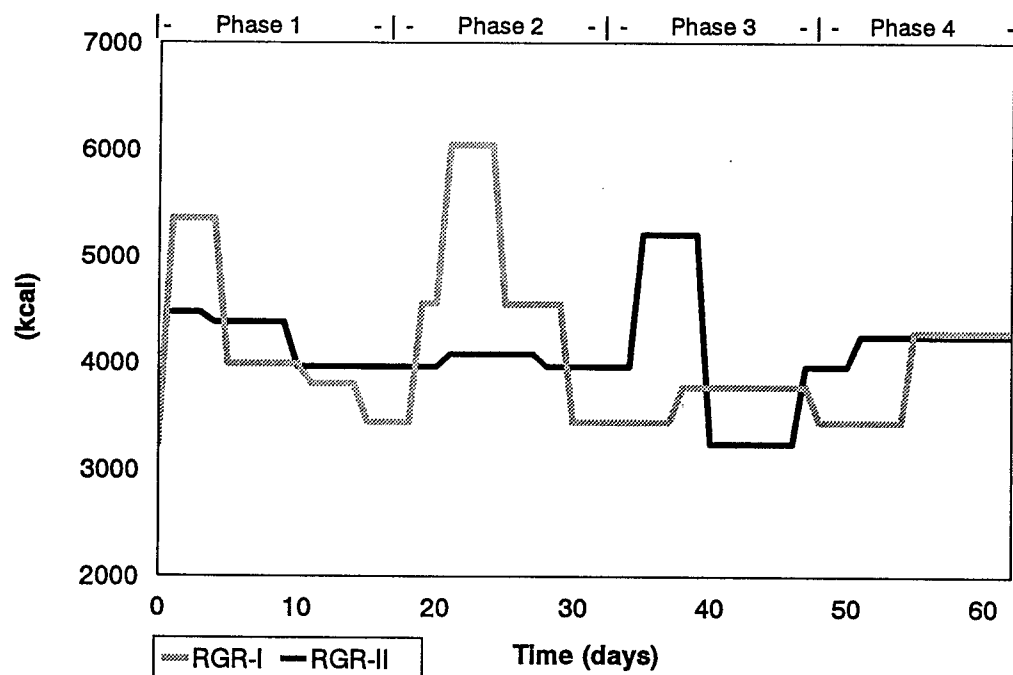
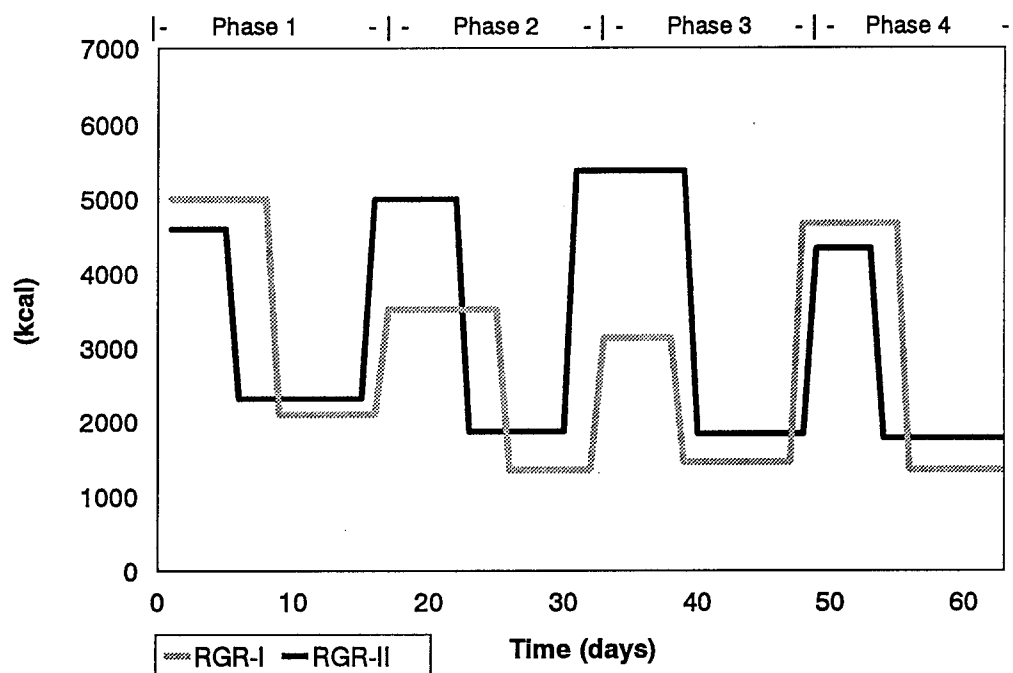


Figure 4.3 Estimated Total Daily Energy Expenditures

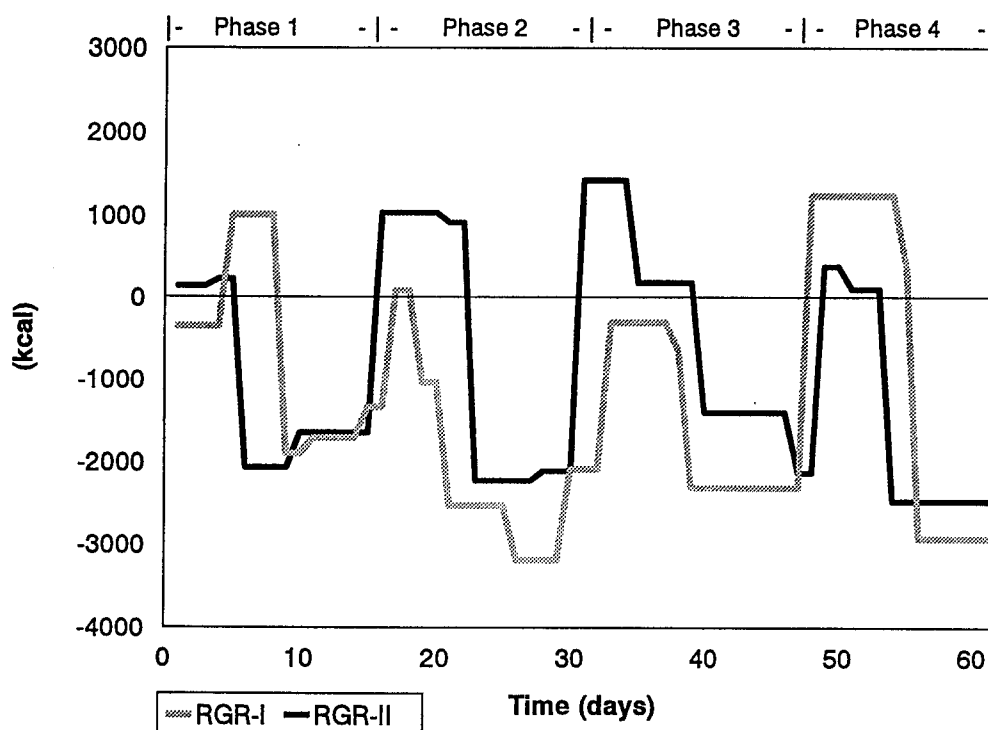


Estimated caloric intake is shown in Figure 4.4. RGR-II was characterized by substantial increases in estimated food intake in garrison, combined with a more modest increase of about 250 kcal/d in food energy intake in the field.

Figure 4.4 Estimated Caloric Intake



Energy balance, i.e., the net difference between energy intake and energy expenditure, is shown in Figure 4.5. Two distinct patterns of energy balance are evident. In RGR-I, the energy balance of the trainees is largely negative until the refeeding at the beginning of the final desert phase (phase 4), and the trainees incur a profound energy deficit in the mountain phase (phase 2). In contrast, energy balance in RGR-II oscillates regularly between positive and negative values during each training phase, and the energy deficit associated with the mountain phase (phase 3 in RGR-II) is less extreme in contrast to RGR-I.

Figure 4.5 Estimated Net Energy Balance

Mean TDEE, food energy intake, and the change in body energy stores, for RGR-I and RGR-II are summarized in Table 4.1.

Table 4.1 Estimated daily energy intake, energy expenditure & energy balance			
	RGR I	RGR II	% Change
TDEE, kcal	4065±840	4090±470	+ <1
Food energy, kcal	2820±1650	3220±1622	+ 14
ΔEnergy balance, kcal	-1200±480	-990±390	- 18

values are means ± S.D.

CONCLUSIONS

The TDEE estimated for both RGR-I and RGR-II was found to be equivalent in both pattern of expenditure and amount expended within similar geographical locations of the training. The initial objective of an overall increase in caloric intake by approximately 15% was achieved as shown in Table 4.1. The near equivalent estimated TDEE between RGR-I and RGR-II along with the increased caloric intake resulted in a 18% improvement in energy balance. These data serve as a basis for the changes in variables such as body composition, and the metabolic markers of metabolism and immune function described in the following chapters.

CHAPTER 5

BODY COMPOSITION, ENDOCRINE MARKERS & STRENGTH PERFORMANCE MEASURES

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INTRODUCTION

The results of the 1991 USARIEM Ranger Nutrition Study (RGR-I) provided the Ranger Training Brigade with objective evidence that the food restriction was excessive (Moore et al., 1992). By the end of the course, Ranger students who completed the course without repeating any phase had reached a transition point between the utilization of most available fat stores and a substantial increase in the catabolism of muscle tissue. Serum hormone changes confirmed the severity of the metabolic responses with thyroid hormone (triiodothyronine) dropping below the normal range to reduce metabolic rate; testosterone, the principal male hormone, immediately declined into the castrate range; and insulin-like growth factor-1 (IGF1), a marker of nitrogen balance declined by more than 50%, a range comparable to severely catabolic patients. A 20% decline in lifting strength correlated with a reduction in fat-free mass (FFM), indicating performance impairments possibly related to muscle catabolism.

This follow-up study was designed to evaluate the efficacy of a modest increase in energy intake in alleviating the severity of catabolism of Ranger students. A second purpose was to describe the recovery of endpoint measures produced by periods of refeeding during the course and following the course. This chapter compares these new data from a class

receiving 15% more kcal per day to the results of RGR-I for metabolic and strength performance measures. A better understanding of the consequences of prolonged energy deficiency, especially of muscle wasting, are important to the military for planning and for the development of strategies to extend the limits of physical performance. The same information may be useful to relief efforts for undernourished civilian populations such as the Somalis (Graham, 1993) and to understanding diseases such as acquired immunodeficiency syndrome where metabolic disturbances prevent nitrogen sparing (Grunfeld & Feingold, 1992).

METHODS

General

The subject characteristics and the design of this study are described in detail in Chapter 2. The volunteers were drawn from a summer class, class 11-92, which approximately matched the time of year in which the RGR-I study group (class 11-91) was studied. Complete measurements were obtained on 50 soldiers at the start and end of the course. Some of these measurements were also made at the end of the second and third phases, and after a "classroom" (refeeding) period in the third phase. Ten of the 50 soldiers returned one week after the end of the course for a single morning blood sample at Fort Benning. Another 10 of the 50 soldiers traveled to USARIEM for detailed measurements five weeks after the end of the course for follow-up evaluation. The results and discussion of the follow-up evaluation appear in Chapter 12. All blood samples were collected between 0500-0800 h, following an overnight fast.

Anthropometric Measurements

Stature was measured at the start of the course with an anthropometer (GPM, Seritex, Inc., Carlstadt, NJ). Nude body weight was measured at each sampling period. Weights and other anthropometric measurements were collected from volunteers at the end of each phase before any refeeding occurred and before breakfast in the intermediate mountain phase sampling period. Five skinfold thicknesses were measured by a single observer, including measurements at the mid-thigh and at the four sites described by Durnin and Womersley (1974). Six circumferences were measured including neck (at the level of the

infrathyroid landmark), biceps (relaxed), forearm, abdomen (at the omphalion), mid-thigh and calf. All limb measurements were made on the right side of the body.

Limb cross-sectional areas were computed from biceps, forearm, thigh and calf circumferences using circular area as an approximation of limb cross-sectional geometry. Muscle cross-sectional areas for upper arm and upper leg were estimated from these areas by subtraction of the area of an outer ring representing fat area calculated from one-half of the measured skinfold thicknesses (the doubled-over subcutaneous fat layer) of the arm (average of biceps and triceps) and leg (mid-thigh). Skinfolts were not measured for the calf or the forearm in this experiment because the subcutaneous fat layer in these regions is negligible in lean young men.

Percent body fat was estimated from the sum of four skinfolts with the equation of Durnin & Womersley (1974) for men aged 20-29 (with body density interpreted using the Siri equation):

$$\% \text{body fat}_{\text{skinfolds}} = 100 \times ((4.95 / (1.1631 - (0.0632 \times \log \Sigma \text{skinfolds}))) - 4.5)$$

and from height, neck and abdominal circumferences, according to the equation from Army Regulation 600-9 (in centimeters):

$$\% \text{body fat}_{\text{AR 600-9}} = 43.74 - (68.68 \times \log(\text{height})) + (76.46 \times \log(\text{abdominal circumference} - \text{neck circumference})).$$

Dual-Energy X-Ray Absorptiometry (DEXA)

Body composition (measured by dual energy X-ray absorptiometry, DEXA) was measured at the beginning and end of the study. The time points relative to the Ranger course were -5 days to -1 day of the start of the course (beginning), and on the last day of the evaluative portion of the course and before any refeeding began (end). Percent body fat (%BF) and bone mineral content were determined using manufacturer-supplied algorithms (Total body analysis, version 3.6, Lunar Corp, Madison, WI). Precision of the measurement is better than $\pm 0.5\%$ of percent body fat (Friedl et al. 1992). Fat-free mass (FFM) and fat weight were determined from %BF and body weight measurements.

Regional body composition estimated by the version 3.6 software is also reported directly in this report (Table 5.2 only). The DEXA-assessed tissue mass closely matches gravimetrically-assessed body weight in normal circumstances, including the start of the Ranger course; however, at the end of the course, total weight, especially total FFM, is overestimated by approximately 3 kg by DEXA. This has been postulated to be the result of physical changes during semistarvation (Friedl et al. 1994). Whether or not this effect is constant throughout the different regions assessed for FFM in regional tissue mass analyses is unknown.

Lifting Strength

Maximal lift capacity was measured with a weight stack machine that simulates the Olympic clean lift and which has been shown to correlate with military tasks such as load carriage and field artillery ammunition loading. A carriage with attached handles slides on two guide rods in the vertical direction. The goal is to raise a weight from the floor (30 cm) to shoulder height. The individual explodes upward extending at the knee and hip, transfers momentum to the upper body, and finishes by dropping under the weight and standing in the upright press position. The volunteers warmed up by lifting 36.3 kg (80 lbs) followed by the addition of weight in increments of 9.1 kg (20 lbs) or 4.6 kg (10 lbs) until a maximum lift ability was achieved (defined as two missed attempts at the same weight). The test-retest reliability of this technique is $r=0.92$ (Sharp & Vogel, 1992) and the measurement has been demonstrated to be highly stable over time.

Jump Test and Calculation of Power

Maximal jump was tested using chalk-marked fingers and a blackboard. With heels flat on the ground, volunteers extended their arms to a maximal reach and marked the board. The jump height was recorded as the difference between this mark and the highest reach achieved in three trials. This was performed twice, with and without counter-movements. Peak power was calculated from each of these two jump procedures by the Harman equation (Harman et al. 1991):

$$\text{Peak power (W)} = (61.9 \times \text{jump height}) + (36.0 \times \text{body weight}) - 1,822$$

Assay of Serum Hormones

Three aliquots of each serum sample were stored frozen at -40 C until processed. Each volunteer's serum samples from the various time periods of the course were tested together in the same assay to remove interassay variations from the interpretation of changes within individuals.

Somatotropic hormones Serum insulin-like growth factor-1 (IGF1) was measured by radioimmunoassay (RIA) after acid-ethanol extraction of samples which had not been previously thawed (Allegro, Nichols Institute Diagnostics, San Juan Capistrano, CA) with intra- and interassay coefficients of variation (CV) of < 5 and 12%. Growth hormone was measured by an avidin coated bead RIA (Allegro HGH, Nichols Institute Diagnostics) with intra- and interassay CV of < 5 and < 10%.

Pituitary-thyroid axis Triiodothyronine (T3) and thyroxine (T4) were measured by RIA (ICN Biomedicals Inc., Costa Mesa, CA). Thyroid binding globulin (TBG) was measured by RIA (Nichols Institute Diagnostics) and thyroid-stimulating hormone (TSH) was measured using a 3rd generation immunoassay with a limit of detection of 0.04 mIU/L (Allegro HS-TSH, Nichols Institute Diagnostics), both with intra- and interassay CVs of <5 and <10%. T4/TBG ratio was calculated as $T4(\mu\text{g/dL})/TBG(\mu\text{g/mL}) \times 10$.

Pituitary-testicular axis Luteinizing hormone (LH) was measured by RIA (ICN Biomedicals Inc.) with intra- and interassay CVs of < 5%. Sex hormone binding globulin (SHBG) was measured by IRMA (Farnos Diagnostica, Oulunsalo, Finland) with intra- and interassay CVs of <5%. Cortisol and testosterone were measured by RIA (Diagnostics Products Corp., Los Angeles, CA) with intra- and interassay CVs < 5%. Free testosterone was calculated from individual serum levels of testosterone, SHBG and albumin with an equation derived from the law of mass action (Sodergard et al. 1982).

Data Analysis

Data were analyzed by paired t-test comparisons between measurements collected at the beginning and at various times during the course. All values are expressed as means $\pm$ standard deviations. Statistical tests with $\alpha \leq 0.01$ were accepted as significant. Data were

analyzed using BMDP statistical software (BMDP Statistical Software, Berkeley, CA).

Comparisons against RGR-I data include recently reanalyzed DEXA data, updated with version 3.6 total body software (DEXA data previously reported was for analyses performed with the version 3.4 software which did not fully account for the effects of body thickness). Endocrine data compared in some of the figures may be more complete for RGR-I assays which were completed after the RGR-I technical report.

RESULTS & DISCUSSION

Body Weight

The mean body weight loss was -10.0 ± 2.8 kg (Figure 5.1) or $-12.6 \pm 2.8\%$ of initial weight (Table 5.1). This compared to $-15.6 \pm 3.1\%$ for RGR-I. The distribution of percent weight loss is shown in Figures 5.1 and 5.2 for RGR-I and RGR-II, respectively. Relative weight changes in RGR-II were shifted closer to 10% (Figure 5.2), a relative amount of weight loss which is often cited as a medically safe limit. Mean body weights for each measurement period for RGR-I and RGR-II are shown in Figures 5.3 and 5.4. The observations made in the middle of the third phase refeeding period demonstrated the previously hypothesized "saw-toothed" pattern of weight loss in response to refeeding. Similar, small increments probably occur in each of the four phases, even as end-of-phase weights progressively decrease.

Body Fat Assessed by DEXA

The distribution of percent body fat of the volunteers was similar for RGR-I and RGR-II study groups at the start of each course (Figures 5.5 & 5.6). The RGR-II group averaged $14.7 \pm 4.1\%$ body fat, which was normally distributed (Figure 5.6). At the end of the course, the mean body fat was $8.4 \pm 2.9\%$ and represented a slightly skewed distribution with only one soldier at the lowest interval of % body fat, and 17 out of 50 soldiers between 4 and 6%. At the end of RGR-I, the distribution was markedly skewed with 11 out of 55 soldiers in this lowest percent body fat interval, and 44 soldiers falling between 4 and 6%. This clearly demonstrates that the modest increase in energy intake in RGR-II provided a significant

Table 5.1 Physical Measurements at the start and end of RGR-II.

Parameter	Start	End	Change	% Change
Body Composition				
Body weight (kg)	78.4±8.7	68.4±7.0	-10.0±2.8	-13±3
--Fat weight (kg)	11.7±3.9	5.8±2.2	-6.0±2.2	-50±12
--FFM (kg)	66.7±6.3	62.6±6.0	-4.0±1.7	-6±2
Body fat _{DEXA} (%)	14.7±4.1	8.4±2.9	-6.0±2.7	-39±14
Body fat _{SKINFOLDS} (%)	17.7±3.5	10.8±2.2	-6.9±2.2	-39±8
Body fat _{AR 800-9} (%)	15.4±3.0	11.1±2.9	-4.3±2.0	-28±12
Skinfolds (mm)				
Biceps	5.3±1.4	3.5±0.6	-1.8±1.1	-32±12
Triceps	10.4±2.9	6.4±1.5	-4.1±1.9	-37±10
Subscap	12.1±2.7	8.2±1.3	-3.9±1.9	-30±9
Suprailiac	19.1±7.4	7.9±2.1	-11.5±6.4	-56±12
Thigh	14.2±5.0	8.6±2.2	-5.8±3.8	-37±14
Circumferences (cm)				
Neck	39.2±1.9	36.3±1.6	-2.8±0.8	-7±2
Upper arm (biceps)	31.6±2.3	28.8±2.0	-2.7±0.9	-9±3
Forearm	28.8±1.5	27.4±1.3	-1.3±0.5	-4±2
Abdomen	84.2±5.3	76.0±4.1	-8.1±3.0	-10±3
Thigh	58.2±3.7	52.7±3.0	-5.5±1.7	-9±3
Calf	38.1±2.3	37.3±2.4	-0.8±1.0	-2±3
Performance measures				
Maximal lift (kg)	81.5±13.3	65.1±10.6	-16.4±7.3	-20±8
Vertical jump (cm) ¹	48.0±7.4	39.9±6.2	-7.8±4.3	-16±9
Vertical jump (cm) ²	44.1±7.2	37.1±5.9	-6.7±5.2	-15±11
Peak power (W) ¹	3972±561	3119±479	-846±253	-21±6
Peak power (W) ²	3730±526	2947±455	-781±313	-21±8

All changes significant ($p < 0.01$), $n = 50$ ¹Jump with counter movement ²Jump without counter movement

Figure 5.1 Distribution of Relative Weight Loss in RGR-I Students

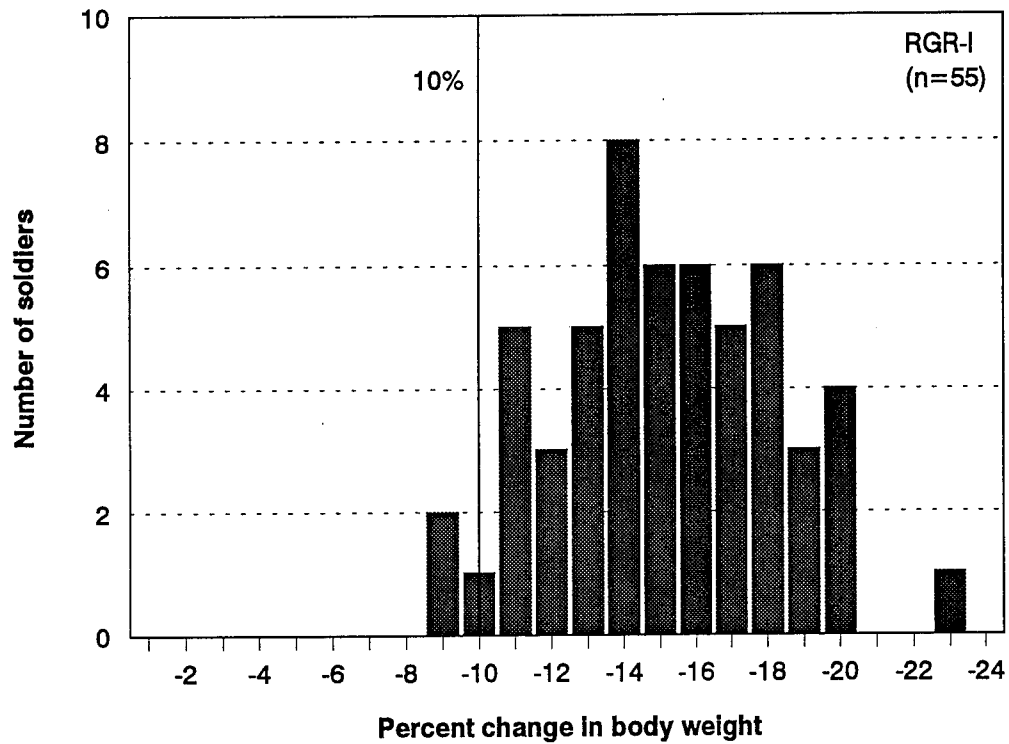


Figure 5.2 Distribution of Relative Weight Loss in RGR-II Students

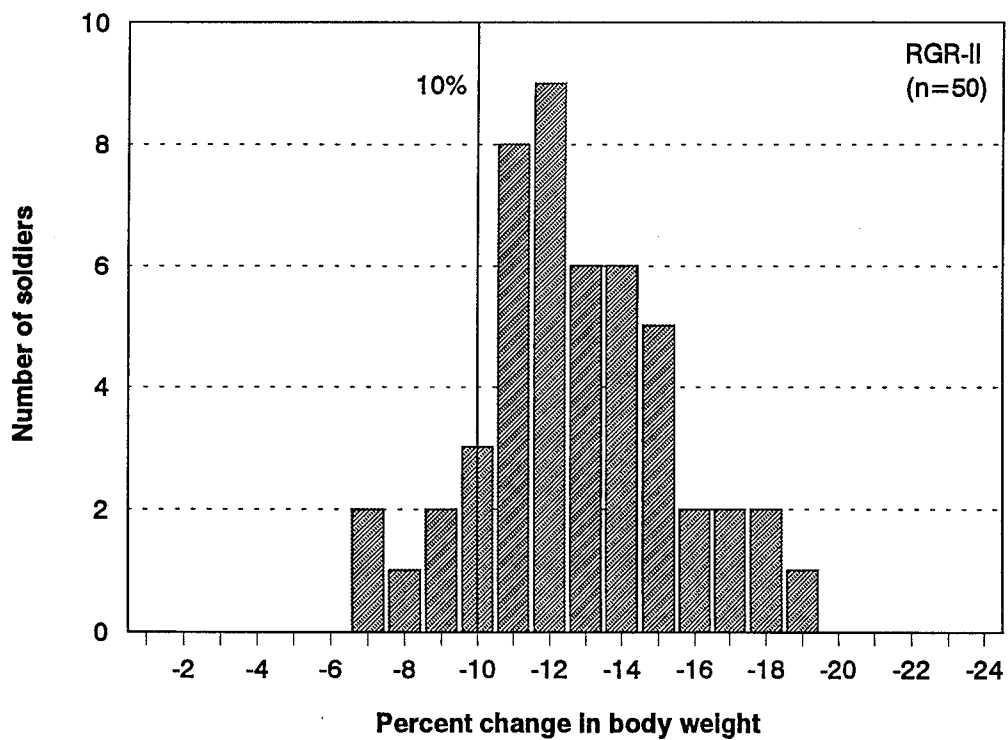


Figure 5.3 Body Weight in RGR-I

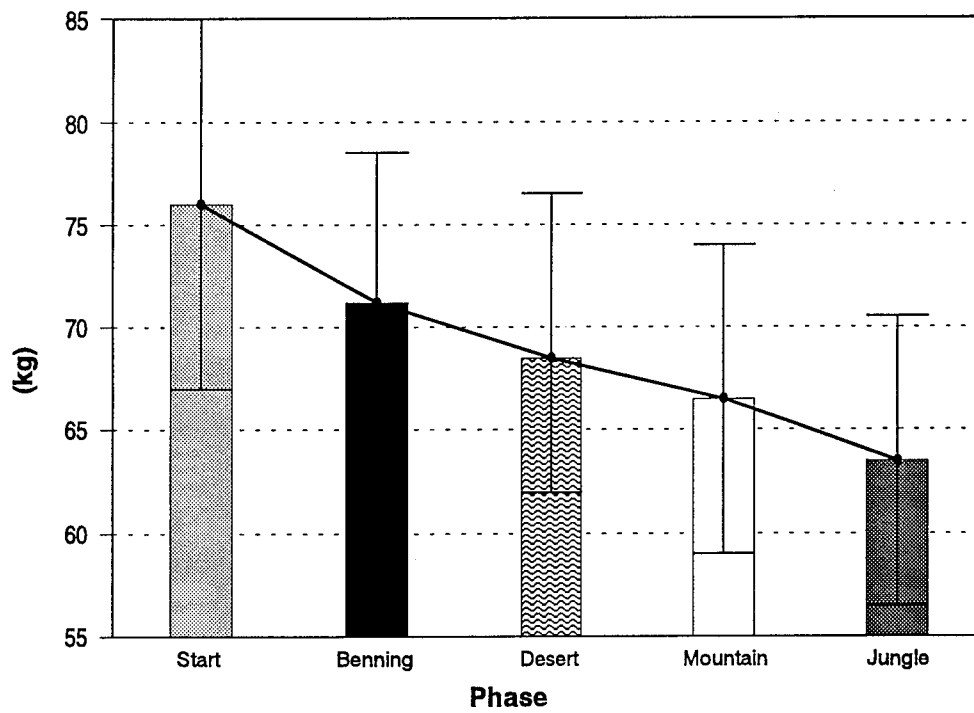


Figure 5.4 Body Weight in RGR-II

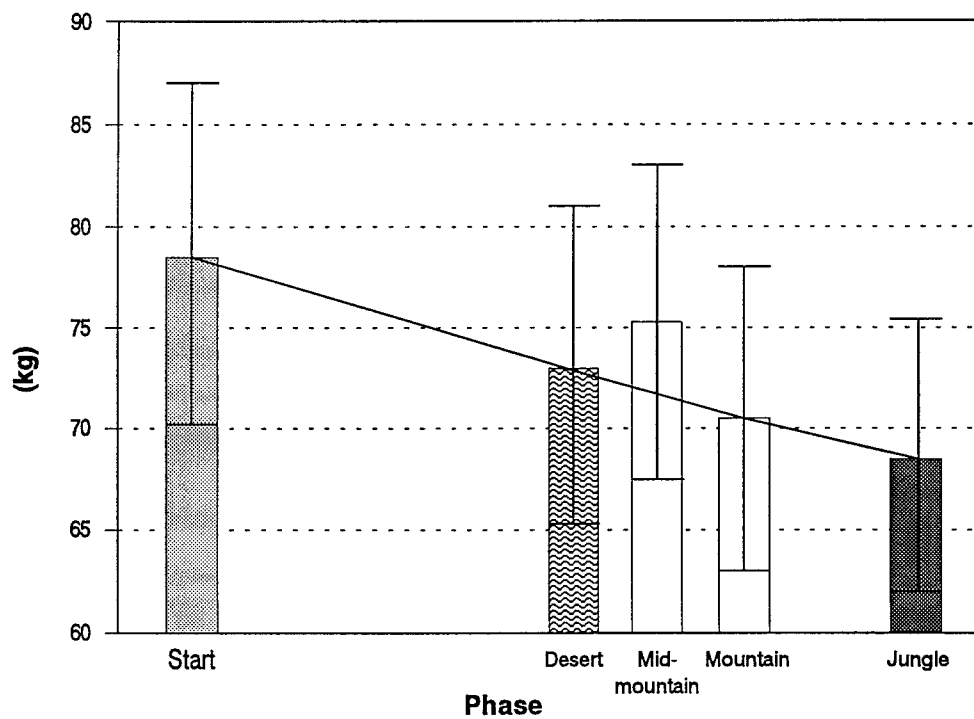


Figure 5.5 Distribution of % Body Fat for RGR-I

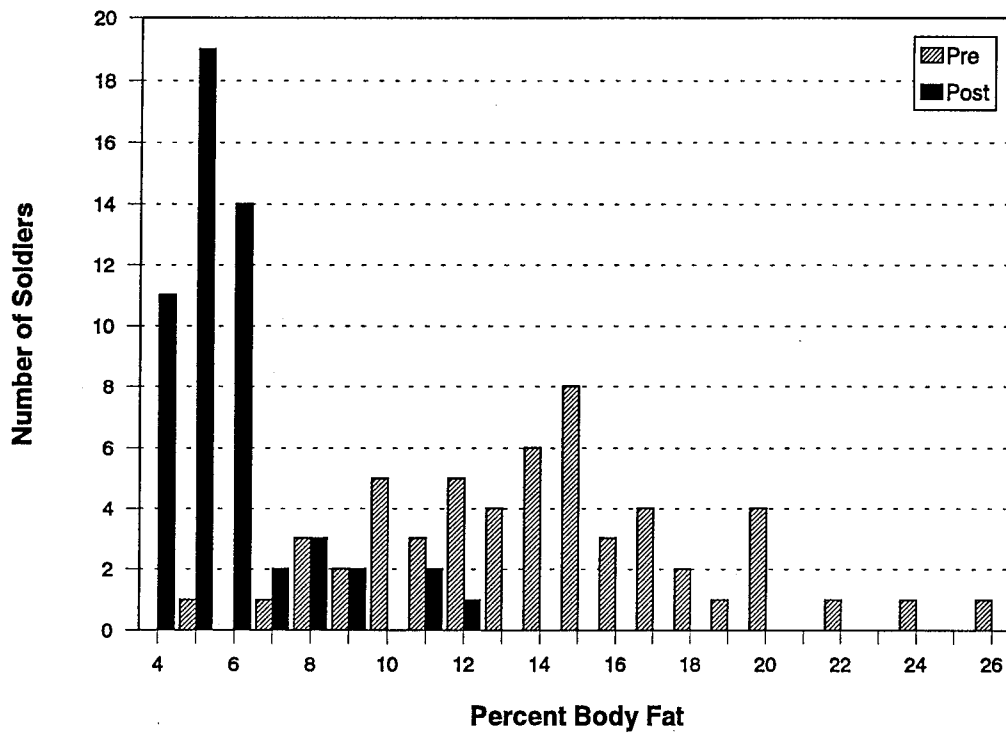
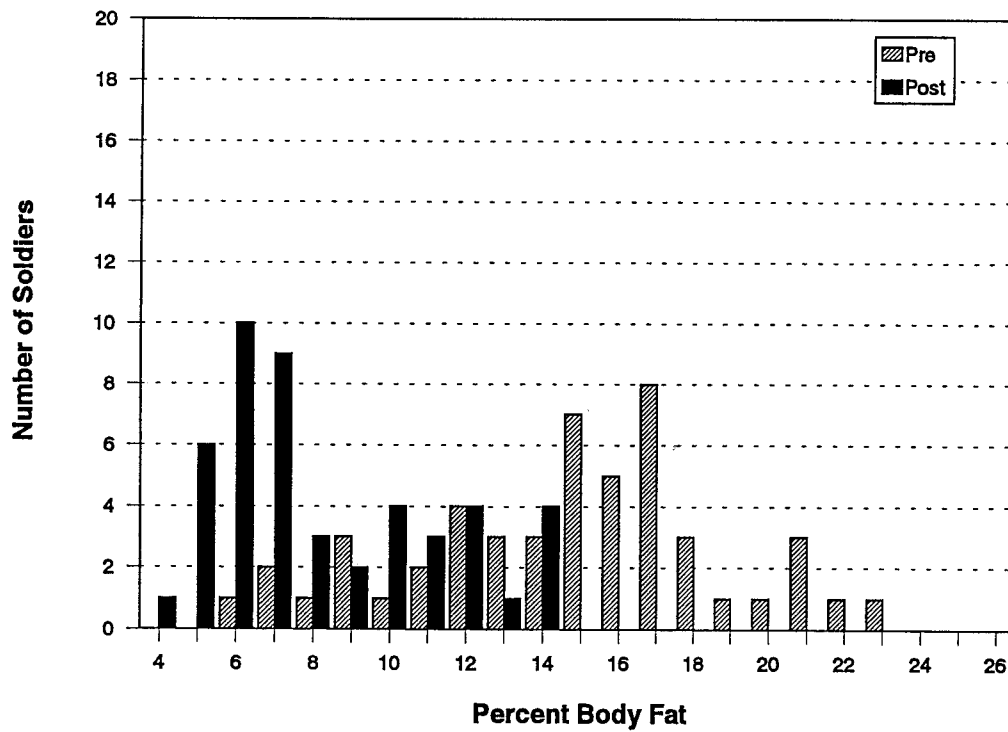


Figure 5.6 Distribution of % Body Fat for RGR-II



benefit to men in the course. Although fat stores were still substantially reduced by the end of the course, the soldiers were not yet driven to the subsequent phase of semistarvation which predominantly involved muscle catabolism. Because they had more fat, they were still able to utilize fat stores for energy (Goodman et al. 1984).

Equal proportions of fat were lost from the arms, legs, and trunk (all 48-50% of initial stores)(Table 5.2). This suggests that adipose tissue in lean young men acts as a single organ in response to semistarvation, instead of specific regions changing more than others; however, the earlier stages of fat loss were not examined.

Fat-Free Mass and Estimates of Limb Muscle Mass

The change in FFM was 4.1 ± 1.7 kg (6.1 ± 2.4 % of initial FFM, Table 5.1). This change was only slightly larger in RGR-I (4.6 ± 2.6 kg, $6.9 \pm 3.6\%$ of initial values). The most important finding in regard to the change in FFM is that no subject lost extreme amounts of FFM; in RGR-I, an extreme response included -20% of initial FFM, or an estimated loss of ~40% of muscle mass. With the additional energy intake in this study, only 3 out of 51 soldiers exceeded losses of greater than 10% of initial FFM (11, 12, 12%), whereas 9/55 exceeded 10% of FFM loss in RGR-I.

Changes in FFM occurred primarily in the non-bone tissue (Table 5.2). As noted in the RGR-I study, there appeared to be a disproportionate loss of arm muscle over leg muscle (Friedl et al. 1993). However, it must be cautioned that these results are suspect because of an error of unknown origin which predicts more tissue than is present (as measured by scale weights) in extremely lean men after a period of semistarvation (Friedl et al. 1994). This may be related to differences in the composition of the FFM after semistarvation which includes a reduced density due to an increased hydration of the FFM (Keys et al. 1950). This could have a pronounced effect on regional FFM estimations because semistarved subjects in the Minnesota study demonstrated clinically apparent edema which occurred primarily in the legs. The most remarkable observation in the regional DEXA

Table 5.2 DEXA-assessed regional body composition estimates and their change at start and end of RGR-II.

Parameter	Start	End	Change	% Change
Lean soft tissue (kg)				
Arm	7.9±1.0	7.0±0.9	-1.0±0.6*	-12±7
Leg	22.5±2.8	20.3±2.2	-2.1±1.6*	-9±6
Trunk	29.6±2.7	30.1±2.9	0.5±1.7	2±6
Total ¹	63.9±6.0	61.3±5.8	-2.6±2.0*	-4±3
Fat (kg)				
Arm	1.1±0.6	0.5±0.3	-0.6±0.4*	-47±20
Leg	4.3±1.5	2.1±0.9	-2.2±1.0*	-50±13
Trunk	5.8±1.9	2.9±1.1	-2.9±1.5*	-48±16
Total	11.9±3.9	6.0±2.3	-5.9±2.6*	-48±13
Bone mineral (kg)				
Arm	0.5±0.1	0.5±0.1	0.0±0.0	0.3±8
Leg	1.4±0.2	1.3±0.2	-0.1±0.1*	-3±4
Trunk	1.2±0.2	1.3±0.2	0.1±0.8	2±7
Total	3.6±0.4	3.6±0.4	-0.1±0.1	0.3±3

*p<0.01, paired t-test

¹The change in total FFM is underpredicted because of an overestimation of FFM by DEXA at the end of Ranger training; this effect of semistarvation presumably affects regional estimations of FFM as well.

change data is the absence of any decline in FFM in the trunk region. This is surprising because in the earliest stages of starvation, major organs and other non-muscle sources are the predominant sites of protein catabolism until a transition to muscle occurs (Goodman et al. 1984). It cannot be determined at present if this is a measurement artifact, or if the truncal FFM is actually maintained in these men by the exercise of Ranger training.

Other Indicators of Body Composition: Skinfold Thicknesses and Circumferences

Body fat estimated from the sum of four skinfold thicknesses verifies the difference in final fat reserve in RGR-I and RGR-II. There was a continued loss of fat in the last two-week phase in RGR-II (Table 5.3), whereas in RGR-I, little additional fat was available and a leveling off of fat loss was indicated by the anthropometric equation. After the in-phase refeeding, mid-point of phase three, fat reserves also demonstrated a slight temporary upturn from the overall downward trend. Anthropometric equations developed against underwater weighing tend to overestimate fat in lean men (Womersley & Durnin, 1977) and, because of the close correlation between DEXA and underwater weighing, would be expected to overestimate DEXA-assessed fat as well.

Body fat estimated using the Army circumference equation for males was less sensitive to the change in body composition than DEXA and skinfold-based equations. This was due to the overestimation of body fat in very lean soldiers at the end of training. While the abdominal measurement was sensitive to the reduction in weight, the neck measurement overpredicted the loss of FFM, reducing the effect of the reduction in abdominal girth in the body fat equation (Table 5.1). The abdominal measurement declined by an average of 8.1 ± 3.0 cm, or 20% less than the average decline in RGR-I. The 2-cm increment which occurred following the mid-phase, highlights the predominance of intra-abdominal fat storage during refeeding. This measurement was made in men who had fasted for 12 hours and was not a reflection of a recent meal.

Neck, biceps, forearm and calf circumferences (anthropometric markers of lean mass) demonstrated modest declines but also reflected a temporary upturn after early phase refeeding as in all the other measurements (Table 5.3). The forearm measurement was added after the RGR-I study to test the hypothesis that tissue of the lower arm was better preserved than in the upper arm during semistarvation, in order to explain the absence of a

Table 5.3 Anthropometric measurements and derived estimates of body composition and cross-sectional areas during RGR-II.

Item	Start	end of Desert	mid-Mountain	end of Mountain	end of Jungle
Body weight (kg)	78.4±8.7	72.9±7.7	75.3±7.5	70.8±7.5	68.4±7.0
Sum of 4 skinfolds (mm)	47.1±13.2	nd [*]	34.7±7.0	28.7±5.5	26.0±4.8
Body fat _{SKINFOLD} (%)	17.7±3.5	nd	14.2±2.4	12.0±2.3	10.8±2.2
Body fat _{AR 600-9} (%)	15.4±3.0	13.0±2.6	14.2±2.9	12.6±2.9	11.1±2.9
Upper arm x-sectional area (cm ²)	80.3±11.0	nd	76.1±9.5	70.3±9.4	66.8±9.0
Upper leg (thigh) x-sectional area (cm ²)	272±33	nd	nd	nd	220±33
Upper arm muscle area ² (cm ²)	68.2±9.3	nd	66.9±8.4	62.2±8.6	59.9±8.0
Forearm muscle area (cm ²)	66.3±6.6	61.7±5.6	65.0±5.8	63.1±6.3	60.2±5.7
Thigh muscle area (cm ²)	230±24	nd	nd	nd	199±20
Calf muscle area (cm ²)	116±14	nd	nd	nd	111±14

nd = not done because of time limitations during data collection.

² "muscle areas" are x-sectional areas including bone; upper arm and thigh include corrections from skinfold thicknesses (see methods).

* indicates difference from starting value, t-test, p<0.05.

Shaded columns represent measurements made immediately following periods of energy restriction.

reduction in grip strength observed in RGR-I (Johnson et al. 1994); we conclude that this is the case. Circumferences of distal extremities (calf and forearm) were the least affected in absolute or relative (-2 and -4%) terms, while circumferences of proximal extremities (upper arm and mid-thigh) showed relative changes (-9 and -9%) comparable to the abdomen. Specific influences to maintain regional muscle mass of the forearm such as carrying a weapon during patrolling may be of some importance; however, the calf was equally preserved relative to the thigh. This relatively small change in the proximal arm indicates that some compensatory mechanism during prolonged semistarvation reduces the catabolic effect of exercise on forearm muscle (Lowry et al. 1984).

A regional analysis of the muscle mass lost from proximal and distal extremities (manual analysis with the "region of interest" function) may provide a more specific answer to the question of changes in the balance of proximal and distal limb tissue.

Estimated Energy Deficit from Body Composition Changes

Based on the changes in stored energy (fat and fat-free mass changes), the average energy deficit was calculated as follows: $56,550 \pm 24,670$ kcal (from the average fat loss) + 4810 ± 1970 kcal (from the average FFM loss) = $61,360 \pm 24,420$ kcal deficit/62 days, or 990 ± 390 kcal/d. This is a significant reduction in the deficit compared to 1200 ± 480 kcal/d observed in RGR-I (t-test, $p < 0.03$). Estimated energy intakes were 2820 and 3220 kcal/d for RGR-I and RGR-II, producing similar or slightly higher values for total estimated energy expenditure (intake + deficit made up from body stores) in RGR-II.

Physical Performance Measures

Maximal lift capacity (MLC) declined by 16.4 ± 7.3 kg or $20 \pm 8\%$ of initial measurements (Figure 5.8). This was similar to the decline noted in RGR-I (16.7 kg) (Figure 5.7); however, there were some differences in the participants of the two classes. In the RGR-II group, there was a cluster of strong and highly trained individuals. This raised the starting average from 77.4 ± 9.6 (RGR-I) to 81.5 ± 13.3 (RGR-II) kg, although it had no effect on the average decrease which occurred in the course. The average lift capacity for soldiers

Figure 5.7 Distribution of Maximal Lift Capacity, RGR-I

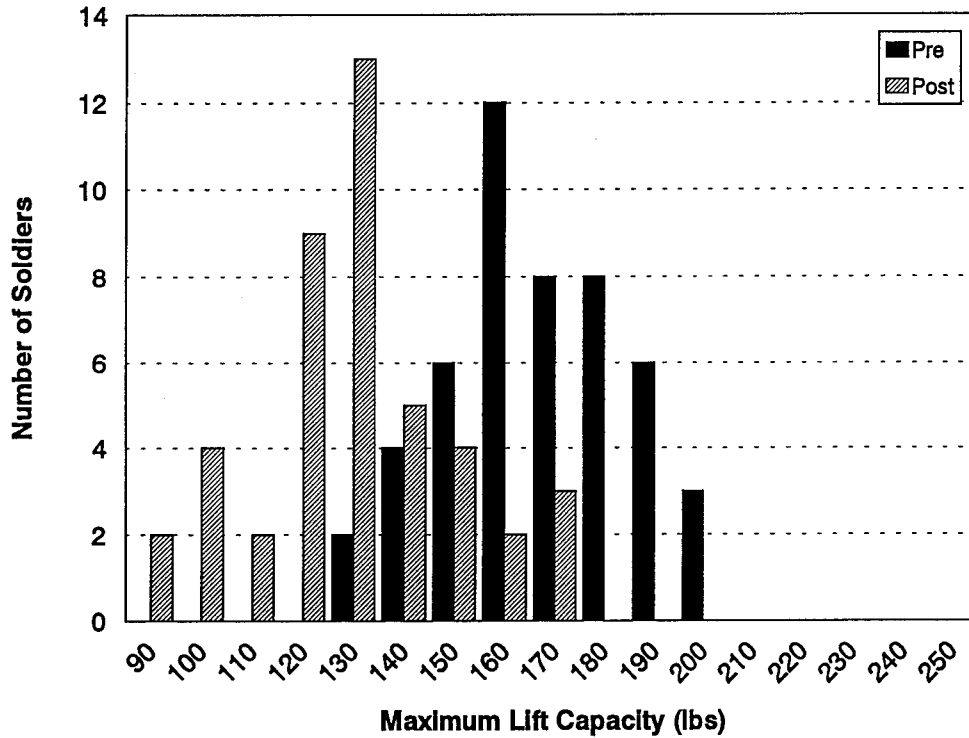
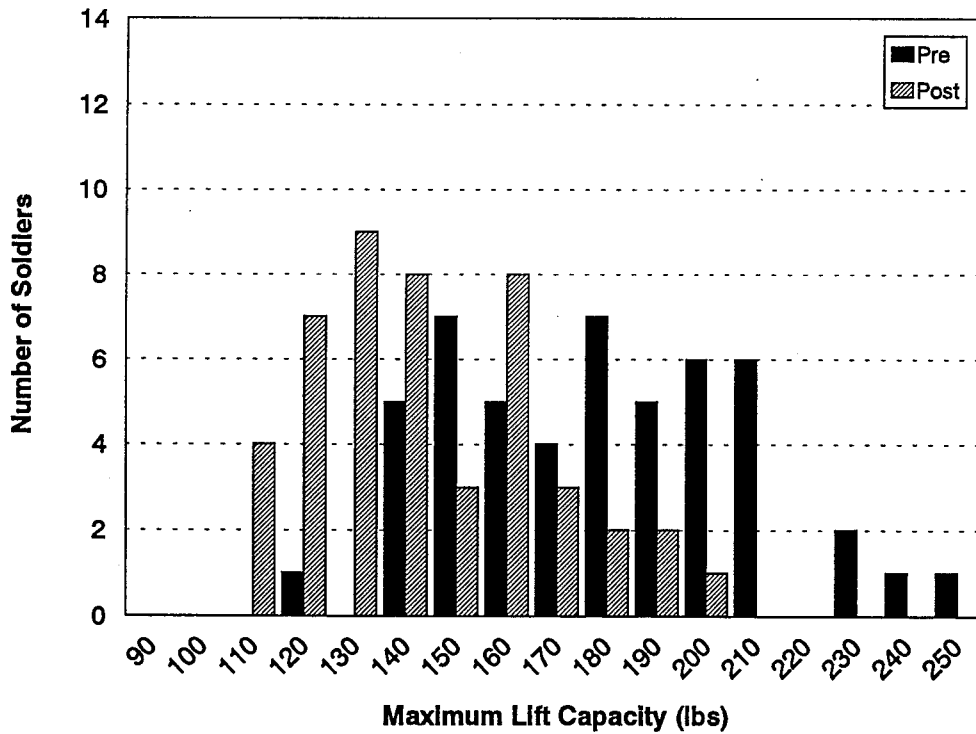


Figure 5.8 Distribution of Maximal Lift Capacity, RGR-II



at the end of Ranger training was comparable to the average for U.S. male soldiers (Sharp & Vogel, 1992).

This consistent reduction in lifting strength suggests that an undefined threshold of metabolic alterations related to this test was again exceeded. There was a correlation between reduction in FFM and in MLC in the previous study ($r=0.41$; $p<0.01$) (Johnson et al. 1994); however, in RGR-II, changes in FFM and MLC were no longer significantly related ($r=0.22$, $p=0.15$). This may be partly because of the smaller range or could be influenced by the presence of a group of more highly trained weight lifting athletes in RGR-II. This difference helps to highlight the conclusion that changes in FFM can only partially explain MLC performance, and neural, hormonal, and motivational factors also play roles. At the start of the course, non-bone lean tissue of the arms, legs, and trunk (assessed by DEXA) correlated reasonably well with MLC ($r=0.49$, 0.41 , 0.41 , in respective order) and with peak power ($r=0.59$, 0.51 , 0.60).

The decline in MLC was paralleled by a similar decline in peak power derived from jump height (with or without counter movement) and body weight (Frykman et al. 1993). MLC and peak power were reasonably well correlated (e.g., start values: $r=0.72$, $n=50$; $\text{MLC [lbs]} = 0.0375 \times \text{peak power [kW]} + 30.3$). This demonstrates that these two physical performance tests measure a similar component of explosive power. This also indicates that a simple jump test (along with body weight) can be used as an expedient indicator of explosive power in field studies.

Endocrine Markers of Nutritional Status: Somatotrophic, Adrenal, Thyroid and Gonadal Axes

Serum IGF1 levels declined by approximately half of the baseline value by the middle of the course (the first sampling period after the start, at the end of the desert phase, Figure 5.9). This was temporarily restored to normal levels within one week of refeeding during the mountain phase (Table 5.4, Figure 5.10). Data for RGR-I are shown in Figure 5.9. This is consistent with refeeding studies where IGF1 has been found to be a reliable and specific nutritional marker (Minuto et al. 1989). Growth hormone levels increased, reflecting an

increased secretory pulse amplitude in appropriate response to a reduced IGF1 inhibitory feedback. These two hormones reflect the presence of negative nitrogen balance during semistarvation (Donahue & Phillips, 1989; Ho et al. 1988; Bolze et al. 1991). Growth hormone and IGF1 have different direct actions on lean tissue and these changes during energy restriction may be adaptive by increasing the efficiency of protein synthesis (Pell & Bates, 1992).

Cortisol levels increased from initial values at six weeks, a delay of two weeks over the point of a significant increase in RGR-I. The glucocorticoid response at six weeks in RGR-II (Figure 5.12) and at four weeks in RGR-I (Figure 5.11) occurred as a similarly low body fat was achieved. The delay in RGR-II suggests that the metabolic stress was reduced. Cortisol has traditionally been used as a generalized stress marker, based on Hans Selye's theory of a generalized adrenal activation, but cortisol serves only as an acute stress marker, declining after the first few days in a multistressor environment such as Ranger training (Aakvaag et al. 1978), and shows no response to other stressors such as heat and acute hunger (Mason, 1974). With longer-term starvation such as anorexia and protein-calorie malnutrition, the adrenal is activated by metabolic stimuli (other than hypoglycemia) (Smith et al. 1975).

The effect of Ranger training on the thyroid-pituitary axis was significantly reduced in RGR-II as demonstrated by T3 levels which declined but remained in the normal range (Figure 5.14). This suggests that metabolic rate was less suppressed in RGR-II (compared to RGR-I, Figure 5.13), and this is also reflected in the previous calculation of an increased daily energy expenditure. This increase in the energy expenditure "ceiling" may improve performance but will also attenuate the reduction in energy deficit. Thus, the better maintained level of T3 indicates a markedly reduced metabolic stress in RGR-II compared to RGR-I. This is a different type of metabolic marker than serum IGF1 because, in the case of thyroid hormones, carbohydrate but not protein refeeding returns T3 levels and metabolic rate to normal in fasted subjects (Azizi, 1978; Hendler et al. 1986). TSH demonstrated an appropriate increase in response to the reduced T3 and T4 levels, with values exceeding 6 mIU/L in ~20% of individuals following the periods of semistarvation; this is commonly used as a clinical indicator of hypothyroidism. However, some studies involving acute starvation, report a decline in TSH which would suggest a centrally-mediated hypothyroidism (Opstad et

Figure 5.9 Serum Insulin-Like Growth Factor-1 Concentrations, RGR-I

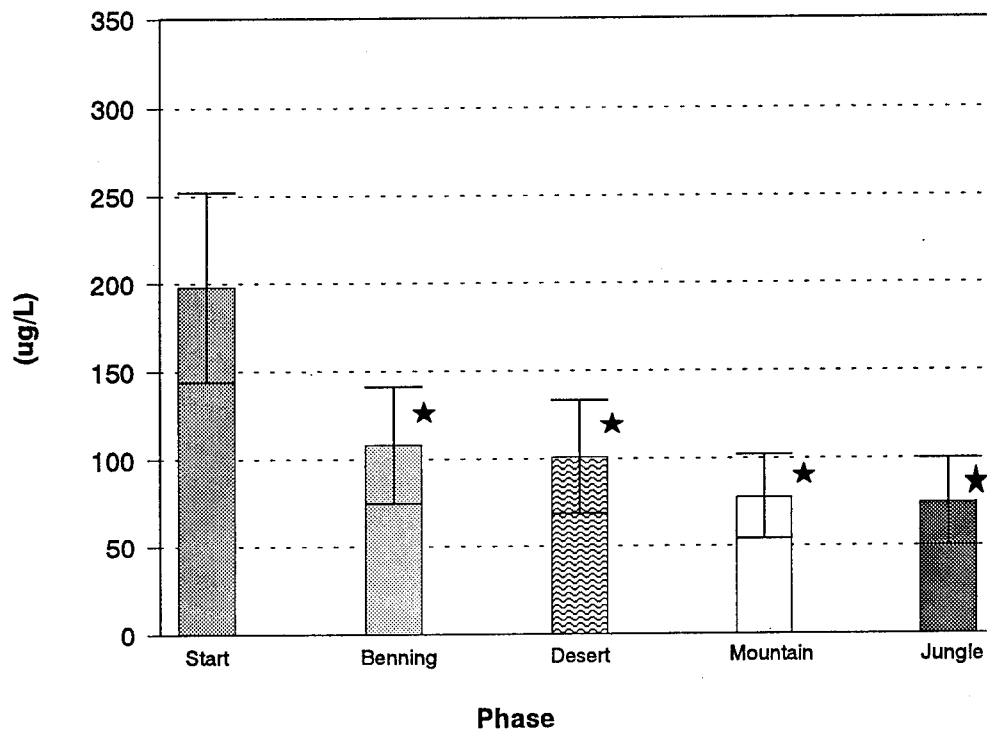


Figure 5.10 Serum Insulin-Like Growth Factor-1 Concentrations, RGR-II

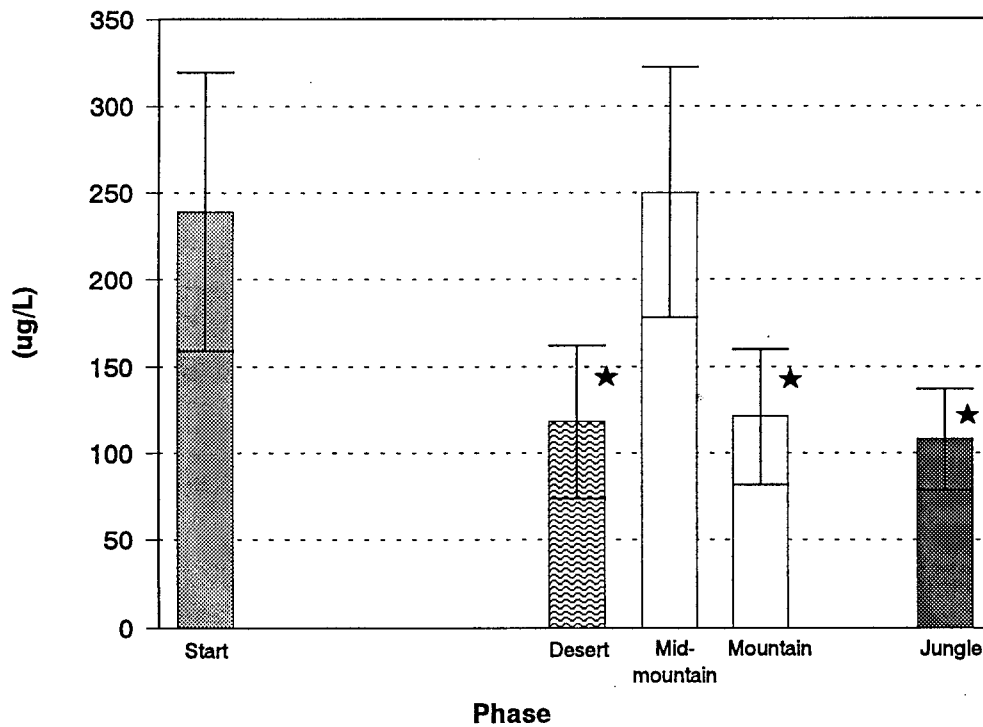


Figure 5.11 Serum Cortisol Concentrations for RGR-I

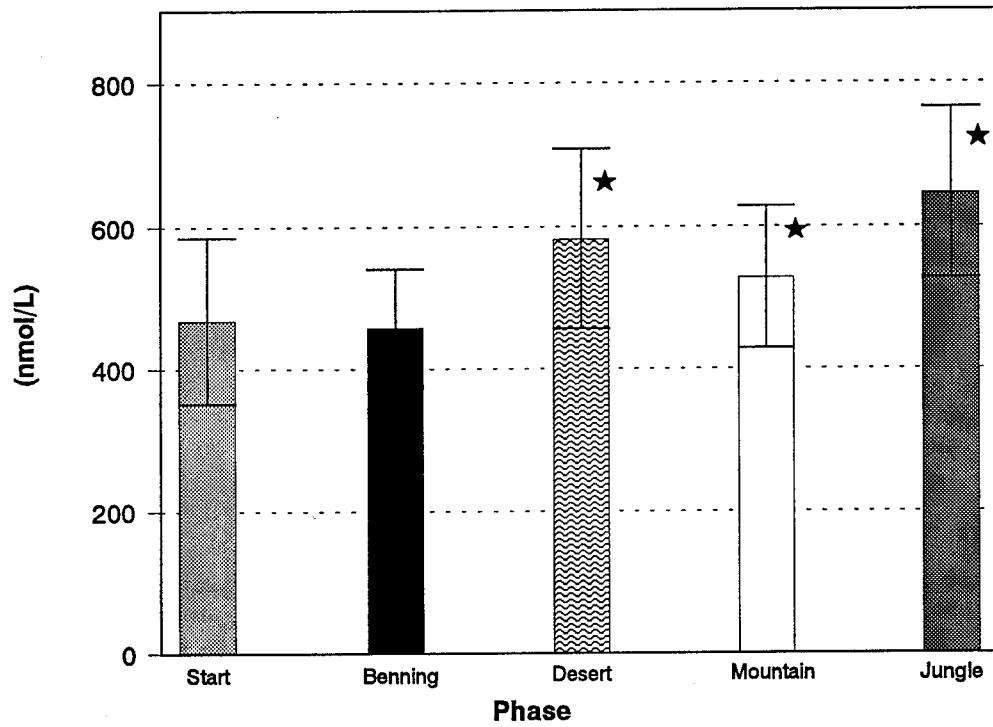


Figure 5.12 Serum Cortisol Concentrations for RGR-II

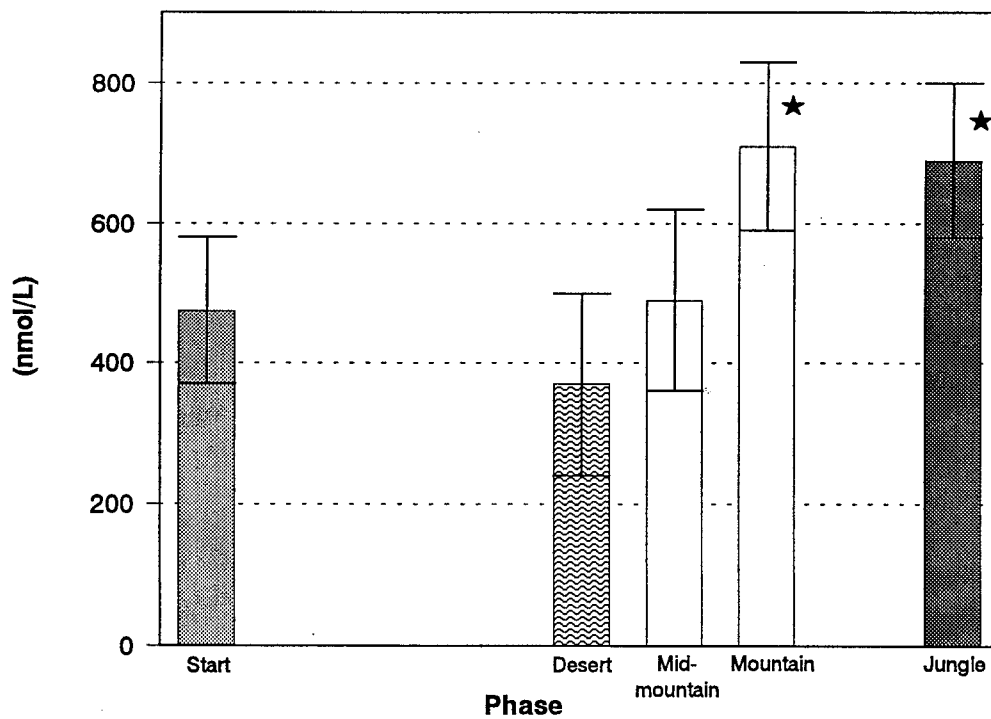


Table 5.4 Serum hormone measurements during RGR-II.

Parameter	Start	end of Desert	mid-Mountain	end of Mountain	end of Jungle
Hepatic-pituitary axis & cortisol					
IGF1 (µg/L)	239±80	118±44*	250±72	121±39*	108±29*
Growth hormone (µg/L)	1.3±1.7	1.0±1.4	0.9±1.2	4.5±3.5*	3.9±2.8*
Cortisol (nmol/L)	469±106	377±119*	495±137	719±128*	692±109*
Thyroid-pituitary axis					
Triiodothyronine (nmol/L)	131±28	109±21*	141±23*	105±20*	101±21*
Thyroxine (µg/dL)	8.1±1.5	7.1±1.4*	7.1±1.8*	7.5±1.4*	6.9±1.3*
TBG (µg/mL)	21.6±3.3	23.1±2.9*	21.9±3.0	23.9±3.7*	24.8±3.5*
T4/TBG ratio	3.8±0.9	2.7±1.2*	3.0±1.4*	3.1±0.9*	2.5±1.1*
TSH (mIU/L)	2.7±1.0	4.4±1.8*	3.5±1.2*	4.4±1.7*	4.7±1.7*
% sample with TSH > 6 IU/L	0	21	2	24	19
Testicular-pituitary axis					
Testosterone (nmol/L)	17.3±4.8	6.9±3.3*	17.2±6.5	4.8±3.2*	3.0±1.8*
SHBG (nmol/L)	26.0±8.7	52.1±21.1*	31.0±9.8*	44.2±15.3*	49.7±17.9*
Free testosterone (pmol/L)	358±115	98±43*	313±150*	72±50*	42±27*
Free testosterone %	2.1±0.5	1.5±0.3*	2.0±0.2*	1.5±0.3*	1.4±0.3*
LH (IU/L)	7.7±2.0	6.9±2.2*	8.1±2.7	5.1±1.1*	4.7±1.5*

notes: shaded columns indicate sampling following a period of restricted rations; values in bold type represent means which fall outside the normal range; *indicates difference from starting value, t-test, p<0.05; T4/TBG ratio calculated as T4(µg/dL)/TBG(µg/mL)×10.

Figure 5.13 Serum Triiodothyronine Concentrations for RGR-I

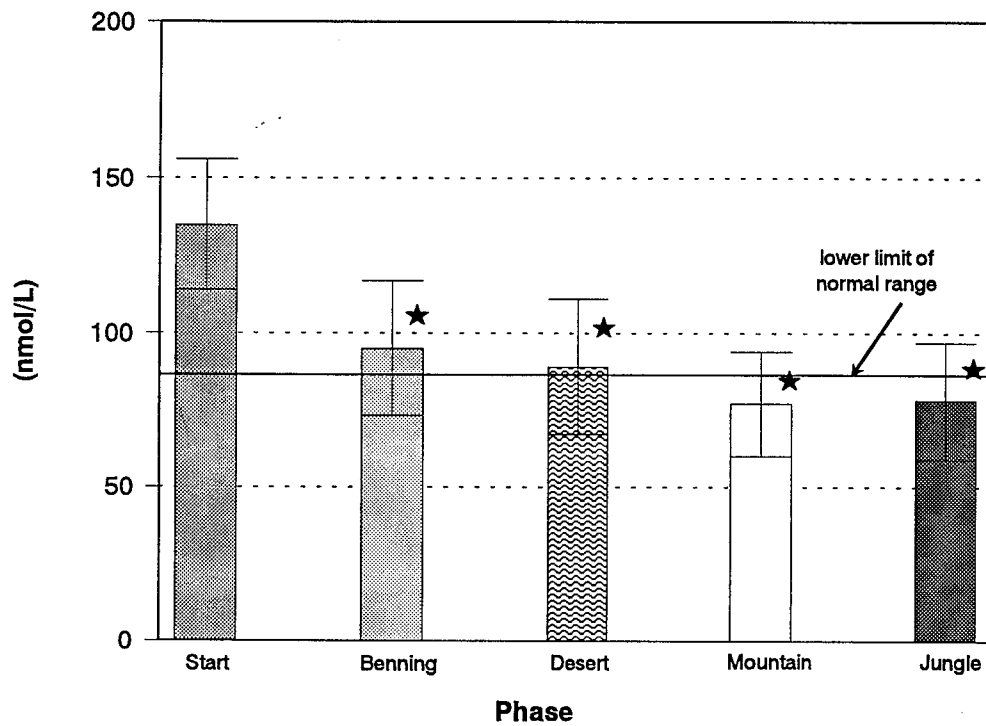
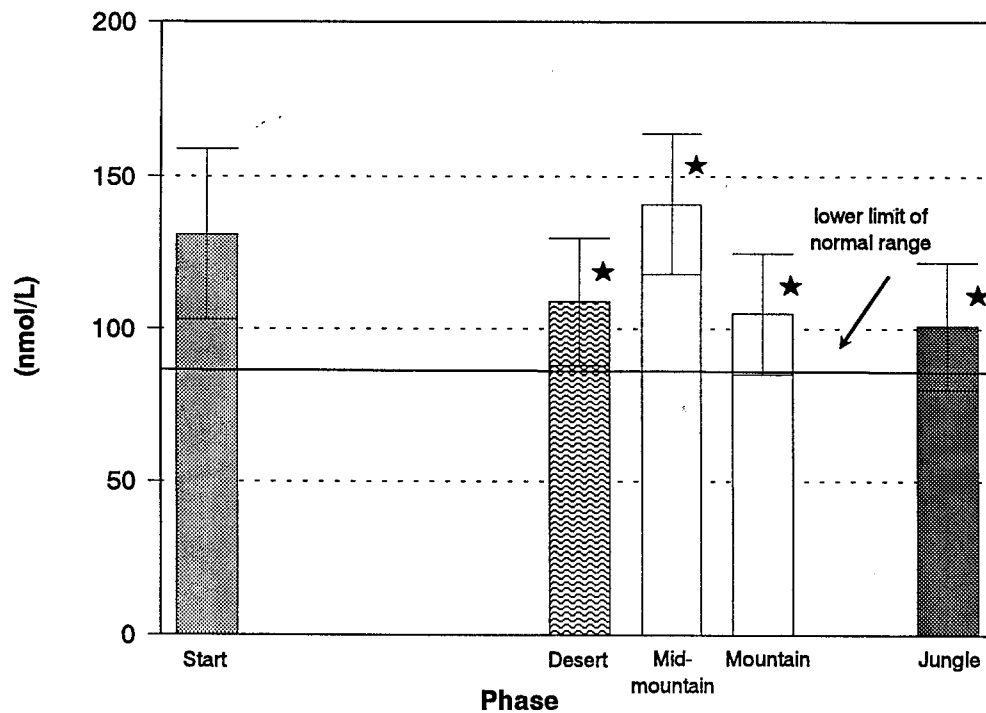


Figure 5.14 Serum Triiodothyronine Concentrations for RGR-II



al. 1984), while others report an increase in TSH (Pimstone et al. 1973). Other stressors in Ranger training have specific effects as well, such as sleep deprivation which has been reported to stimulate TSH secretion (Palmblad et al. 1979). The responses observed in Ranger training certainly include the well-established effect of energy deficit on peripheral conversion of T4 to T3 (Vagenakis et al. 1977). The data for TBG are shown for RGR-I and RGR-II in Figures 5.15 & 5.16, respectively. TBG levels increased (Figure 5.16), presumably in response to decreased circulating insulin levels which may stimulate hepatic production of isoforms less resistant to metabolic clearance, in a regulation similar to that of SHBG (Plymate et al. 1988). This magnified the effect of the small but significant decline in T4 in terms of a decline in the bioavailable (free) T4 (reflected in the T4/TBG ratio).

Testosterone declined to levels well below the normal male range in RGR-II (Figure 5.18) as in RGR-I (Figure 5.17), with prompt recovery of this generalized stress marker following the early phase refeeding during the mountain phase. A similarly profound decline in serum testosterone has been reported to occur within 48 hours after the start of Norwegian Ranger Training involving sleep deprivation, food restriction, high levels of energy expenditure and psychological stress (Aakvaag et al. 1978); sleep has a small effect on increasing the suppressed testosterone levels (Opstad & Aakvaag, 1983). This profound suppression is different from the positive response to physical conditioning in Finnish Basic Trainees (Remes et al. 1979) or the acute rise in testosterone following several successful simulated jumps in the Norwegian Army Parachute School (Davidson et al. 1978). The large rise in SHBG (Figures 5.19 & 5.20), in response to reduced insulin and testosterone during semistarvation (Aakvaag et al. 1978; Plymate et al. 1988), further reduced the bioavailable testosterone as indicated by the decline in calculated non-SHBG-bound testosterone levels (Table 5.4). In contrast to the other measured pituitary hormones (growth hormone and TSH), LH was significantly reduced indicating that the reduced testosterone is primarily a central hypogonadism (i.e., a hypothalamically-mediated stress response). LH pulse frequency is reduced during acute fasting (Cameron et al., 1991), but a primary hypogonadism (i.e., direct effects at the testicular level) in fasting men has also been suggested (Klibanski et al. 1981; Kyung et al. 1985; Opstad & Aakvaag, 1983); there is no effect of endurance exercise with adequate energy intakes on LH secretion (Rogol et al., 1984).

Figure 5.15 Serum Thyroid-Binding Globulin Concentrations for RGR-I

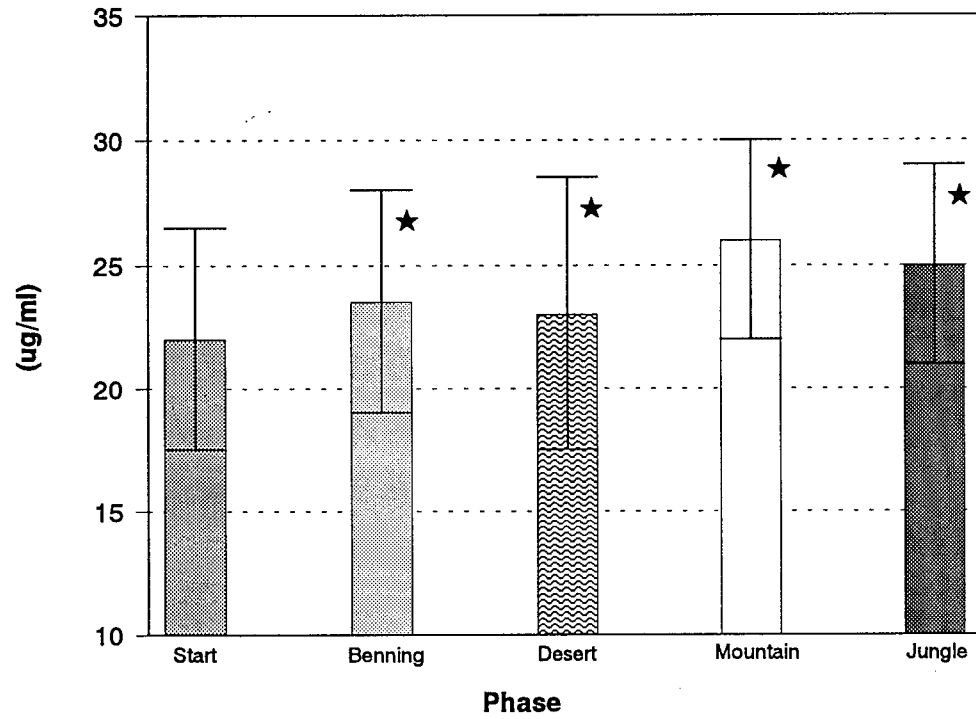


Figure 5.16 Serum Thyroid-Binding Globulin Concentrations for RGR-II

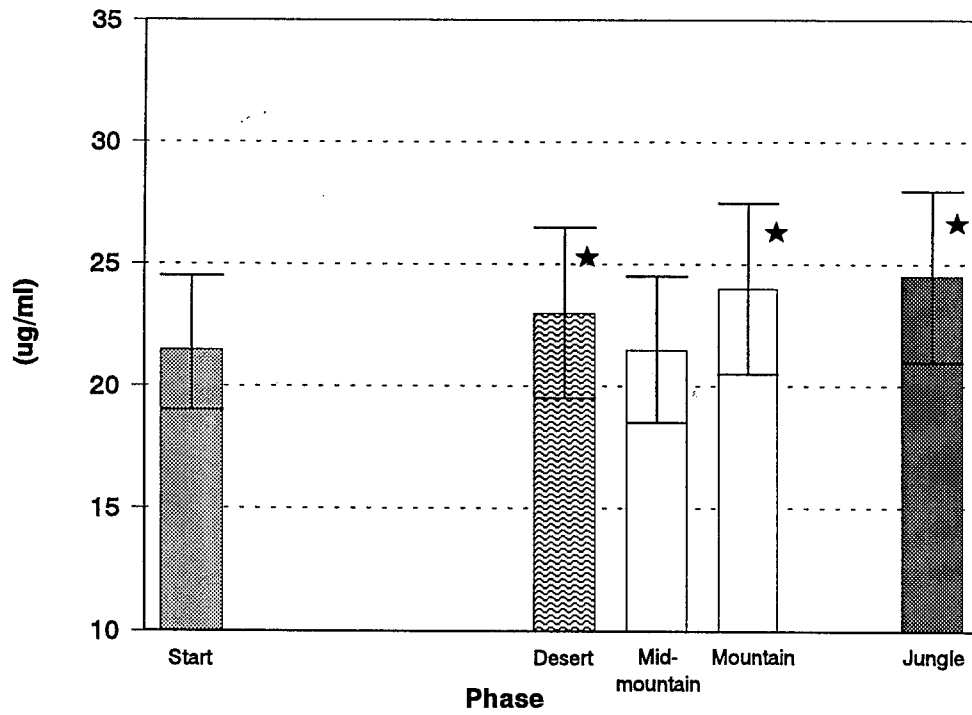


Figure 5.17 Serum Testosterone Concentrations for RGR-I

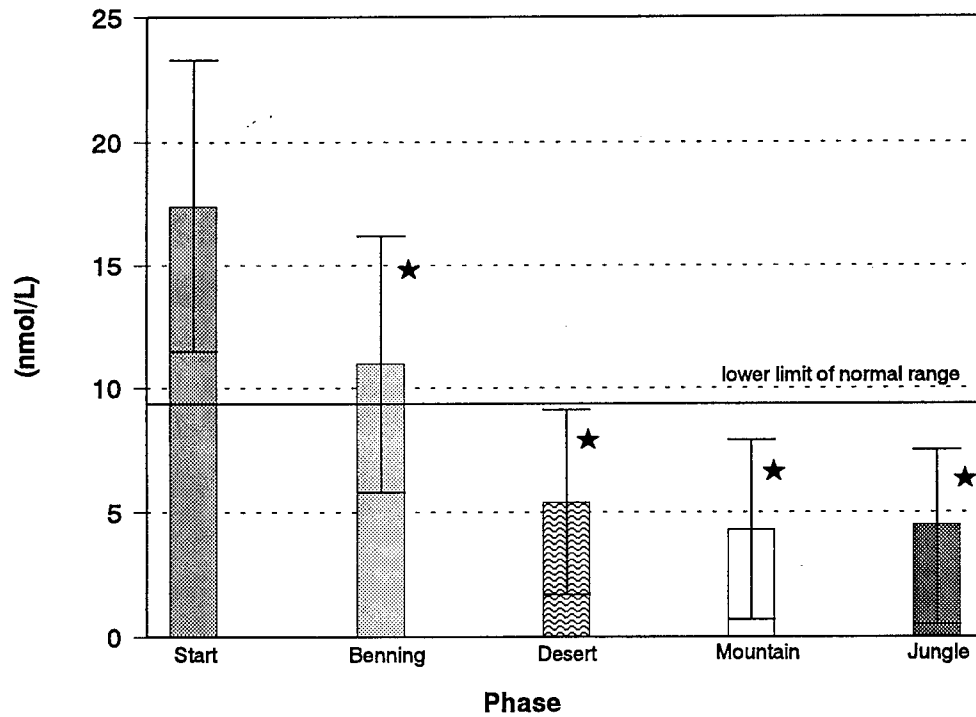


Figure 5.18 Serum Testosterone Concentrations for RGR-II

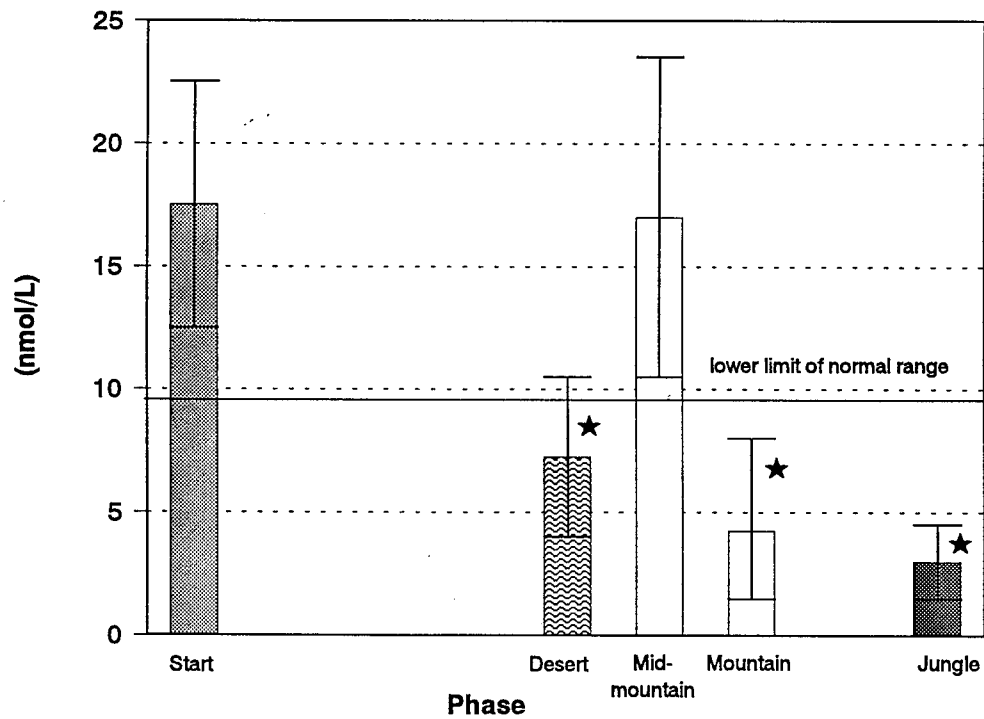


Figure 5.19 SHBG Concentrations RGR-I

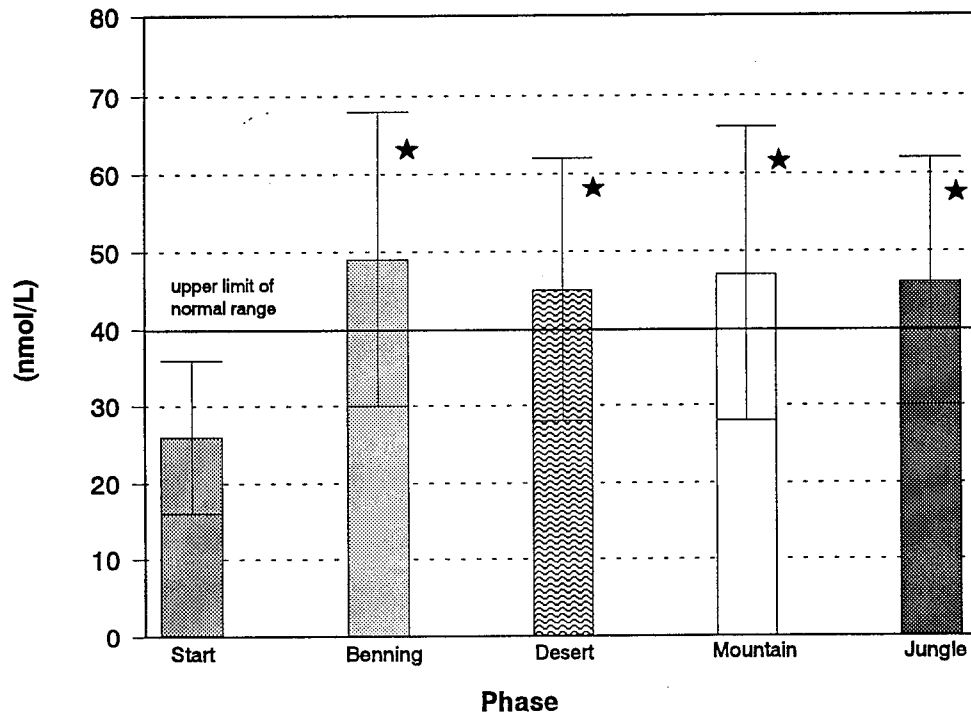
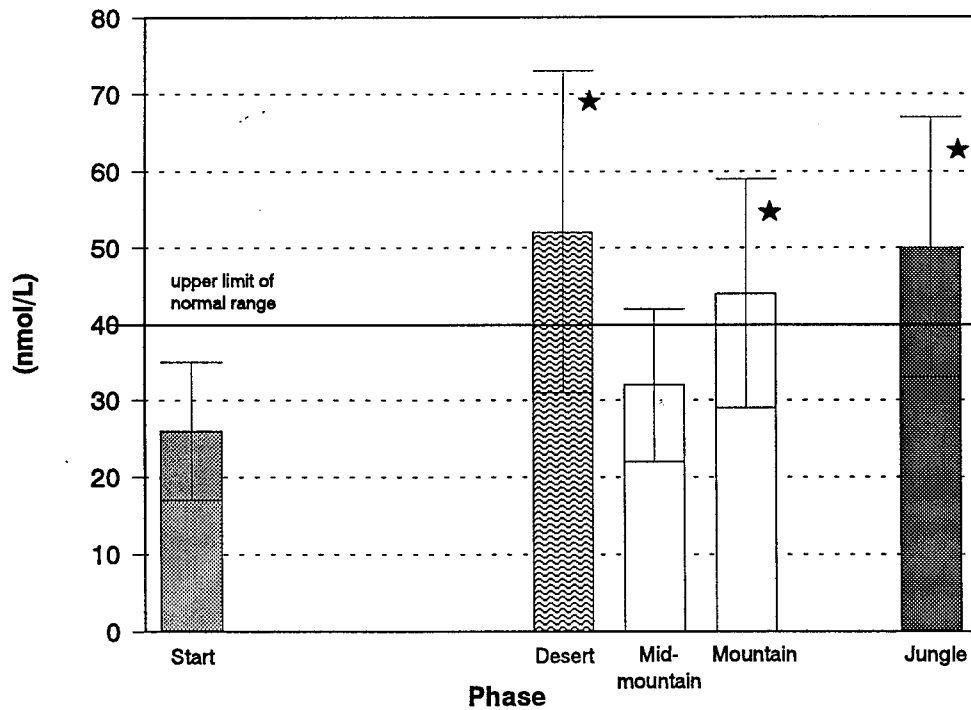


Figure 5.20 SHBG Concentrations RGR-II



In summary, the serum hormones measured in RGR-II indicate a prominent stress response (e.g., male sex hormone levels in the castrate range), including the presence of a significant metabolic stressor (e.g., reduced IGF1); these responses can be primarily attributed to energy rather than sleep deprivation (Opstad, 1991). There is a clear indication that the response to the metabolic stressor has been attenuated in RGR-II, as demonstrated by smaller suppression of T3 and a delayed rise in cortisol.

Correlations between Hormonal Markers and Strength

Efforts to correlate changes in serum hormones with changes in performance measures were fruitless. This comes as no surprise for several reasons. For hormones with a high pulsatility, concentrations in a single blood sample indicate only an average of randomly sampled pulses (e.g., growth hormone), and even when the pulse amplitude is markedly suppressed (e.g., LH), a serum concentration only indicates changes at one point in a cascade of regulatory events. Changes in serum concentrations of the hormones do not necessarily reflect tissue levels, nor do they directly reflect the level of anabolism achieved in any given individual (Griggs et al., 1985).

In RGR-I, there was a strong inverse relationship between weight loss and serum IGF1 concentrations measured at the end of Ranger training (Moore et al., 1993). IGF1 treatment has been shown to maintain lean tissue in rodents during starvation (O'Sullivan et al., 1989) and cancer cachexia (Ng et al., 1992). However, strength performance depends on more than muscle mass, including neuromuscular factors, other aspects of metabolism and psychological motivation; thus, pinpoint assessment of specific serum hormones in this study provides useful markers of metabolic status but will not predict strength performance.

Declines in T3 and testosterone are expected to change the composition of muscle fiber types with preferential catabolism of fast twitch (Type IIA) fibers (Goldspink & Ward, 1979; Krotkiewski et al., 1980) during energy restriction leading to a greater proportion of slow twitch muscle fibers (Russell et al., 1984). Henriksson (1990) and his colleagues have demonstrated a specific decline in Type IIA fibers during military exercises with energy deficiency and with dynamic exercise of long duration and low intensity (skiing with a backpack for 30 km/day). This has theoretical implications for the type of physical

performance which would be most affected by semistarvation. Strength measurements such as those made in this study are more likely to be affected than endurance exercise.

CONCLUSIONS

Weight loss was modestly attenuated (~1/6th less than in RGR-I) by the increased energy intake. This relatively small difference had a marked effect on metabolic status because it was sufficient to keep the men from entering the next phase of starvation involving increased catabolism of lean mass. At the end of training, the soldiers in this study still had some fat stores, even though they were significantly reduced; in RGR-I, most men had depleted their fat stores. This difference between studies was also reflected in expedient indicators such as skinfold thicknesses and abdominal circumference. Thyroid hormones, primarily T3, remained within the low normal range, indicating that the regulation of metabolic rate was closer to normal than in RGR-I where T3 was reduced below normal. The greater total energy expenditure estimated for this group (compared to RGR-I) may also be a reflection of a more normal metabolism.

There was only a slight attenuation of the decline in FFM (-6.0% of initial, compared to 6.9% in RGR-I), probably representing an inevitable FFM loss which accompanies the large reduction in fat weight. This similarity to RGR-I reiterates how small the nutritional intervention really was, even though it had a profound physiological benefit. The benefit stems from keeping the soldiers just below the threshold for marked muscle catabolism which would occur subsequent to depletion of fat stores. In RGR-I, soldiers finished the course just over this threshold and were metabolically poised for cachexia. The delayed rise in cortisol in response to declining fat stores indicates that the feeding intervention extended these soldiers' energy stores by roughly two weeks (one phase of training); the same estimate is obtained from the change in body energy stores.

Dynamic muscular strength assessed by maximal lift capacity and by power from jump performance demonstrated significant decrements which are only moderately associated with declines in muscle mass. The decrement in maximal lift capacity was nearly identical to the decrement observed in RGR-I, again indicating how small the intervention was, or reflecting

an all-or-none decline in performance associated with restricted feeding. A dichotomous response was also demonstrated in serum testosterone (a generalized stress marker). There is no direct relationship between strength decrements and serum testosterone (or other measured hormone) levels.

All measures had returned to normal within approximately one month (five weeks) following the end of Ranger training, with the exception of rebound changes in body fat and several other metabolic markers. In the ten soldiers assessed, fat had increased by 40% above normal levels, and binding proteins (SHBG and TBG) were suppressed in response to hypercaloric intakes. Data obtained in RGR-I suggests that hyperphagia peaks at approximately one month, and all parameters are normal by six months post-training (and probably sooner). As body composition and metabolic rate normalizes, sleep quality is also expected to be restored (Shapiro et al., 1990).

Reduced circulating levels of hormones such as T3 and testosterone, as observed here, inevitably lead to suggestions that artificially restoring these to the normal range may offer some physiological or psychological advantage to the individuals. Such an intervention could actually be detrimental to the health and safety of the soldier. Both T3 and testosterone increase metabolic rate and increase lipolysis; declining action by these hormones during food restriction is a physiologically appropriate response which helps to preserve energy stores (Winpheimer et al., 1979). Even the altered fast-to-slow muscle fiber ratio provoked by this alteration in thyroid and possibly testosterone, has been suggested to be a useful adaptation to starvation because of the slow-twitch fiber capacity for fatty acid oxidation (Henriksson, 1990). Other effects cannot readily be predicted but at least one experiment suggests a reason for caution in thyroid replacement. Rats infected with streptococcal pneumonia demonstrate a decline in circulating thyroid hormones and action (Little, 1985a); when replaced to normal thyroid levels, morbidity and mortality in the rats substantially increase rather than decrease (Little, 1985b). Coincidentally, streptococcal pneumonia has been a problem pathogen for Ranger students (Riedo et al., 1991) but this and other types of infections could conceivably be promoted with hormone replacement treatment. Stimulating endogenous responses to IGF1 or administering recombinant IGF1 will preserve body weight and presumably FFM, but at the risk of currently unknown costs to other adaptive mechanisms involved in turning down metabolism. A more promising

approach to pharmacological intervention would be to block maladaptive responses which occur in a subset of soldiers who lose excessive amounts of FFM. This calls for further investigation to identify catabolic factors such as interleukin-1 and tumor necrosis factor (which may inappropriately increase in some individuals during Ranger Training), and to determine if simple interventions such as administration of indomethacin can be effective (Beisel, 1992; Belizario et al., 1991).

Expedient tests which are minimally intrusive, and can be performed with limited resources, can be recommended for future evaluation of such metabolically stressful scenarios. These would include weight, the four skinfolds used in the Durnin-Womersley body fat equation, abdominal circumference to estimate changes in body composition, a simple jump test (with counter movement allowed) to calculate peak power as an indicator of dynamic strength, and measurement of serum T3 and testosterone as markers of energy deficit and overall stress levels, respectively. All of these tests yielded dramatic results, and can serve as a benchmark of metabolic severity to which future studies can be compared.

CHAPTER 6

DURATION & PATTERNS OF SLEEP

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Walter Reed Army Institute of Research

INTRODUCTION

Technological advances have resulted in military operations being conducted twenty-four hours per day for extended periods of time. During these operations, soldiers are required to perform maximally despite chronic, and sometimes severe, sleep restriction. Most laboratory studies focus on the effects of a few days of total sleep deprivation, rather than on extended periods of sleep restriction. Field studies may include both total sleep deprivation and sleep restriction, but rarely last longer than several days for any particular soldier. Consequently, little is known about the effects of, or the recovery process following, chronic sleep restriction. The eight-week U.S. Army Ranger training course uses sleep restriction as one of the stressors applied throughout the course and provides a model for chronic sleep restriction under stressful conditions similar to conditions found during actual combat operations.

The continuity of sleep theory proposed by Naitoh and Angus (1987) suggests that the restorative value of sleep depends on the quality, as well as the quantity, of sleep. Little is known about the impact of chronic sleep restriction under stressful conditions on quality and restorative value of sleep. Specifically, the quality of sleep obtained during Ranger training has not been assessed. Some stressors, such as food restriction, that are an integral part of Ranger training may affect the quality and restorative value of sleep during Ranger training.

This study compared sleep quantity and quality measures for two classes going

through the Ranger training course. The 1992 class differed from the 1991 class in caloric intake and in the order of the four phase locations (Ft. Benning, desert, mountain, and jungle) during training. Sleep quantity and quality were also assessed during the first five days following course completion to assess the short-term recovery process.

METHODS

Sleep was measured indirectly in 32 students throughout the Ranger training course by measuring movement activity with the Walter Reed Activity Monitoring System (AMS). When subjects were dropped from the course, other students were issued monitors in their place. Activity monitors were issued at the beginning of each phase and collected at the end of each phase. Between phases, data was downloaded from the monitors, batteries were changed, and the monitors were reprogrammed for the next phase. Monitors also were issued to nine of the students for five days immediately following graduation from the 1992 class.

Equipment

The AMS is comprised of a wrist activity monitor and a data programming and reading device. The wrist monitor measures approximately 1.75 in by 1.25 in by 0.45 in and weighs less than 40 grams. This new model of activity monitor is smaller and more comfortable to wear than previous models. It contains a 3 V lithium battery, accelerometer, and a microprocessor. Motion is detected by a ceramic bimorph beam mechanically attached and electrically connected to an internal circuit board.

Because limited battery life allows data collection for only 12 days, half of the monitors (16) were programmed to start on the first day of a phase and run for approximately 12 days. The other 16 monitors were programmed to start collecting data on the ninth day of each phase and collected data through the last day of each of the phases.

Activity monitoring has been used extensively to assess sleep in ambulatory subjects, and corresponds closely to standard laboratory measures of sleep using EEG recording (Mullaney, et al., 1980, and Webster, et al., 1982). Action software, Version 1.24, (Ambulatory Monitoring, Inc.) was used to score sleep episodes. The algorithm used by this software to score sleep was based on the work of Dr. Cole and Dr. Kripke at the University of California at San Diego and San Diego VA Medical Center. This set of criteria for scoring sleep episodes was consistent with EEG measures of sleep 90% of the time. Activity monitors slightly overestimate sleep time, because periods of awake immobility cannot be distinguished from periods of sleep. Therefore, this bias produced a conservative estimate of sleep loss in this study.

Measures

Activity monitoring produced several dependent measures of sleep. Total time asleep for each 24 hour period was computed. The mean daily quantity of sleep for each phase was then calculated for each subject, and these means were used in the statistical analyses.

In addition, four indices of sleep quality were computed for each sleep period lasting at least one hour. However, if a subject was scored as awake for 30 consecutive minutes, or longer during a sleep period, the period was scored as two separate sleep periods. This procedure was used to avoid scoring activities such as guard duty as disrupted sleep.

The first index of sleep quality was the percent of time during each sleep period that was scored as sleep. The second index was movement expressed as the percentage of minutes in a sleep period in which movement occurs ($\#$ of minutes with movement/total $\#$ of minutes in a sleep period $\times$ 100). The third index was fragmentation, expressed as the percentage of periods with no movement lasting 1 minute ($\#$ of 1 minute periods with no movement/total number of periods without movement lasting any length of time $\times$ 100). An increase in this index reflected an increase in the proportion of short periods of immobility to all periods of immobility. The fourth was a global index of sleep disruption and was the sum of the second and

third indices. This measure reflected both the proportion of time spent moving during sleep, and the frequency of periods of movement in sleep. These quality of sleep indices have been used to discriminate people with normal sleep patterns from people with Sleep Apnea Syndrome (SAS), which results in disrupted sleep patterns and daytime sleepiness (Aubert-Tulkens, Culee, et al., 1987). Weighted means based on the length of sleep periods were calculated for each subject for each phase for each of the four sleep quality indices, and these means were used in their respective analyses.

Analyses

The dependent sleep measures were analyzed using two factor (2 x 4) ANOVAs. Although there were three factors (class, phase location, and phase number), only class and one of the other two factors were independent of each other. Significant two-factor ANOVA's were followed by two-tailed t-tests. Only descriptive statistics are presented for the recovery period due to the small sample size. (Data was retrieved from only six of the nine monitors.) Descriptive statistics are expressed as mean and standard deviation.

RESULTS

Sleep Quantity

The average daily amount of sleep during the RGR-II class did not differ from the sleep obtained during the RGR-I class, which indicates that increasing caloric intake and changing the order of phase locations had no impact on the amount of sleep students obtained throughout the Ranger training course. Students in RGR-I averaged 3.59 hours of sleep per day throughout the course, and students in RGR-II averaged 3.61 hours of sleep per day. However, quantity of sleep was affected by the phase number, or length of time spent in the course ($F(3,140) = 6.80, p < .001$). Average daily sleep increased to 4.33 hours during the final phase of Ranger training, compared to average sleep during the other three phases of the course that ranged

from 3.25 to 3.45 hours per day. (See Table 6.1 & Figure 6.1.) This increase occurred even though the location of the final phase changed from the desert in 1991 to the jungle in 1992. Because the location of the phases had no impact on the amount of time the students slept (See Figure 6.2), increases in the amount of sleep during the final phase probably reflected the length of time students spent in the course, rather than consistent differences in the training plan for sleep. This increase in phase four sleep also may explain the interaction between class and location found for sleep quantity ($F(3,140) = 5.60, p < .002$). The average daily sleep was highest during the desert phase in RGR-I when the desert phase was the fourth phase, and sleep was highest during the jungle phase in RGR-II when the jungle phase was the fourth phase (See Table 6.1).

Table 6.1 Mean Daily Sleep (Hours)

PHASE LOCATION				
Class	BENNING	DESERT	MOUNTAIN	JUNGLE
RGR- I	3.78 (0.64) [1]	4.12 (0.57) [4]	3.27 (1.66) [2]	3.12 (0.43) [3]
RGR-II	3.45 (0.71) [1]	3.41 (1.38) [2]	3.25 (1.05) [3]	4.33 (0.81) [4]
BOTH	3.54 (0.70)	3.71 (1.16)	3.26 (1.26)	3.89 (0.91)

* Values are means with standard deviations in parentheses.

Values in brackets are the phase numbers.

Figure 6.1 Mean Daily Sleep by Phase Number

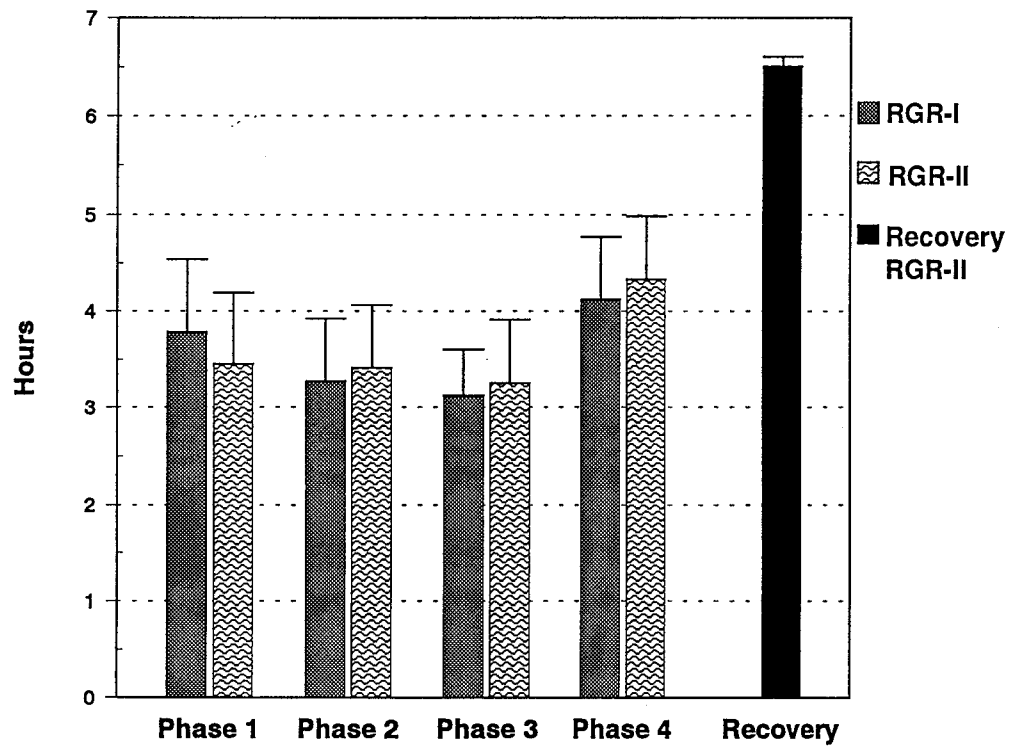
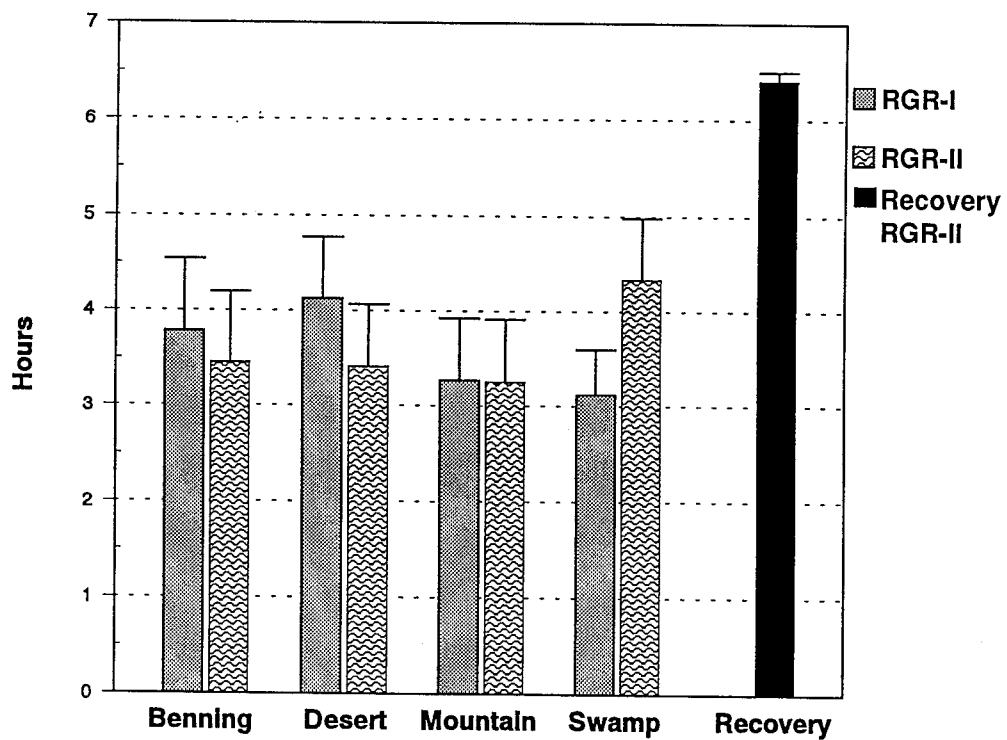


Figure 6.2 Mean Daily Sleep by Location



In summary, quantity of sleep was not influenced by the increase in caloric consumption during the 1992 class, nor was quantity of sleep different at any of the four phase locations. However, sleep increased during the final phase of the course, probably as a result of the length of time students had spent in the course.

Quality of Sleep

Quality of sleep is defined as the percent of sleep period scored as sleep. Students slept between 85 and 89% of the time during sleep periods. Neither phase location, nor phase number affected this measure. Although students in RGR-II slept a lower percentage of time during the sleep periods than students in RGR-I ($F(1,136) = 3.19, p < .04$), the small effect size (2%) and the relatively large sample size indicate that these results should be interpreted with caution.

Movement Index

The percentage of time spent moving during sleep periods was not affected by class, phase location, or phase number. (See Figure 6.3.) These values were also consistent with values reported for normal volunteers in the literature (Aubert-Tulkens et al., 1987).

Fragmentation Index

Sleep fragmentation was not affected by class (the nutrition manipulation) or by phase number. Sleep fragmentation increased slightly during the desert phase ($F(3,136) = 3.19, p < .3$) (See Figure 6.4), however the fragmentation index during the desert was similar to the value reported for normal volunteers by Aubert-Tulkens and coworkers (1987).

Global Index

Because the global index is a sum of the movement and fragmentation indices, the effect found for phase location ($F(3,136) = 2.69, p < .05$) reflects the increase in sleep fragmentation found in the desert phase.

Figure 6.3 Movement Index of Sleep Quality

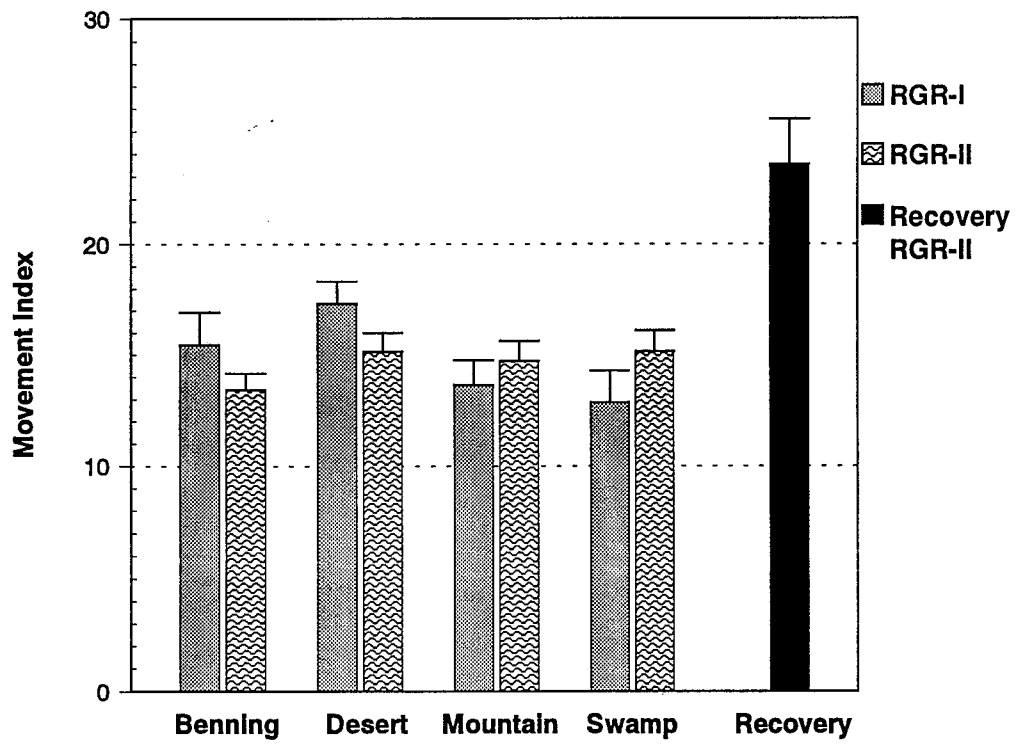
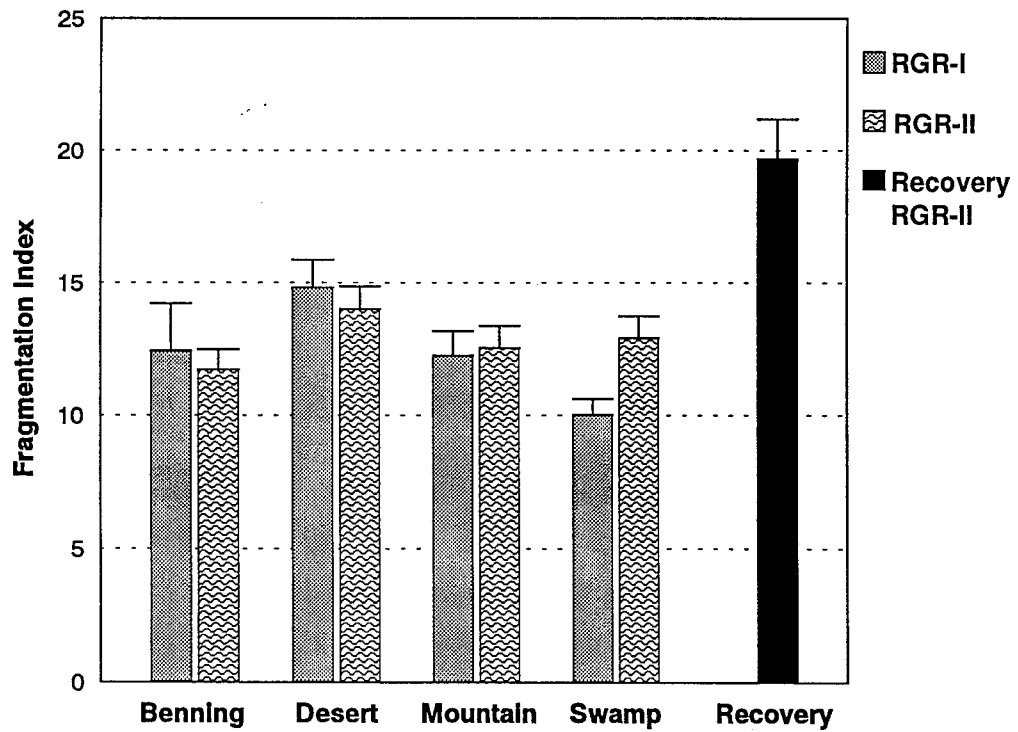


Figure 6.4 Fragmentation Index of Sleep Quality



Recovery

Students averaged 6.46 hours sleep per day during the first 5 days of recovery (See Figure 6.1.), which is slightly less than the 7 - 8 hour norm. Recovery sleep also appeared to be of poor quality compared to sleep during the course (global indices of sleep quality were 43.22 and 27.43, respectively). During recovery, six students who completed the 1992 class slept 74% of the time during sleep periods, compared to 86.58 % of the time for students of the same class while in Ranger training. The movement index was higher during recovery than during the course (23.53 and 14.60, respectively). (See Figure 6.3.) The fragmentation index also was higher during recovery than during the course (19.68 and 12.81, respectively). (See Figure 6.4.)

CONCLUSIONS

Sleep quantity and quality were remarkably consistent across classes going through Ranger training. Even the increase in caloric intake in RGR-II did not affect sleep quality or quantity significantly. Students in RGR-I and II averaged 3.6 hours of sleep per night throughout the course, and the quality of this sleep was similar to the quality of sleep reported for normal volunteers in another study (Aubert-Tulkens et al., 1987). The 3.6 hour average for RGR-I and II was slightly higher than the 3.2 hour average reported by Pleban and coworkers (1990) for a ranger school class starting in 1987. This difference appears to be due to the increase in sleep during the final phase of the 1991 and 1992 classes. Since the final phase was conducted at different locations for the two classes, the increase in sleep was probably not due to consistent differences in training policy concerning sleep at the different phase locations.

One possible explanation is that Ranger trainees reach some limit of tolerance for sleep restriction or general course conditions during the fourth phase. This limit may cause the Ranger trainees to fall asleep more often during times they should be awake, or may cause such a widespread reduced level of functioning in the students that the Ranger cadre individually increase the scheduled sleep time. The findings of Pleban and coworkers are consistent with this hypothesized limit. The students in the

1987 class did not sleep more during the final phase of the course. However, these students also had a 2 week break for the holidays following their first phase of training. As a result, their final phase was only their third consecutive phase of the course and would not be expected to increase sleep quantity.

Some soldiers who have completed Ranger training have reported sleep disturbances extending for some time after the Ranger training course. Data from the first 5 days of recovery are consistent with these reports. Instead of sleeping more than the 7 - 8 hour norm, as would be expected during recovery from sleep restriction, these subjects slept less than the norm. In addition, sleep quality was worse during recovery than at any time during the course, as well as worse than normal values that have been reported in the literature. Recovery sleep disturbances may impair performance and prevent recent graduates of the Ranger training course from performing maximally for some undetermined length of time following the course. Further research is needed to validate that sleep disturbance occurs during recovery, to determine the length of time any disturbance is evident, and to determine any impact on performance following the Ranger training course. In addition, possible causes of any disturbance, including disruptions in circadian rhythms, should be investigated.

CHAPTER 7

COGNITIVE PERFORMANCE

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INTRODUCTION

Food deprivation, short of complete starvation, does not appear to significantly degrade cognitive performance. The classic study of food deprivation was conducted by Keys et al. (1950). In that study, consumption levels approximated 50% of requirements producing a 25% weight loss over 24 weeks. No meaningful changes were found in objectively measured cognitive performance, although subjects repeatedly reported memory lapses, inability to concentrate, obsessive behaviors, apathy and lethargy. *Post hoc* analyses of behavior during famines (CMNR, 1986; Graham, 1993; Keys et al., 1950), confirm that non-cognitive behaviors of lethargy, helplessness, and hypochondria disrupt cognitive performance, but do not prevent individuals from functioning in an intelligent and purposeful manner when the opportunity arises to procure food or escape the situation.

Military field studies of food restriction are also a testimony to the resilience of cognitive performance. Johnson et al. (1982) reviewed a series of American, British, Canadian, and Australian studies of the minimum number of calories per day needed to sustain military performance while on 4-day to 14-day patrols in temperate and extreme environments. For example, Crowdy et al. (1982) studied the impact of 12 days of food restriction on soldiers during arduous training and testing in the Malaysian jungle. The food-restricted group's consumption averaged 47% of requirements, creating a weight loss of 6%. Performance on marksmanship, vigilance, arithmetic, and coding tests did not significantly change from baseline across the 12

days of testing. In general, weight losses of 6% or less, over periods of 10 to 45 days, produce no meaningful degradation in cognitive performance (Askew et al., 1986; Askew et al., 1987; Hirsch et al., 1984; Hirsch et al., 1993; Johnson et al., 1982; Lichton et al., 1988; Popper et al., 1987; Roberts et al., 1987; USA, 1986). Moreover, moderate levels of under-consumption often enhance cognitive performance for 3 to 15 days.

In contrast, recent studies of Ranger training (Moore et al., 1992; Pleban et al., 1990; Popp, 1992), where weight losses were 10-20% over a period of 60 days, suggest that cognitive performance is degraded significantly (see also Opstad et al., 1978; Rognum et al., 1986). Unlike the typical laboratory or field study, Ranger training combines food restriction with strenuous exercise, sleep restriction, danger, and personal evaluation. Anecdotal accounts of performance during Ranger training often include descriptions of soldiers who walk around in a daze, disassociated from their surroundings, unable to safely cope with simple physical or intellectual obstacles. Previous studies of Ranger training have neither systematically sampled cognitive performance across phases of the course nor sampled it at the same time that physiological and physical performance measures were being made. The RGR-II study was designed to systematically quantify changes in cognitive performance as a function of sustained exposure to a multi-stressor environment.

METHODS

Materials

Food restriction and sleep restriction in Ranger training leads to uncomplicated energy deficiency, rather than malnutrition or neurochemical substrate depletion (Moore et al., 1992). Consequently, the changes in cognitive performance most likely to be seen were presumed to be transient inattentiveness, indifference, and confusion rather than severe lapses in memory, reasoning, or information processing capacity (Askew et al., 1987; Calloway, 1982; Cohen et al., 1982; CMNR, 1986; Gorsky et al., 1983; Keys et al., 1950; Krueger et al., 1992; Webb, 1982). Four measures of

cognitive function were chosen for their sensitivity to the predicted changes; relevance to common military tasks; ability to measure different aspects of cognitive function; minimal practice requirement; and ease of administration. The decoding, reasoning, and pattern-recognition tasks are commonly used to assess cognitive performance under stress (CMNR, 1986; Krueger et al., 1992). The memory task was based on tasks traditionally used to analyze methods of processing meaningful information (Craik et al., 1975). All tasks were investigator-paced to create a realistic sense of urgency and to assess the incidence and severity of deficits in speed and accuracy.

Decoding Military communication tasks often require soldiers to use a decoding chart to translate arbitrary symbols into meaningful digits at a glance. These tasks test information-processing capacity and attention to detail. The decoding task is often referred to as a "symbol-digit substitution" task and has been used in earlier studies of Ranger training (Pleban et al., 1990; Popp, 1992). It measured the soldier's ability to rapidly transform geometric symbols into numbers using a decoding chart. Soldiers were given 90 seconds to complete 120 items.

Memory Military operations often require memorization of seemingly random lists of words, such as challenge and password combinations; code names for objectives, units, and landmarks; etc. Successful storage and retrieval of these lists requires effective mnemonic strategies, which often require substantial conscious effort. The memory task used in this study measured the soldier's ability to quickly memorize a list of key words embedded in mnemonic text and recognize whether words in a subsequent list were from the previously memorized group of words. The first portion of the memory task (30 sec) contained nine simple sentences (e. g., "The girl put the glass on the table."). There were always two key words in each sentence (e. g., "glass" and "table") and sentences had a repetitive format, which made the key words easy to identify. The second portion of the memory task (60 sec) consisted of a list of 15 words. Words from the previous list of sentences required a "yes" answer and new words required a "no" answer (e. g., a correct answer to "mug" would be "no" and a correct answer to "table" would be "yes"). The list always contained nine words from the previous 18 and six distractors for a total of 15 (this relationship was not discussed with the soldiers). Another task (reasoning) was administered during

the delay between the presentation of the sentences and the recognition testing, to control the length of the delay and to prevent rehearsal.

Reasoning Military operations often require the soldier to quickly determine the logical or temporal sequence of events from a hastily written message. Substantial conscious effort is often required and errors in judgement can be costly. The reasoning task measured the soldier's ability to rapidly determine whether a one sentence description of the order of a simultaneously presented pair of letters was logically true or false. In this task, the soldier reads a message ("A is not followed by B"), determines its meaning ("B should come first"), compares the meaning of the message to a two-letter combination ("AB"), decides whether the statement describes the order of the two-letter combination, and marks the statement "true" or "false" (false, in this example). Soldiers were given 60 seconds to complete 25 items. This task was always administered in between the first and second portions of the memory task.

Pattern Recognition Military map-reading tasks often require soldiers to rapidly detect arbitrary symbols embedded in larger symbols. Map-reading requires the ability to separate figure from ground and to attend to detail. The pattern-recognition task was a variant of tasks commonly referred to as a "match-to-sample" or "hidden figures" task. The task measured the soldier's ability to quickly decide whether a sample figure was embedded in any of four other simultaneously presented figures. A correct answer to a test figure which contained the sample figure was "yes." A correct answer to a test figure which did not contain the sample figure was "no." Soldiers were given 60 seconds to complete six items (each item presented a sample and four test figures).

Procedure

Performance on each of the cognitive tasks was measured at six time-points during the course: in the week prior to the beginning of Ranger training, at the end of the Benning and desert phases, during the middle of the mountain phase, and at the

end of the mountain and jungle phases. A different, but parallel, version of each task was used each time the tasks were administered, to minimize practice effects. Performance was measured in places and at times convenient to the Ranger cadre and frequently coincided with blood draws, body composition measurements, or physical performance measurements.

The four tasks were administered in a fixed order which provided control over the length of exposure to each task and the ability to practice or rehearse the task. Instructions for the task were always read to the soldiers immediately prior to administration of the task. The test session began with the memory task. Soldiers were given 30 sec to identify and memorize the 18 key words. This was immediately followed by administration of the reasoning task (60 sec), which was immediately followed by the recognition-test portion of the memory task (60 sec). This sequence of events was repeated twice more in quick succession. Once the three memory tasks and the three reasoning tasks had been administered, soldiers were given 90 sec to complete the decoding task, followed by 60 sec to complete the pattern recognition task. Soldiers were closely monitored to insure that they followed instructions and remained awake. On-the-spot corrections were made as necessary.

RESULTS

Data from the 51 soldiers who finished all four phases of the course during RGR-II were analyzed. Graduates and non-graduates of RGR-II were included in the analyses. Data were analyzed initially to determine the degree of change in performance at each phase. For each of the cognitive tasks, data on performance across phases of the course (baseline, Benning, desert, mountain, and jungle) were analyzed using the one-way repeated measures analysis of variance provided in SuperANOVA (v 1.11, Abacus Concepts). *A priori* pairwise comparisons of means were made using the least squares means technique (Kirk, 1968) provided in SuperANOVA. The second set of analyses were, like the first set, one-way analyses of variance followed by *a priori* pairwise comparisons. This set was used to determine the degree of intraphase recovery of performance (anticipated due to the increase in

food and sleep typically provided during the first week of the mountain phase).

Performance in the middle of the mountain phase was compared to performance at the end of the desert phase and at the end of the mountain phase.

Data Transformation

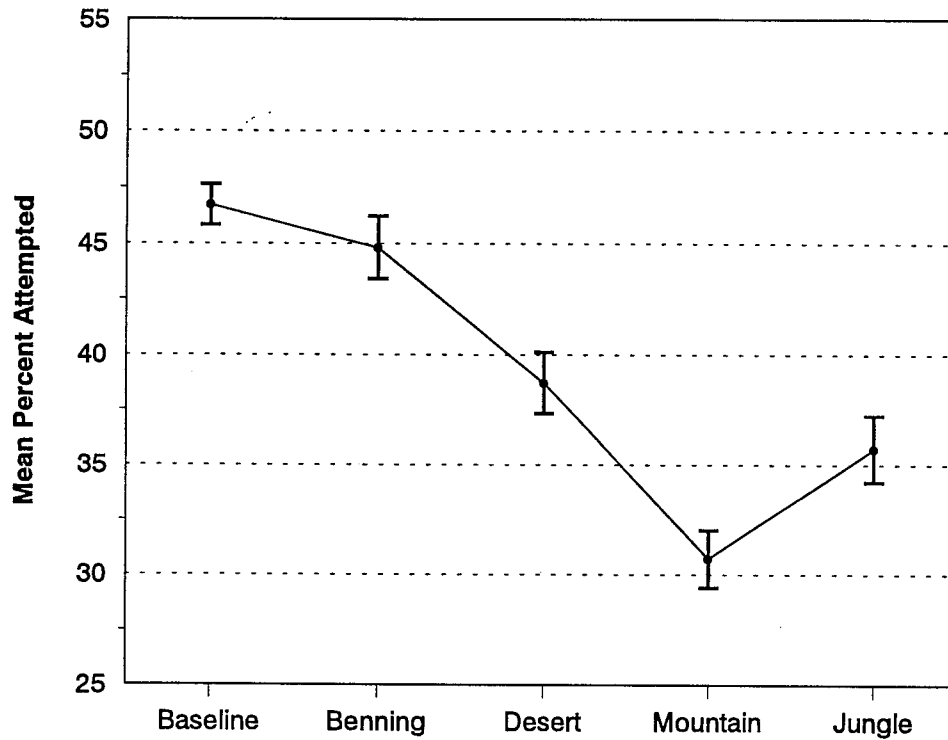
All four tasks were scored for number of items attempted in the allotted time and number of items answered correctly in the allotted time. The resulting scores were converted to percent measures in order to make it relatively easy to compare performance across tasks. A measure of accuracy, "percent correct," was obtained on the decoding task by dividing the number of items decoded correctly by the total number of items decoded. A measure of speed, "percent attempted," was obtained on the decoding task by dividing the number of items attempted in the allotted 90 seconds by the total number of items presented (120). A measure of accuracy was obtained on the memory task by dividing the number of items recognized correctly (whether "yes" or "no") by the total number of items answered. A measure of speed was obtained on the memory task by dividing the number of items attempted in the allotted 60 seconds by the total number of items presented (15). Data on the three trials of the memory task were averaged to yield a single score for the session (for each of the dependent variables, percent correct and percent attempted). A measure of accuracy was obtained on the reasoning task by dividing the number of items judged correctly by the total number of items answered. A measure of speed was obtained on the reasoning task by dividing the number of items attempted in the allotted 60 seconds by the total number of items presented (25). Data on the three trials of the reasoning task were averaged to yield a single score for the session. A measure of accuracy was obtained on the pattern recognition task by dividing the number of items matched correctly (whether "yes" or "no") by the total number of items answered. A measure of speed was obtained on the pattern recognition task by dividing the number of items attempted in the allotted 60 seconds by the total number of items presented (24).

Changes In Cognitive Performance Across Phases

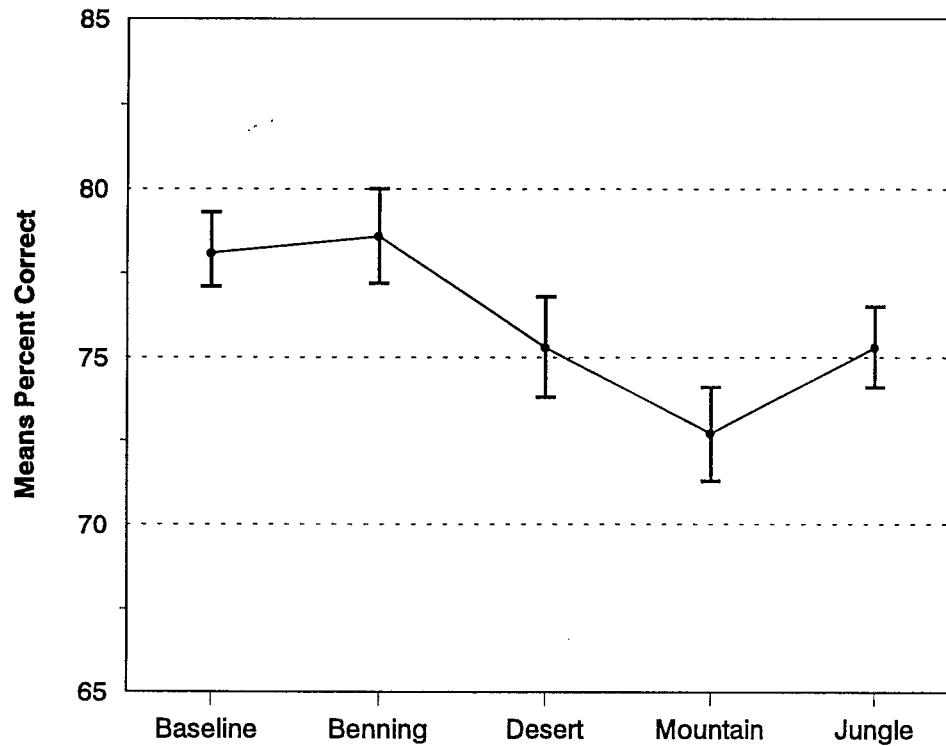
Decoding Performance on the decoding task showed the traditional speed for accuracy trade-off. Near-perfect accuracy was maintained across all administrations of the task. However, speed on the task was compromised in order to maintain accuracy, $F(4, 200) = 52.21, p = .0001$, indicating that information processing capacity was compromised by the stresses of the course. As shown in Figure 7.1, speed was significantly impaired at the end of the desert, mountain, and jungle phases (see Table 7.1). Performance at the end of the jungle phase was significantly better than at the end of the mountain phase (but was still significantly worse than at the end of the desert phase).

Memory It was clear from observing soldiers during the administration of the task, that they had more than sufficient time (60 sec) to answer all the items on the recognition test portion of this task. In spite of this, accuracy declined over time (see Figure 7.2), $F(4, 200) = 5.16, p = .0006$. Evidently, 30 sec was not enough time to memorize all the words presented at the beginning of the task and was certainly not enough time to permit a speed-accuracy trade-off as basic memory functions were compromised by the stress of Ranger training. Accuracy on the memory task was significantly impaired at the end of the desert, mountain, and jungle phases (see Table 7.2). The last three phases were not significantly different from each other.

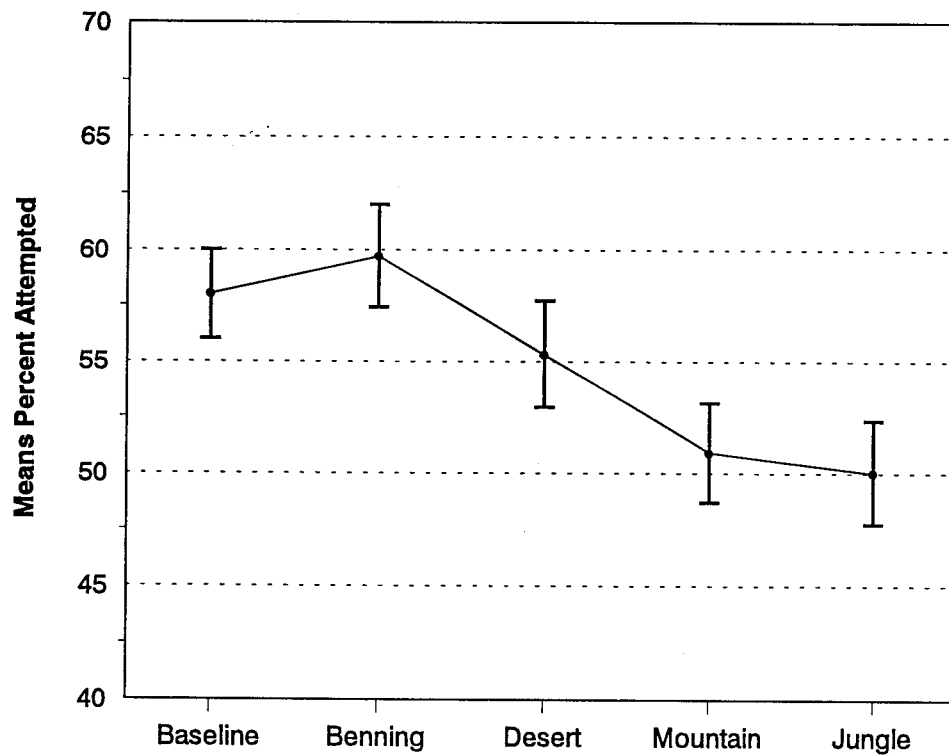
Reasoning Performance on the reasoning task also showed the traditional speed for accuracy trade-off, indicating that logic processing ability was degraded. Accuracy on the task was maintained at 80-85% across all administrations, $F(4, 200) = .889, p = .47$, while speed declined (Figure 7.3), $F(4, 200) = 9.96, p = .0001$. Speed was significantly impaired at the end of the desert, mountain, and jungle phases (see Table 7.3). Speed at the mountain and jungle phases was significantly worse than at the desert. Mountain phase and jungle phase were not significantly different from each other (see Table 7.3).

Figure 7.1 Mean Percent Attempted ($\pm$ SE) on the Decoding Task at each Phase**Table 7.1** Least Square Mean Comparisons of Cell Means across Phases for the Percent Attempted Variable on the Decoding Task (p values)

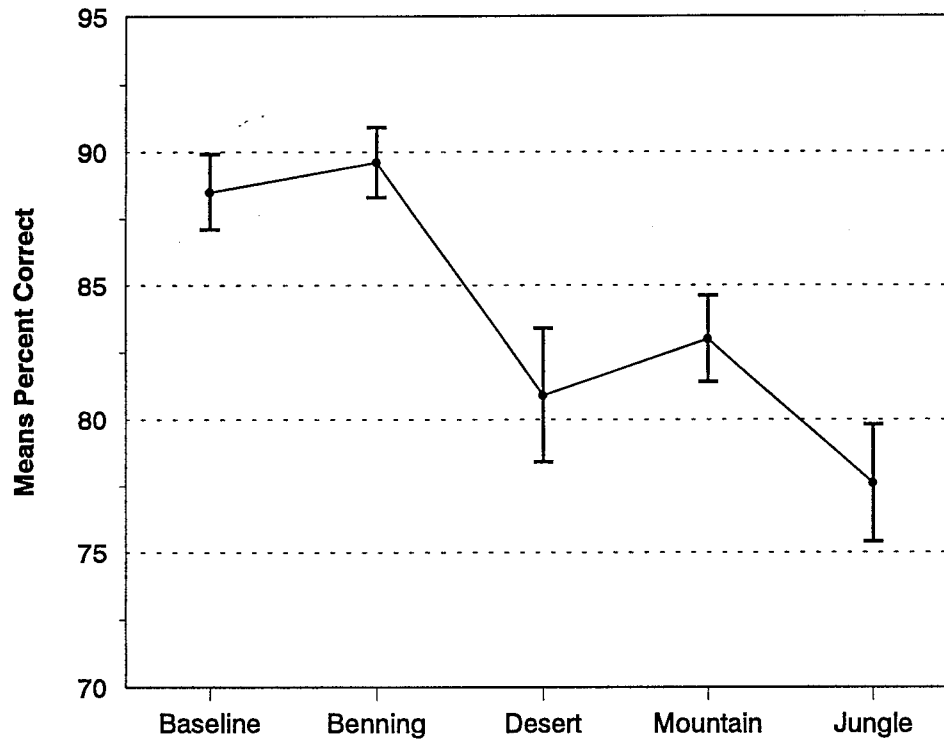
	Baseline	Benning	Desert	Mountain	Jungle
Baseline		0.1400	0.0001	0.0001	0.0001
Benning			0.0001	0.0001	0.0001
Desert			0.0001	0.0001	0.0188
Mountain					0.0001
Jungle					

Figure 7.2 Mean Percent Correct ($\pm$ SE) on the Memory Task at each Phase**Table 7.2** Least Square Mean Comparisons of Cell Means across Phases for the Percent Correct Variable on the Memory Task (p values)

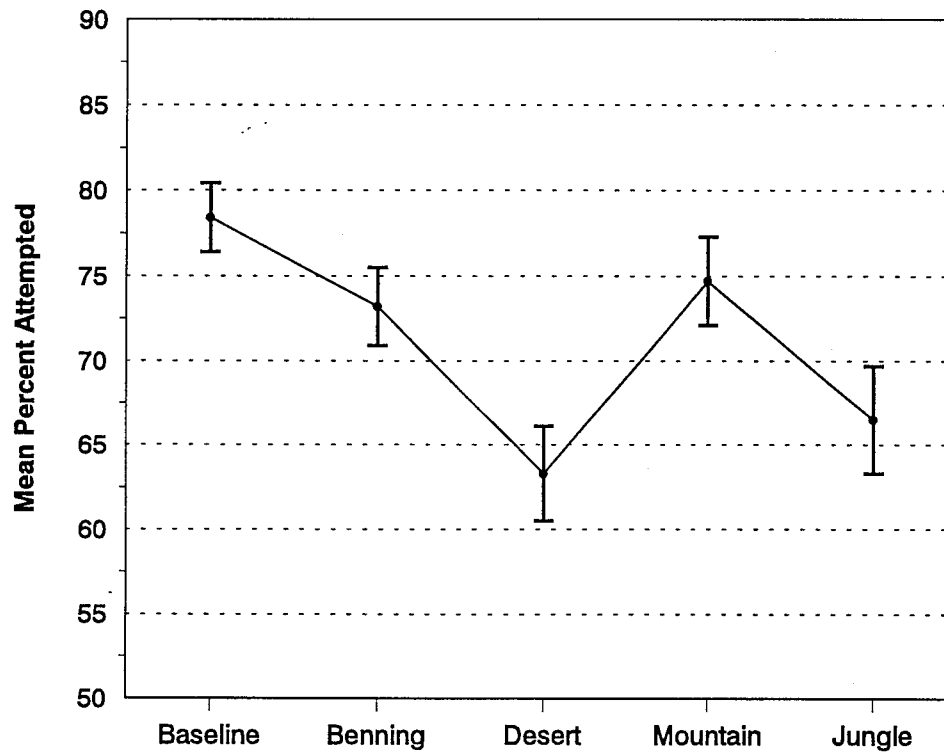
	Baseline	Benning	Desert	Mountain	Jungle
Baseline		0.7721	0.0606	0.0004	0.0561
Benning			0.0306	0.0001	0.0282
Desert				0.0820	0.9729
Mountain					0.0881
Jungle					

Figure 7.3 Mean Percent Attempted ($\pm$ SE) on the Reasoning Task at each Phase**Table 7.3** Least Square Mean Comparisons of Cell Means Across Phases for the Percent Attempted Variable on the Reasoning Task (p values)

	Baseline	Benning	Desert	Mountain	Jungle
Baseline		0.3885	0.1595	0.0003	0.0001
Benning			0.0239	0.0001	0.0001
Desert				0.0219	0.0057
Mountain					0.6295
Jungle					

Figure 7.4 Mean Percent Correct ($\pm$ SE), Pattern Recognition Task at each Phase**Table 7.4** Least Square Mean Comparisons of Cell Means across Phases for the Percent Correct Variable on the Pattern Recognition Task (p values)

	Baseline	Benning	Desert	Mountain	Jungle
Baseline		0.6195	0.0009	0.0146	0.0001
Benning			0.0001	0.0034	0.0001
Desert				0.0365	0.1382
Mountain					0.0171
Baseline					

Figure 7.5 Mean Percent Attempted ($\pm$ SE), Pattern Recognition Task each Phase**Table 7.5** Least Square Mean Comparisons of Cell Means across Phases for the Percent Attempted Variable on the Pattern Recognition Task (p values)

	Baseline	Benning	Desert	Mountain	Jungle
Baseline		0.0576	0.0001	0.1791	0.0001
Benning			0.0003	0.5752	0.0138
Desert				0.0001	0.2296
Mountain					0.0026
Jungle					

Pattern Recognition Both speed and accuracy were impaired on the pattern recognition task, $F(4, 200) = 10.29$, $p = .0001$ for the accuracy measure (Figure 7.4) and $F(4, 200) = 10.51$, $p = .0001$ for the speed measure (Figure 7.5). The data suggest that the fundamental ability to rapidly recognize an embedded figure or other degraded visual information was severely compromised during Ranger training. Accuracy was significantly impaired at the end of the desert and jungle phases (Table 7.4). Speed was similarly impaired (Table 7.5).

Intraphase Recovery Of Cognitive Performance

Decoding Although no significant recovery of function was shown on the mid-mountain test (Figure 7.6), the decline in performance was temporarily halted, $F(2,100) = 32.89$, $p = .0001$. Performance at mid-mountain did not differ from that at desert ($p = .53$) and was better than performance at the end of the mountain phase ($p = .0001$).

Memory Significant recovery of function (equal to baseline) was shown on the mid-mountain test, $F(2,100) = 7.34$, $p = .0011$ (Figure 7.7). Performance on the mid-mountain test was significantly better than performance at either the desert ($p = .0457$) or the mountain test ($p = .0002$).

Reasoning Performance on this task continued to decline during the first half of the mountain phase, $F(2, 100) = 8.56$, $p = .0004$ (Figure 7.8). Performance on the mid-mountain test was worse than at the desert test ($p = .0001$) and not different from the mountain test ($p = .09$).

Pattern Recognition Significant recovery of function was shown (Figures 7.9 and 7.10) at the mid-mountain test on the accuracy measure, $F(2,100) = 22.33$, $p = .0001$ and on the speed measure, $F(2,100) = 16.65$, $p = .0001$. Accuracy on the mid-mountain test was greater than on either the desert ($p = .0001$) or the mountain test ($p = .0001$). However, on the speed measure, performance at mid-mountain was greater than at desert ($p = .0001$) and the recovery was maintained for the mountain test ($p = .37$).

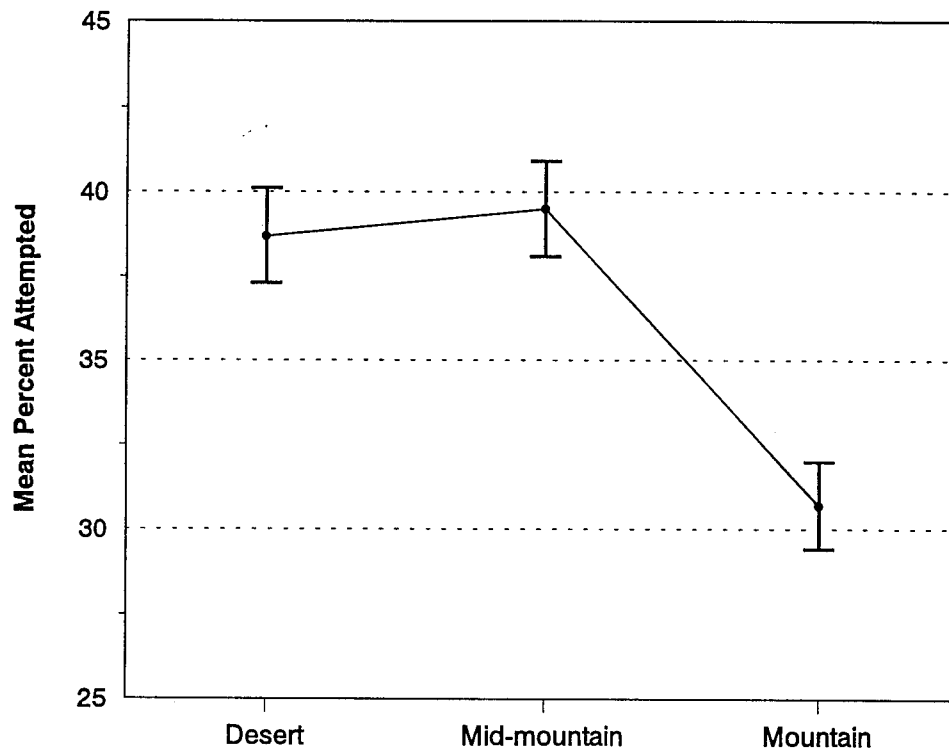
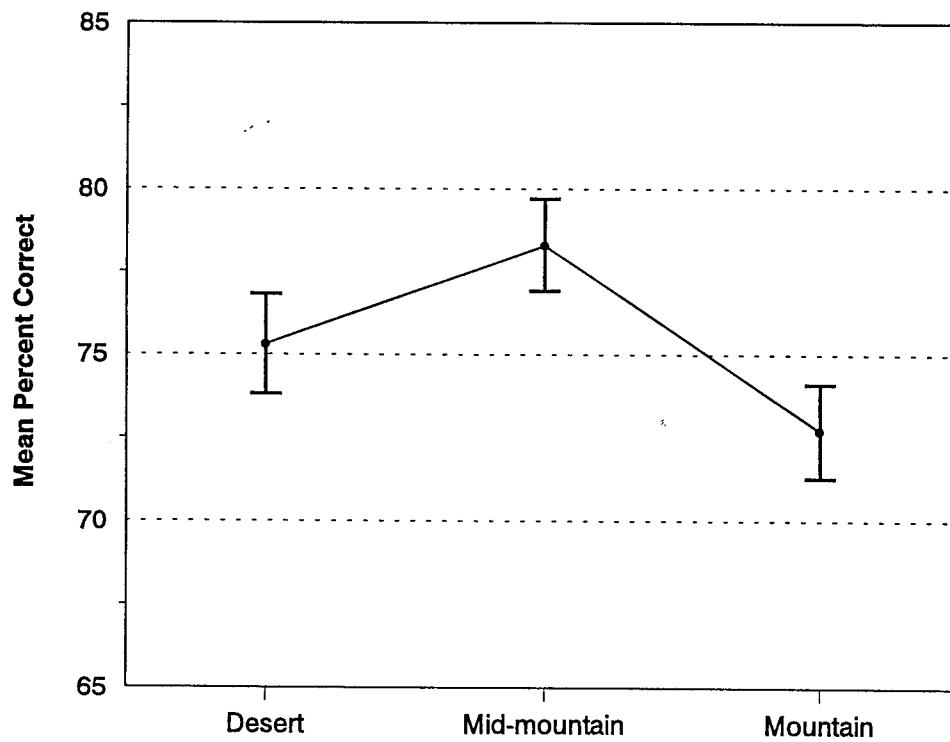
Figure 7.6 Mean Percent Attempted ($\pm$ SE) on the Decoding Task, Mid-Mt Phase**Figure 7.7** Mean Percent Correct ($\pm$ SE) on the Memory Task, Mid-Mt Phase

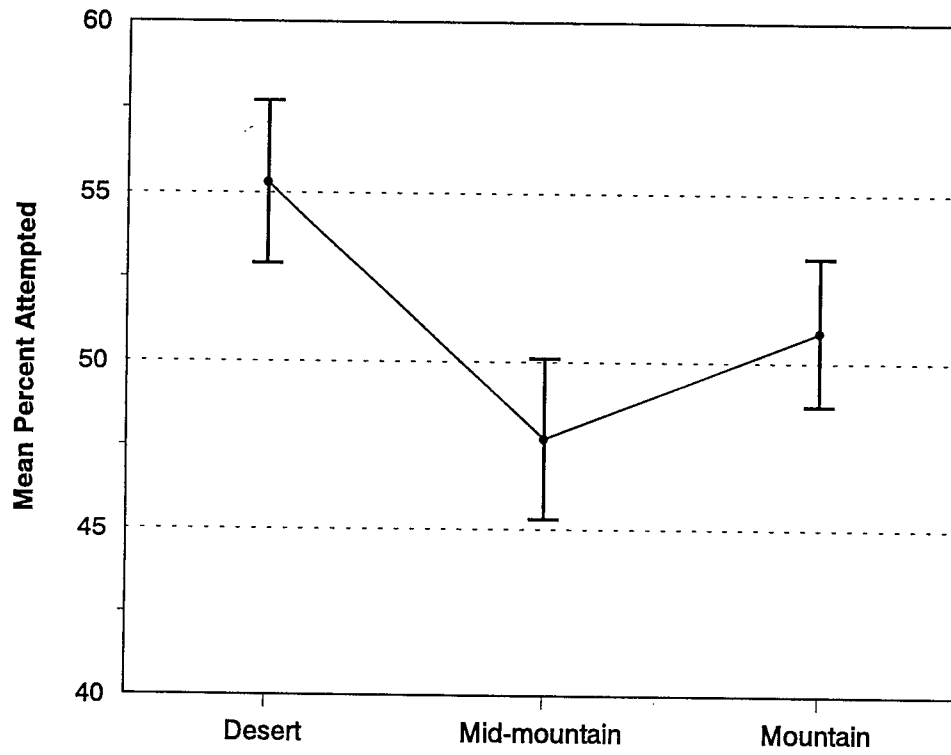
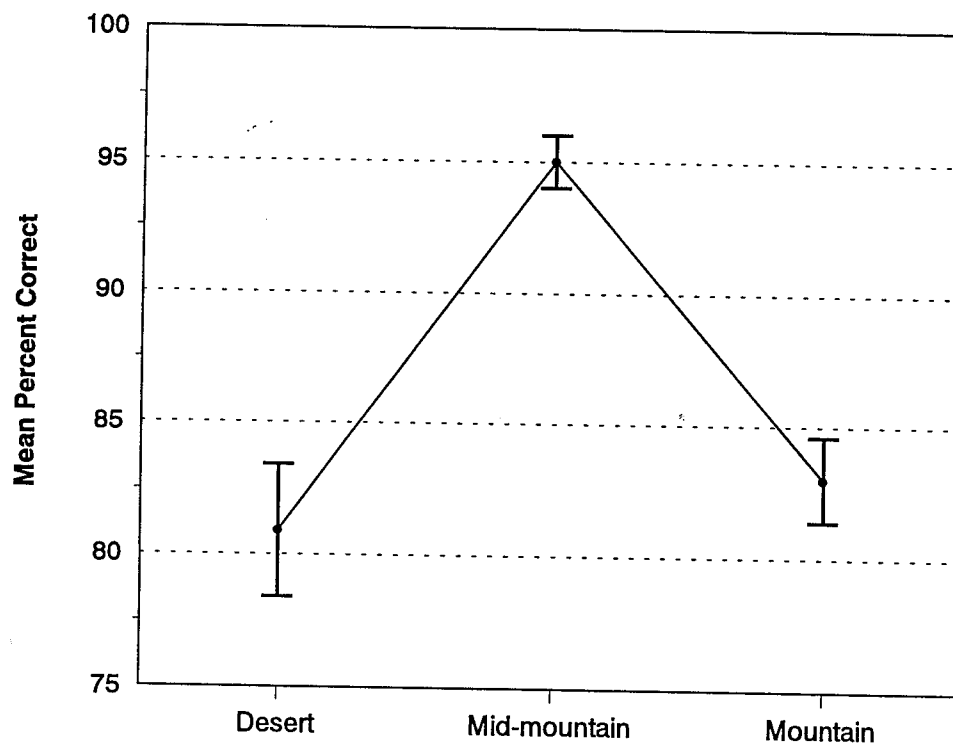
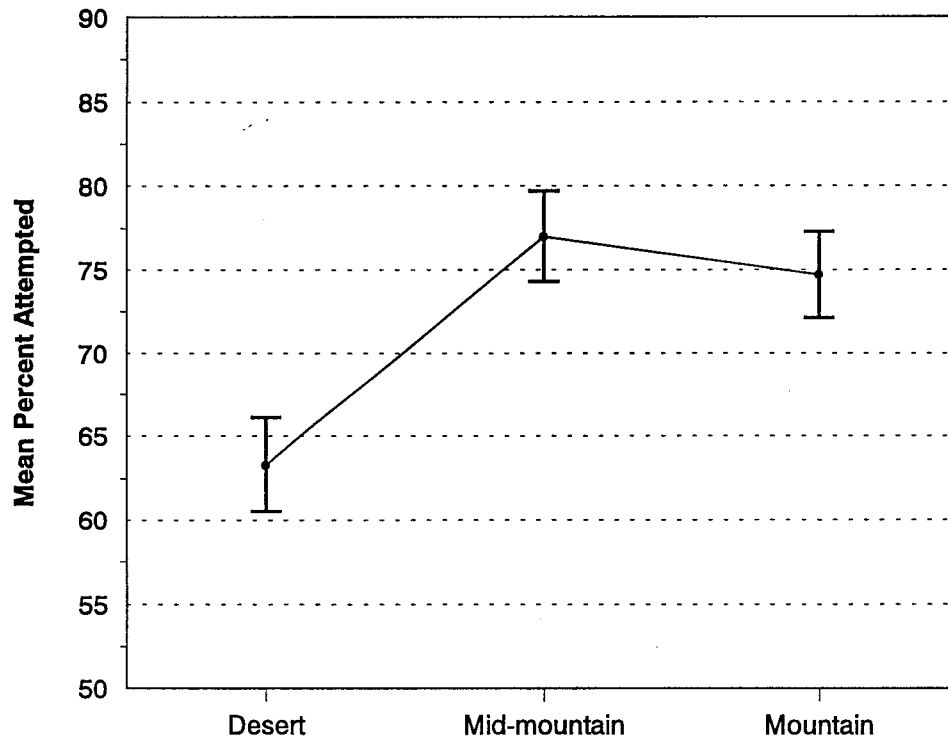
Figure 7.8 Mean Percent Attempted ($\pm$ SE), Reasoning Task, Mid-Mt Phase**Figure 7.9** Mean Percent Correct ($\pm$ SE), Pattern Recognition Task, Mid-Mt Phase

Figure 7.10 Mean Percent Attempted ($\pm$ SE), Pattern Recognition Task, Mid-Mt Phase



CONCLUSIONS

At the end of the Benning phase there was no significant change in performance on any task. This finding is consistent with previous research demonstrating that weight losses of less than 6% are not associated with any impairment in cognitive performance. This can not be attributed to a lack of stress in the first phase of the course. Attrition rates were quite high in this phase as soldiers adjusted to the physical and emotional stress of Ranger training. The most likely explanation is that soldiers were able to compensate by drawing on their mental and physical reserves. This conclusion is supported by the physiological and immunological data collected at the end of the Benning phase in an earlier study of Ranger training (Moore et al., 1992).

Average speed on the decoding task (the task where speed was the most critical) was 8% to 16% less than baseline at the end of the desert, mountain, and jungle phases, while average performance on the reasoning speed parameter was 3% to 8% less than baseline at those phases. The fact that baseline levels of accuracy were maintained beyond the end of the Benning phase on the decoding and reasoning tasks is evidence that the "finishers" who made up this group were highly motivated, willing to work hard to sustain performance under duress. On the memory and pattern recognition tests, where the time allotted to the task prevented an effective speed-accuracy trade-off, average accuracy at the end of the desert, mountain, and jungle phases was 3% to 10% less than baseline. The performance deficits on these four tasks are small on an absolute scale, but constitute a 10% to 34% decline in relative performance (late-phase performance / baseline performance x 100). Deficits that large on fundamental cognitive functions are indicative of high levels of stress.

There was evidence of recovery of function at the mid-mountain phase (and at the end of jungle phase) associated with increases in sleep and food allotments. Recovery was most obvious on the pattern recognition task, a task on which no speed-accuracy trade-off occurred, and not apparent on the reasoning task, a task on which a speed-accuracy trade-off was used effectively. It is clear that this recovery was short-lived and was typically insufficient to prevent a further cumulative decline when restrictions on food and sleep were re-instituted. Again this supports the conclusion that soldiers had no mental or physical reserves on which to draw during the second half of the course.

These data should be put into perspective. Observations of Ranger cadre about the average performance of a class during the final phase of the course will not match those reported here. The data in this study are from soldiers who had endured the stress of Ranger training for eight weeks. They formed only 25% of the soldiers in the course. All other students in the final phase of RGR-II were "recycles," who at that point had endured only two to five continuous weeks of Ranger training. "Recycles" are not likely to show deficits in cognitive performance, just as the "finishers" of RGR-II showed no deficits early in the course.

In summary, although there were some differences in the pattern of impairment across the four tasks used, the degree of impairment indicated a pervasive decline in cognitive function. Despite the fact that these data represent a worst-case scenario, Ranger cadre should be encouraged to consider the trade off between the level of stress and the loss of cognitive function, learning and retention. These brief respites will not substantially alter the overall experience of sustained stress that characterizes Ranger training, but will provide an increased margin of safety and training effectiveness.

CHAPTER 8

CLINICAL CHEMISTRIES AND BIOCHEMICAL MARKERS OF NUTRITION

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INTRODUCTION

Despite the severe caloric deprivation with concomitant loss of body weight and body fat documented in RGR-I, no vitamin or mineral deficiencies were shown, regardless of whether the students were using vitamin/mineral supplements. Although the subjects in RGR-I lost an average of 4.6 kg of fat-free mass (FFM) along with increases in indicators of protein catabolism (i.e., increased urea nitrogen), all indicators of protein nutrition were within normal ranges.

All the measures of nutritional status measured in RGR-I were also assessed in RGR-II. One improvement in the clinical chemistry analysis in RGR-II was our ability to perform a complete blood count (CBC) analysis at the field laboratory test sites. Prior to the initiation of the study, a Coulter Counter was purchased. The CBC analyses for RGR-I were performed in the various military hospital laboratories at each of the training sites. Performing the CBC analysis at the test site on the same piece of equipment provided more accurate and precise blood cell analyses.

METHODS

Blood Samples

Blood samples were collected from each subject at the time periods shown in Table 2.2. On the days prior to the collection of blood samples, soldiers were reminded that no food or fluid (except plain water) was permitted after 2100 hours the evening preceding the blood draws, giving at least an 8 hour fast prior to the blood draw. These instructions were followed for all the blood draws except the desert phase. The blood draw at the end of desert phase was not a fasted sample.

Subjects were asked to drink one liter of water prior to sleep the night before each blood draw in order to help assure euhydration. Our personal observation was that water discipline is well enforced by the Ranger Instructors.

Five vacuum tubes were drawn on each subject as shown in Table 8.1. Immediately following the separate blood draws, 2 ml blood samples were obtained as shown in Table 8.1. Serum (or plasma) and cells that were separated were frozen until shipment on dry ice to the various analytical laboratories.

Listed below is a general outline of the blood analyses performed on the blood samples taken from each tube.

Tube 1: Serum was obtained by centrifugation (1200 x g, 15 min, 10°C). The analyses listed in Table 8.2 were performed on serum samples. These biochemical analyses were performed by Pennington Biomedical Research Center, Baton Rouge, LA (PENN).

Tube 2: Hematology measurements were determined on location using a Coulter JT Blood Analyzer (Coulter, P.O. Box 2145, Hialeah, Florida 33012). This analyzer determined hemoglobin, mean packed volume, mean corpuscular volume, white cell count, red cell count, platelet count, %lymphocytes, %monocytes and %granulocytes (CBC). After the CBC was completed, plasma and red cells were

separated by centrifugation (1200 x g, 15 min, 10°C). The red blood cells (RBC) were washed three times in physiological saline. Analyses shown in Tables 8.3 and 8.4 were performed by PENN.

Tube 3: Blood collected in this tube was transported to the USDA, Vitamin and Mineral Nutrition Laboratory, Beltsville Agriculture Research Center, Beltsville, MD (USDA). Whole blood samples were obtained and incubated with immunofluorescence monoclonal antibodies as described in Chapter 9. Preparation of whole blood cultures for lymphocyte blastogenesis and cytokine secretion was conducted (Kramer et al., 1990). Measurement of T-lymphocyte soluble IL-2 receptors, T-lymphocyte and monocyte secreted IL-2 and IL-6 and concentrations of plasma IL-2 and IL-6 were performed using commercially prepared and standardized ELISA kits. Details of the methodology are given in Chapter 9. An aliquot of plasma was obtained from this tube for copper and zinc analyses by atomic absorption spectrophotometry as described below.

Tube 4: Endocrine markers were determined by the Occupational Health and Performance biochemical laboratory at USARIEM using radioligand assay procedures. Testosterone, cortisol, thyroxine, triiodothyronine, thyroid binding globulin, thyroid stimulating hormone, growth hormone, sex hormone binding globulin (SHBG), and immunoreactive LH were measured by direct radioimmunoassay or immunoradiometric assays. Insulin-like growth factor one (IGF1) was measured by radioimmunoassay after extraction procedures. All of these measurements were performed using 5 mls of serum. The details of the methodology are outlined in Chapter 5.

Tube 5: Whole blood samples were obtained and used for analyses of monocyte and granulocyte function as described in Chapter 10.

Table 8.1 Blood Sample Processing Table

Tube	Aliquot	Analysis Lab	Analyses
Tube 1. 15 ml Red Top	2 ml serum 2 ml serum 2 ml serum	Pennington Labs Pennington Labs Pennington Labs	See Table 8.2 Transferrin, Prealbumin Retinol Binding Protein Vit C
Tube 2. 7 ml Purple Top/w EDTA	1 ml whole blood 2 ml RBC 2 ml plasma	USARIEM Pennington Labs Pennington Labs	B ₁₂ , Folate CBC RBC Transketolase RBC Glutathione Reductase RBC Glutamate Oxaloacetate Transaminase Glycerol, NEFA ¹ , AST ² , ALT ³ Ferritin, Vit D, RBC Folate
Tube 3. 15 ml Blue top/w heparin (Trace Element Free)	2 ml whole blood 2 ml plasma 2 ml whole blood	USDA Beltsville USDA Beltsville USDA Beltsville	Immune Function Zn, Cu Flowcytometry
Tube 4. 15 ml Red Top	2 ml serum 2 ml serum 2 ml serum	USARIEM USARIEM USARIEM	IGF1, Testosterone, Cortisol T3, T4, TSH, TBG LH, SHBG, GH
Tube 5. 7 ml PurpleTop/w EDTA	3 ml whole blood 4 ml whole blood	WRAIR WRAIR	Monocyte Function Granulocyte Function

¹ Non-esterified Fatty Acids ² Aspartate Aminotransferase ³ Alanine Aminotransferase

Table 8.2 Serum Analyses

<u>Test</u>	<u>Method</u>	<u>Wavelength</u>
Albumin	bromocresol purple	600 nm
Blood Urea Nitrogen	urease/glutamate dehydrogenase	340 nm
Calcium (total)	arsenazo III	650 nm
Ceruloplasmin	nephelometry	
Chloride	ion specific electrode	
Cholesterol	chol est/chol ox/peroxidase	520 nm
CO ₂	ion specific electrode	
GGT ⁽¹⁾	gamma glutamyl p-nitroaniline	410 nm
Glucose	hexokinase/G6PDH	340 nm
HDL Cholesterol	(see note 1.)	
β-Hydroxybutyrate	β-hydroxybutyrate dehydrogenase	340 nm
Immunoglobulins, (IgA, IgG, IgM)	turbidimetry	
Iron	ferrozine	560 nm
LDH ⁽²⁾	lactate to pyruvate	340 nm
Magnesium	calmagite	520 nm
Phosphorus	molybdate	520 nm
Potassium	ion specific electrode	
Pre Albumin	nephelometry	
Sodium	ion specific electrode	
Total Bilirubin	modified Jendrassik-Groff	560 nm
Total Iron Binding	MgCO ₃ /ferrozine	560 nm
Total Protein	biuret	560 nm
Transferrin	nephelometry	
Triglycerides	lipase/glycerol kinase/G1PDH	520 nm
Uric Acid	uricase/peroxidase	520 nm

note 1. HDL-cholesterol was determined after dextran sulfate (MW 50,000) precipitation with DMA reagent and analyzed for cholesterol.

⁽¹⁾Gamma Glutamyl Transferase ⁽²⁾Lactic Dehydrogenase

General Chemistry Analyses

Table 8.2 and Table 8.3 show the analyses performed on serum and plasma, respectively. Analyses were performed using a Beckman Synchron CX5 automated chemistry analyzer (Beckman Instruments, Fullerton, CA) with manufacturer recommended reagents. All the chemistries were performed concurrently with approved quality control assurance procedures using BioRad unassayed chemistry controls and monthly comparisons made in an interlaboratory quality assurance program. The laboratory (PENN) is a participant of the College of American Pathologists Survey Program.

Table 8.3 Plasma Analyses

<u>Test</u>	<u>Method</u>	<u>Wavelength</u>
ALT ¹	aspartic acid/ α -keto glutaric acid/malate dehydr	340 nm
AST ²	alanine/ α -keto glutaric acid/ LDH	340 nm
Glycerol	glycerokinase/pyruvate kinase/LDH (Sigma)	
Immuno-globulin (IgE)	turbidimetry	
Lactate	lactate dehydrogenase (Sigma)	
NEFAs ³	acyl CoA synthase/oxidase/peroxidase (Wako)	560 nm

¹Alanine Aminotransferase ²Aspartate Aminotransferase ³Non-esterified Fatty Acids

Markers of Protein Metabolism

Serum transferrin, ferritin, prealbumin, and retinol binding protein were determined using IRMA kits (BioRad) with standards prepared against the 1st International Standard.

Markers of Vitamin Status

Vitamin B₁₂ and folate were measured in EDTA plasma using a combined radioreceptor assay (BioRad). The procedure used a boiling step to eliminate interferences by binding with intrinsic proteins, and ⁵⁷Co and ¹²⁵I were counted following competition for binding with intrinsic factor (Vitamin B₁₂ or binding proteins (folate). The same method was used for determination of RBC folate on samples treated with ascorbic acid at the time of collection.

Vitamin B₁ (Thiamin), B₂ (Riboflavin) and B₆ (Pyridoxal) were assayed indirectly by stimulation of appropriate enzymes in RBC hemolysate (Table 8.4). The assays were based on methods developed by Bayoumi (1976) and Vuilleumier (1990) and adapted to the Beckman Synchron CX5. Percent stimulation was determined as:

$$100 \times \frac{\text{activity with vitamin} - \text{activity without vitamin}}{\text{activity without vitamin}}$$

The hemolysates were produced from red blood cells collected from EDTA treated blood. The RBC were washed twice with normal saline and frozen until analysis. Samples were thawed and hemolyzed by adding 2 mls of cold deionized water per 500 ul of RBC. Hemoglobin was determined on the hemolysate and samples were then diluted with deionized water to achieve a final hemoglobin concentration of approximately 1 g/dL.

Table 8.4 Vitamin Status Markers

<u>Vitamins</u>	<u>Assay Method</u>
Pyridoxal	in vitro stimulation of erythrocyte glutamate oxaloacetate transaminase activity by pyridoxal-5'-phosphate
Riboflavin	in vitro stimulation of erythrocyte glutathione reductase activity by flavin adenine dinucleotide
Thiamin	in vitro stimulation of erythrocyte transketolase activity by thiamin pyrophosphate

Using an IA kit (Incstar Corporation) with acetonitrile extraction, 25-hydroxy-vitamin D in serum was determined in duplicate. Specificity of the antibody was equal for the D2 and D3 forms; cross-reactivity for 1,25 dihydroxy compounds and cholesterol were 5% and 1%, respectively.

Markers of Trace Element Status

Aliquots of plasma were obtained from Tube 3 shown in Table 8.1 and analyzed for copper and zinc concentration. This tube was an acid washed, trace element free vacutainer tube. Three hundred microliters of thawed plasma were mixed with 600 ul of 20% trichloroacetic acid solution, in triplicate. After vortexing, the samples were incubated at 96° for 45 minutes. Samples were centrifuged and the resulting supernatant was analyzed by atomic absorption spectrophotometry.

Normal Limits

Appendix C shows the normal limits, reporting units and reference for all the blood analyses performed for this study.

Statistical Analyses

Statistical analyses of the data were performed using the SAS software (SAS Institute, Inc., SAS Campus Drive, Cary, NC 27513). When the F values from the ANOVA analysis were found to be significant at $P < 0.05$, a Dunnett's Test (Dunnett, 1954) was used to determine which means were significantly different from baseline values. Data contained in tables are mean values ± 1 standard deviation.

RESULTS

Although there were some statistically significant differences in the serum electrolyte and macro-mineral concentrations (Table 8.5), all mean values were within normal reference ranges. Mean serum albumin, creatinine, glucose, total protein and uric acid remained within the normal reference ranges during the course of the study (Table 8.6). Normal albumin and total protein concentrations suggest that there was not a severe inadequate protein intake during the study. This is further supported by normal concentrations of more sensitive indicators of protein nutritional status: prealbumin, and retinol binding protein. However, the elevated blood urea nitrogen concentrations suggest higher than normal level of protein catabolism. This is supported by the body composition data that show a mean loss of 9 lbs of fat free mass over the 8 weeks of training.

Nonesterified fatty acids and B-hydroxybutyrate were shown to increase above their normal reference ranges as the study progressed (Table 8.6). These values are in agreement with the observed decrease of body fat from a mean level of 14% to 6% over the 8 weeks and the estimated energy deficits shown in Figure 4.4. Increased fatty acid mobilization was reflected by these markers and reached the highest levels during the end of the mountain phase, corresponding to the period of highest energy deficit. It is of interest that these markers of metabolism responded to decreased energy deficit during the mid-mountain phase (Figure 8.1).

Table 8.5 Indices of Electrolyte & Macro-Minerals Status

Item	Ref. Value	Unit	Baseline	Desert	Mid- Mountain	Mountain	Jungle
n			51	48	48	45	49
Ca	2.1- 2.6	mmol/L	2.5± 0.1	2.4*± 0.1	2.6*± 0.1	2.6*± 0.1	2.5± 0.0
Cl	101- 111	mmol/L	105.4± 2.1	107.8*± 2.4	104.6± 0.9	101.7*± 2.2	102.3*± 2.5
K	3.6- 5.0	mmol/L	4.1± 0.3	4.1± 0.3	4.4*± 0.3	4.3*± 0.4	4.4*± 0.5
Mg	0.7- 1.0	mmol/L	1.0± 0.0	1.0*± 0.0	1.0*± 0.1	1.0*± 0.1	0.9*± 0.0
Na	135- 145	mmol/L	138.9± 1.6	141.4*± 1.8	140.6*± 1.2	138.3± 0.1	138.8± 1.8
TCO2	21- 31	mmol/L	27.6± 2.2	25.5*± 1.7	28.8*± 0.9	27.4± 1.5	31.0*± 1.9

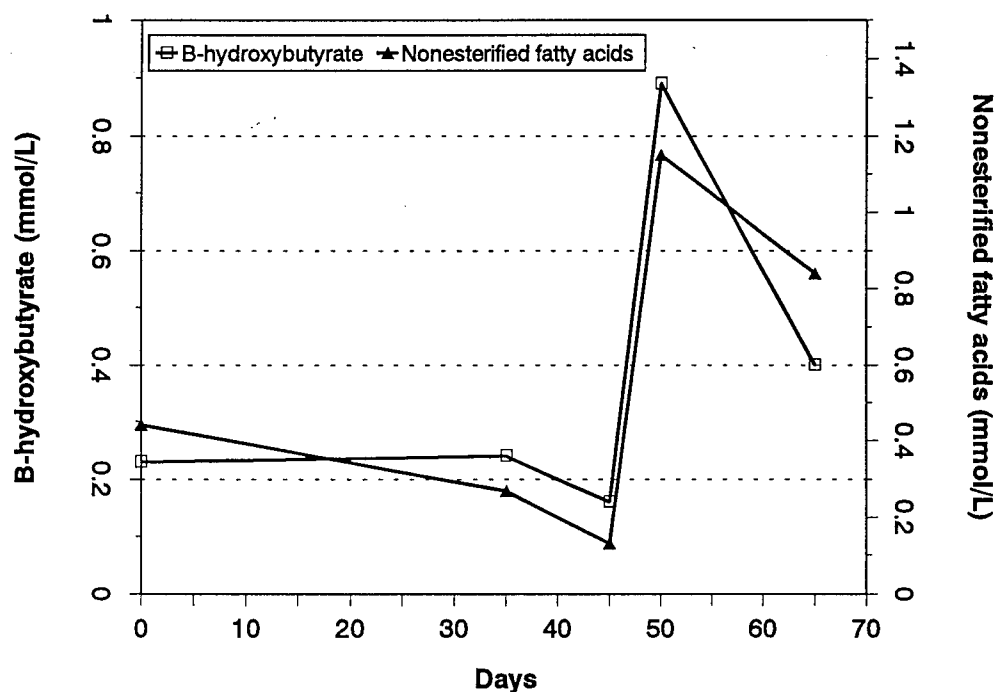
*Significantly different from baseline at P<0.05.

Table 8.6 Indices of Energy & Nitrogen Metabolism

Item	Ref.	Unit	Baseline	Desert	Mid-Mt.	Mountain	Jungle
n			51	48	48	45	49
Albumin	3.2- 5.5	g/L	4.6± 0.3	4.7± 0.3	4.4*± 0.3	4.9±* 0.4	4.9*± 0.4
βhydroxy- butyrate	0.00- 0.42	μmol/L	0.23± 0.13	0.24± 0.11	0.16± 0.08	0.89*± 0.50	0.40*± 0.14
Cerulo- plasmin	1.57- 3.96	μmol/L	1.89± 0.23	2.09*± 0.27	1.95± 0.27	2.31*± 0.31	2.20*± 0.38
Creatinine	53- 115	μmol/L	104± 11	109± 11	100± 10	102± 12	106± 12
Glucose	3.9- 5.8	mmol/L	4.3± 0.6	4.4± 1.2	4.6± 0.3	4.4± 0.7	4.7*± 0.6
Glycerol	61- 232	μmol/L	110.4± 51.0	122.8± 75.8	60.3± 18.2	144.8± 76.4	125.4± 40.9
Lactate	0.3- 1.3	mmol/L	2.3± 0.6	3.2*± 0.7	3.1± 0.5	2.8*± 0.6	2.6*± 0.5
Nonester- ified fatty acids	0.10- 0.60	mmol/L	0.4± 0.2	0.3*± 0.2	0.1*± 0.1	1.2*± 0.4	0.8*± 0.3
Prealbumin	17-42	mg/dL	25.9± 3.8	19.9*± 2.6	28.6*± 3.3	19.6*± 3.1	21.5*± 3.2
Retinol Binding Protein	3.0- 6.0	mg/dL	4.6± 0.7	3.7*± 0.4	4.9*± 0.5	3.6*± 0.4	3.7*± 0.5
Total Protein	6.7- 8.2	g/L	7.1± 0.5	7.4*± 0.4	7.1± 0.4	7.8*± 0.6	7.8*± 0.4
Urea Nitrogen	5.0- 12.9	mmol/L	12.9± 2.4	15.6*± 2.9	14.4*± 1.9	14.4*± 3.6	13.8± 2.4
Uric Acid	0.15- 0.43	mmol/L	0.4± 0.1	0.4*± 0.1	0.3*± 0.0	0.4*± 0.1	0.5*± 0.0

*Significantly different from baseline at P<0.05.

Figure 8.1 Changes in Serum Concentrations of Indicators of Fatty Acid Metabolism



Serum lactate concentrations were elevated above normal reference range and remained elevated throughout the study. Lactate is the end product of anaerobic glycolysis and was once believed to reflect oxygen deficiency in muscle. It is now known that well oxygenated muscles can actually produce large amounts of lactate (Holloszy, 1988). The elevated levels of serum lactate at all time points measured reflect the prolonged and intense energy expenditure demonstrated by the doubly-labeled water method. Lactic acid levels will vary dramatically with physical activity and can easily rise within 4 to 6 mmol/L with moderate physical activity. Therefore, although the levels shown in Table 8.6 are above Pennington's normal reference range, the levels are not severely elevated for the activity level of Ranger students.

Table 8.7 shows the changes in concentrations of four serum enzymes measured during the study. ALT, AST and GGT are ubiquitous enzymes which are involved in protein metabolism. The highest concentrations of these enzymes are found in liver and muscle. While ALT and GGT remained within normal reference ranges, AST increased significantly above the normal upper limit during the end of the mountain phase. This increase is most likely related to the changes in free fat mass. The AST concentration decreased significantly to baseline values near the end in response to the decreased energy deficit, mid-mountain phase. LDH is an enzyme that catalyzes the interconversion between lactic and pyruvic acids. The enzyme is found at highest concentrations in the liver and muscle tissue. The increased serum levels shown in Table 8.7 correspond to the increase in serum lactate discussed above. The progressive increase in serum cholesterol (Table 8.8) is most likely a consequence of the decrease in thyroid hormone shown in the endocrine analyses.

Indicators of iron metabolism are shown in Table 8.9 and are displayed graphically in Figure 8.2. The data indicate that the training did not adversely impact iron metabolism. The decrease in serum iron early in the training was most likely due to iron redistribution which occurred as a result of the acute phase response to the stress of the training.

Changes in plasma zinc and copper concentrations are shown in Figure 8.3. Both zinc and copper concentrations increased significantly in the last two phases of the study. The increase in copper along with the copper binding protein, ceruloplasmin, would be expected for an acute phase response to the stress of the training. However, during an acute phase response, plasma zinc concentrations usually decrease (Cousins, 1986). Metallothionein, a zinc binding protein, has been shown to increase in the liver during an acute phase response, causing sequestering of zinc in the liver. A possible source of the increased plasma zinc could have resulted from muscle catabolism. However, there was no statistically significant correlation between plasma zinc increase and fat free mass decrease ($r^2 < .10$). The observed increase in plasma zinc is particularly interesting considering that the observed increase is after a period of low zinc intake (Table 8.10).

Table 8.7 Enzymic Markers of Hepatobiliary & Muscle Metabolism

Item	Ref. Value	Unit	Baseline	Desert	Mid- Mountain	Mountain	Jungle
n			51 (50)	48 (48)	48 (49)	45 (46)	49 (50)
ALT ¹	10-60	IU/L	16.9 ±6.3	23.9* ±4.9	36.3 ±24.9	38.8 ±14.9	32.5 ±10.6
AST ²	10-42	IU/L	35.2 ±14.6	39.7 ±10.3	36.4 ±17.0	54.5 ±25.6	41.3 ±15.6
Bilirubin	3.4- 17.1	µmol/L	20.6 ±13.8	17.1* ±8.6	22.5* ±10.4	17.3* ±5.8	21.2 ±8.0
GGT ³	7-64	IU/L	14.8 ±4.2	14.3 ±2.6	18.9 ±4.5	17.2 ±4.1	15.5 ±3.3
LDH ⁴	91- 180	IU/L	180.0 ±39.0	230.6* ±31.4	200.6 ±34.5	243.6* ±58.0	236.0* ±52.1

¹Alanine Aminotransferase ²Aspartate Aminotransferase ³Gamma Glutamyl Transferase ⁴Lactic Dehydrogenase *Significantly different from baseline at P<0.05.

Table 8.8 Indices of Cholesterol Metabolism

Item	Ref. Value	Units	Baseline	Desert	Mid- Mountain	Mountain	Jungle
n			51	48	48	45	49
Cholesterol	3.6- 5.2	mmol/L	4.1± 0.7	4.2± 0.7	4.7*± 0.6	4.5*± 0.8	5.1*± 0.9
HDL-cho.	0.7- 1.7	mmol/L	1.2± 0.2	1.6*± 0.3	1.7*± 0.4	2.1*± 0.4	2.1*± 0.4
Triglyceride	40- 150	mg/dL	83.6± 20.6	111.4*± 22.0	100.6*± 29.9	52.1*± 5.8	57.4*± 7.8

*Significantly different from baseline at P<0.05.

Figure 8.2 Hematology and Iron Status

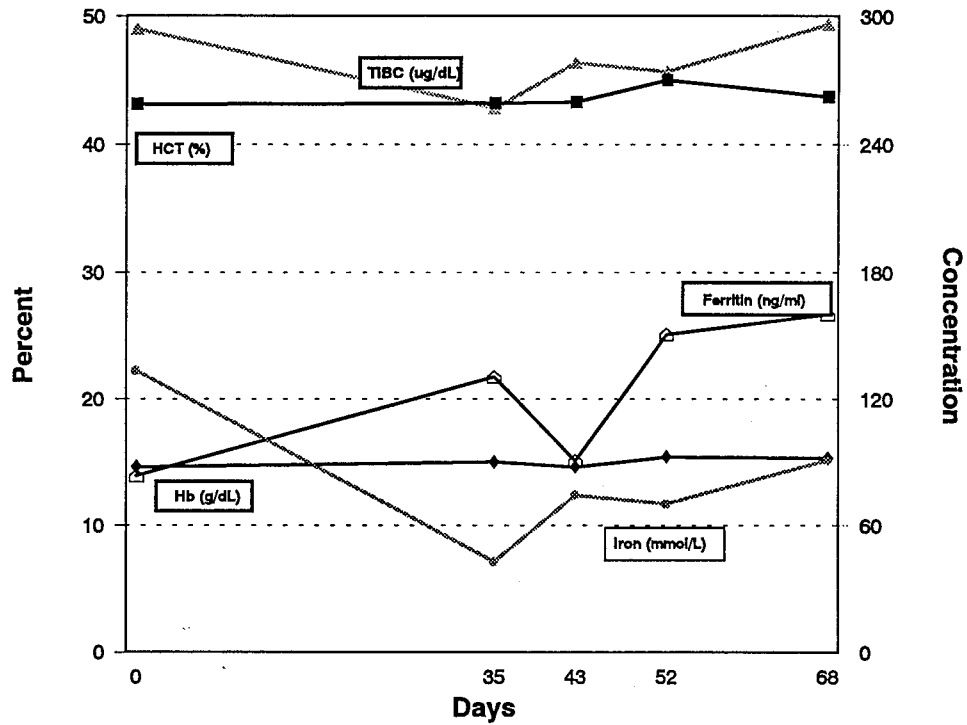


Figure 8.3 Plasma Zinc, Copper, and Ceruloplasmin

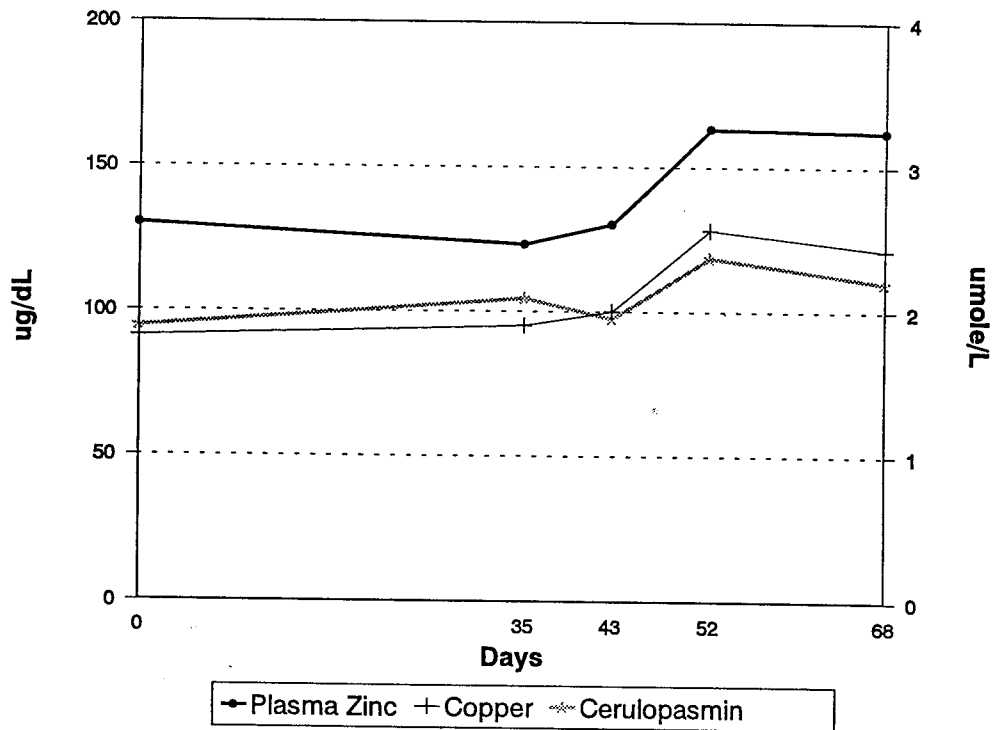


Table 8.9 Indices of Iron Metabolism

Item	Ref. Value	Unit	Baseline	Desert	Mid- Mountain	Mountain	Jungle
n			51	48	48	45	49
Ferritin	22.0- 447	ng/ml	83.0± 47.0	130*± 58	55± 91	150*± 70	167*± 68
Hb	12.1- 17.2	g/L	14.6± 0.2	15.0± 0.1	14.6± 0.1	15.4*± 0.1	15.3*± 0.1
Hct	36.1- 50.3	%	43.1± 0.5	43.3± 0.4	43.3± 0.4	45.1*± 0.4	43.7± 0.3
Iron	9.0- 29.0	μmol/L	24.0± 6.0	7.6*± 2.3	13.0*± 5.5	12.5*± 4.1	16.3*± 5.6
MCH	27.6- 33.3	pg	30.4± 0.1	31.1*± 0.2	30.8± 0.3	30.8± 0.1	31.4*± 0.14
MCV	82.2- 97.4	fL	89.6± 0.4	89.9± 0.4	90.6± 0.4	90.2± 0.41	89.5± 0.3
RBC	3.9- 5.7	×10 ¹² /L	4.8± 0.4	4.8± 0.3	4.8± 0.3	5.0*± 0.3	4.9± 0.3
TIBC	54.0- 75.0	μmol/L	53.0± 7.0	46.0*± 6.0	50.0± 5.0	49.0*± 6.0	53.0± 5.0
Trans- ferrin	32.1- 54.6	μmol/L	37.3± 4.2	35.4± 4.8	37.4± 4.0	36.8± 5.3	37.5± 3.8

*Significantly different from baseline at P<0.05.

Table 8.10 Estimated Daily Trace Mineral Intake by Phase

Phase	Copper, mg	Iron, mg	Zinc, mg
Benning	2.0	20.4	13.3
Desert	2.2	19.9	15.2
Mid-mountain	2.6	33.1	23.6
Mountain	1.0	12.4	9.2
Jungle	1.3	15.3	12.4
MRDA ¹	1.5-3.0 (RDA)	18.0	15.0

¹ Military Recommended Dietary Allowance

Indices of vitamin status are presented in Table 8.11. The mean values were calculated from all 51 subjects that completed the study. A survey taken at the start of the training showed that 31 of the 51 subjects said they planned on using vitamin supplements during the 8 weeks of training. We did not survey for compliance at the end of the study. The results from a 2x5 (vitamin use x time) ANOVA showed no significant difference between those that stated they planned to use vitamin supplements and those that said they did not plan to use supplements (Vit A: $P=.99$, Vit C: $P=.65$, Vit D: $P=.98$, Riboflavin: $P=.39$, Thiamin: $P=.82$, Vit B₆: $P=.96$, Vit B₁₂: $P=.54$, Folate: $P=.85$). All the indices of vitamin status were significant for time, $P<0.01$. Further statistical analyses of the vitamin assessment data was completed on all 51 subjects.

Despite some statistically significant differences during the training, all indices of vitamin status were within normal reference values with the exception of vitamin C. The elevated concentration of vitamin C, particularly at the end of the jungle phase, is most likely due to the high concentration of vitamin C in some items of the MRE. Values below the normal reference ranges were determined to indicate possible individual vitamin E deficiencies that may have been masked when mean values were evaluated. Only four values for vitamin C were outside normal reference ranges at the baseline measurements. However, these values were in the normal

Table 8.11 Indices of Vitamin Status

Serum/ Plasma	Ref Value	Units	Baseline	Desert	Mid- mountain	Mountain	Jungle
Vit B ₁	<23	% stimu	20± 9	17± 7	11± 5	18± 7	18± 6
Vit B ₂	<76	% stimu	6±5	6±5	7±5	10*± 5	11*± 7
Vit B ₆	<130	% stimu	91± 18	87± 17	86± 15	83± 16	77*± 13
Vit B ₁₂	171- 840	pmol	334± 112	419± 118	354± 90	524*± 170	528*± 165
Vit C	28-85	umol/L	71± 29	84*± 19	71± 20	72± 21	158*± 16
Vit D 25-OH-D3	25- 126	nmol/L	99± 16	97± 3	91± 18	118*± 24	110*± 20
Serum Folate	4.6- 36.6	nmol/L	13.4± 6.7	13.1± 4.4	13.0± 5.1	19.3± 6.8	12.3± 5.0

*Significantly different from baseline at P<0.05.

range at the end of the desert phase and continued to stay normal through the remainder of the study.

The mean total white blood cell count and the three part differential determined by the Coulter Counter are shown in Table 8.12. The strenuous exercise caused a significant increase in circulating white blood cells. A leukocytosis response to exercise is a well documented phenomena (Fry 1992, Tvede 1989). The significant increase in granulocytes and decrease in lymphocytes at the end of the desert, mountain and jungle phases is also in agreement with the literature. There was a significant return to baseline levels in these cell types during the reduced energy deficit period at mid-mountain phase.

Serum concentrations of immunoglobulins, IgA, IgG, and IgM remained within normal reference ranges during the course of the study (Table 8.13). This

Table 8.12 White Blood Cell Differential Analysis

Item	Ref. Value	Unit	Baseline	Desert	Mid- Mountain	Mountain	Jungle
Total WBC	3.6- 9.6	$\times 10^9/L$	6.6 $\pm$ 1.6	7.0 $\pm$ 2.6	7.2 $\pm$ 2.7	7.9* $\pm$ 2.1	7.8* $\pm$ 2.5
Lymphocyte	1.2- 3.4	$\times 10^9/L$	2.3 $\pm$ 0.5	2.0* $\pm$ 0.4	2.3 $\pm$ 0.5	1.8* $\pm$ 0.4	2.1* $\pm$ 0.5
Granulocyte	1.4- 6.5	$\times 10^9/L$	3.7 $\pm$ 1.2	4.4* $\pm$ 1.1	4.2 $\pm$ 1.2	5.6* $\pm$ 1.8	5.2* $\pm$ 2.3
Monocyte	0.1- 0.6	$\times 10^9/L$	0.6 $\pm$ 0.3	0.7 $\pm$ 0.2	0.7 $\pm$ 0.4	0.5* $\pm$ 0.2	0.6 $\pm$ 0.2

*Significantly different from baseline at $P < 0.05$.**Table 8.13** Serum Immunoglobulin Concentrations

Item	Ref. Value	Unit	Baseline	Desert	Mid- Mountain	Mountain	Jungle
IgA	69-382	mg/dL	215.6 $\pm$ 94.4	232.2 $\pm$ 101.0	225.1 $\pm$ 96.7	245.6 $\pm$ 116.8	255.0 $\pm$ 113.4
IgE	0-120	IU/dL	467.2 $\pm$ 1287.2	674.1 $\pm$ 1541.5	689.3 $\pm$ 1471.7	887.3 $\pm$ 1702.9	884.6 $\pm$ 1732.4
IgG	723- 1685	mg/dL	1059.5 $\pm$ 215.4	1114.0 $\pm$ 242.7	1033.8 $\pm$ 189.4	1162.5 $\pm$ 254.7	1140.0 $\pm$ 245.0
IgM	63-277	mg/dL	131.2 $\pm$ 61.9	127.1 $\pm$ 53.9	136.8 $\pm$ 60.8	140.8 $\pm$ 67.7	148.6 $\pm$ 64.5

*Significantly different from baseline at $P < 0.05$.

would be expected because serum immunoglobulin concentration has been shown to be unaffected even by severe malnutrition (Biesel, 1979). The data must not be interpreted as an indication of maintenance of intact normal humoral responses. Affective humoral immunity depends on the interaction of many cell types and on

metabolically active substances other than immunoglobulins.

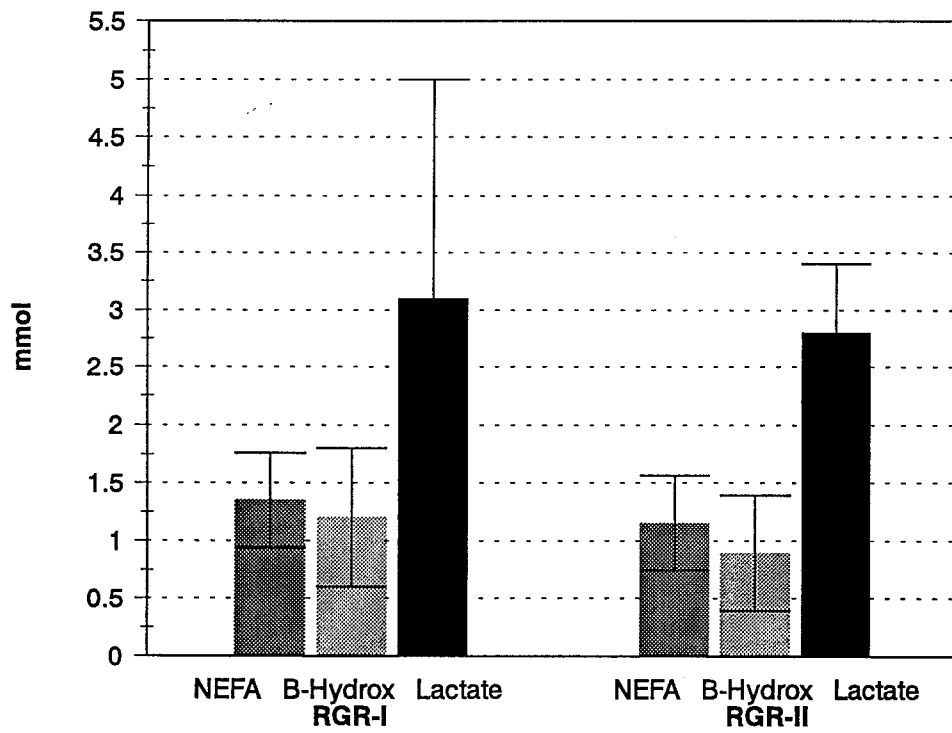
The immunoglobulin, IgE is produced in response to allergic reaction. The mean serum value was elevated during the study and showed wide variation between individuals. Generally, the concentrations of non-IgE antibodies in the blood vary little from person to person, whereas those of IgE antibodies can vary considerably (Lichenstein, 1993). Given the exposure to pollen, insect bites and bee stings during Ranger training, it is not surprising to find such elevated concentrations of IgE in some individuals.

CONCLUSIONS

The pattern of changes in the various metabolic markers measured during the 8 weeks of training is consistent with changes in body composition and the semi-starvation regimen. Although a number of the markers were shown to be outside of the normal reference ranges, there is no reason to consider any of these observations as 'life threatening.' Many of the markers responded by end of the 7 day 're-feeding' period at the mid-mountain phase. Despite the caloric restriction, it appears that the subjects received adequate nutrition with respect to protein, vitamin and mineral intakes.

The main objective of this study was to increase the caloric intake by 15% above the intake observed in RGR-I. The goal was to decrease the impact of the training on body composition and immunological status while still providing a stressful level of food deprivation. Stringent statistical analysis between the two studies is difficult, particularly because of the difference in the order of the training phases. However, some observations comparing the chemistry data between RGR-I and RGR-II suggest that there was a beneficial effect of the increased caloric intake. Figure 8.4 compares three of the metabolic markers between both studies at the end of the mountain phase, the phase with the greatest energy deficit. All three markers, non-esterified fatty acids, β -hydroxybutyrate, and lactic acid, were affected by the caloric intervention.

Figure 8.4 Comparison of Selected Metabolic Markers, end of Mt. Phase



CHAPTER 9

IMMUNE FUNCTION I: Lymphocyte Proliferation & Subset Analysis; Interleukin Production

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INTRODUCTION

One of the earliest demonstrations that exercise may have an effect on immunological functions was reported by Rusch and Kline (1944). They demonstrated that growth of transplanted tumors in vigorously exercised rats were reduced compared to sedentary controls. During the past seven years there has been an increasing interest in the effect of exhaustive exercise on immunological function (Morgan et al., 1990, Newsholme E.A., 1993, Stone, M.H., 1991). Physical fatigue may contribute to increased susceptibility to illness. Some athletes appear to suffer high rates of certain illnesses, such as infectious mononucleosis (Foster et al., 1982) and upper respiratory illness (Berglund and Hemmingsson, 1990). Frequent illness has also been observed in athletes experiencing "overtraining", a condition characterized by prolonged fatigue and due primarily to excessive training (Mackinnon, 1992).

It is difficult to take research results from civilian athletes and apply these to soldiers performing exhaustive exercise under the additional stress of simulated combat conditions. The RGR-I study was unique in the fact that it was a comprehensive study involving the interaction of physiological, psychological, and nutritional effects on immune function. The *in vitro* proliferative response of peripheral blood lymphocytes from the volunteers was severely depressed from baseline values at each time point studied after the start of the training. Because of the multi-stressor conditions imposed on the volunteers during the Ranger training, it is impossible to

associate the suppressed immune response solely with the severe negative energy balance.

This chapter will discuss the immunological assessment of volunteers during the RGR-II study concerning an attempt to increase the caloric intake 15% above the intake estimated during RGR-I. Additional methodology was used in RGR-II in an attempt to further define the effect of Ranger training on host defense mechanisms.

METHODS

Immunological Procedures

Blood collection At each collection point, a 7 mL blood draw was collected, for immune studies, in sodium heparinized (143 USP units) VACUTAINER (Becton Dickinson VACUTAINER Systems, Rutherford, NJ; No. 6527, Lot No. 1Y061) tubes. The blood was held at ambient temperature (18-27°C) for 20 h prior to preparation for *in vitro* cell culture, and staining and fixation of leukocyte subsets. Delayed processing was done to equalize the time for transport of the blood from the training sites to the immunology laboratory in Beltsville, Maryland. Plasma for IL-6 quantitation was removed from packed cells at 30-32 h post-collection. The plasma samples were held at -70°C until analysis.

The blood was diluted 1:4 and 1:2 with RPMI-1640 tissue culture medium (Sigma Chemical Co., St. Louis, MO) in polystyrene tubes (FALCON^R, No. 2003, Becton Dickinson Labware) for preparation of lymphocyte proliferation and interleukin production cultures, respectively. The RPMI-1640 contained L-glutamine at 2.0 mmol/L and penicillin-streptomycin at 100,000 U/L and 0.1 mg/L, respectively; referred to hereon as RPMI-1640. The *in vitro* cultures for lymphocyte proliferative responsiveness received in order: 100 μ L of RPMI-1640 per well of round bottom 96-well tissue culture plates (Corning Glass Works, Corning, NY., Cat. No. 258550), 50 μ L of RPMI-1640 alone (background) or with designated amounts of phytohemagglutinin-M (PHA-M; Sigma Chemical Co., St. Louis, MO., Cat. No. L-2646,

Lot No. 49F-40221) or pokeweed mitogen (PWM; Sigma, Cat. No. L-9379, Lot No. 66F-9530). The cultures contained a final volume of 200 μ L, with the final blood dilution at 1:16.

Proliferative activity in vitro was based on median DNA incorporation of tritiated thymidine (methyl- ^{3} H; specific activity 6.7 μ Ci, 248 GBq/mmol, DuPont, New England Nuclear, Boston, MA) by cells in triplicate cultures without (background) and with stimulant. PHA-M was added to the cultures at 0.25, 0.5, 1, 2, 4, 8 and 16 μ g per culture. PWM was added to the cultures at 0.025, 0.05, 0.1, 0.2, 0.4, 0.8 and 1.6 μ g per culture. Cell cultures stimulated with PHA-M were incubated for 72 h, and those with PWM for 144 h, at 37°C in a 5% CO₂, 95% humidified air incubator. ^{3} H-thymidine (1.0 μ Ci; 37 KBq) was added to each culture 24 h prior to termination. The cell cultures were harvested onto 12-well filtermats (Skatron Inc., Sterling, VA., Cat. No. 11731). The filter discs with 4.5 mL of scintillation fluid (READY-SAFE^R, Cat. No. 158735, Beckman, Columbia, MD) were counted in a beta scintillation counter (Beckman LS 3801). Proliferative activity of lymphocytes is expressed as mean DPM plus standard error.

Cells for interleukin production were cultured in the same manner as those for proliferation, except that the cultures contained 50 μ L of blood diluted at 1:2 with RPMI-1640, with a final dilution per culture at 1:8. The unstimulated cultures (background) received RPMI-1640 alone, and the stimulated cultures received 2.0 μ g of PHA-M. The cultures were incubated for 48 h, at which time the supernatants from each set of six cultures were collected, pooled, and stored at -70°C until assayed.

Interleukin-2 (IL-2) and soluble IL-2 receptors (IL-2R) were determined in supernatants from unstimulated (background) and PHA-M stimulated cultures by INTERLEUKIN-2 ELISA KITS (DuPont Co., Wilmington, DE; Cat. No. NEK-057, Lot No. C1049) and CELLFREE IL-2R Test Kits (T-CELL DIAGNOSTICS, Inc., Cambridge, MA; Cat. No. CK1024, Lot No. 5139), respectively. Interleukin-6 (IL-6) was determined in supernatants from unstimulated and PHA-M stimulated cultures, and plasma by Quantikine Immunoassay (R&D SYSTEMS, Minneapolis, MN; Cat No. D6050, Lot No. 9244181).

Lymphocyte subset markers for CD3, CD4, CD8 and CD19 were analyzed with a FACStar Flowcytometer (Becton Dickinson Immunocytometry Systems, Mountain View, San Jose, CA), using the whole blood lysis method and dual color immuno-florescence assay. The results are presented as percent and absolute number of cells per uL of whole blood.

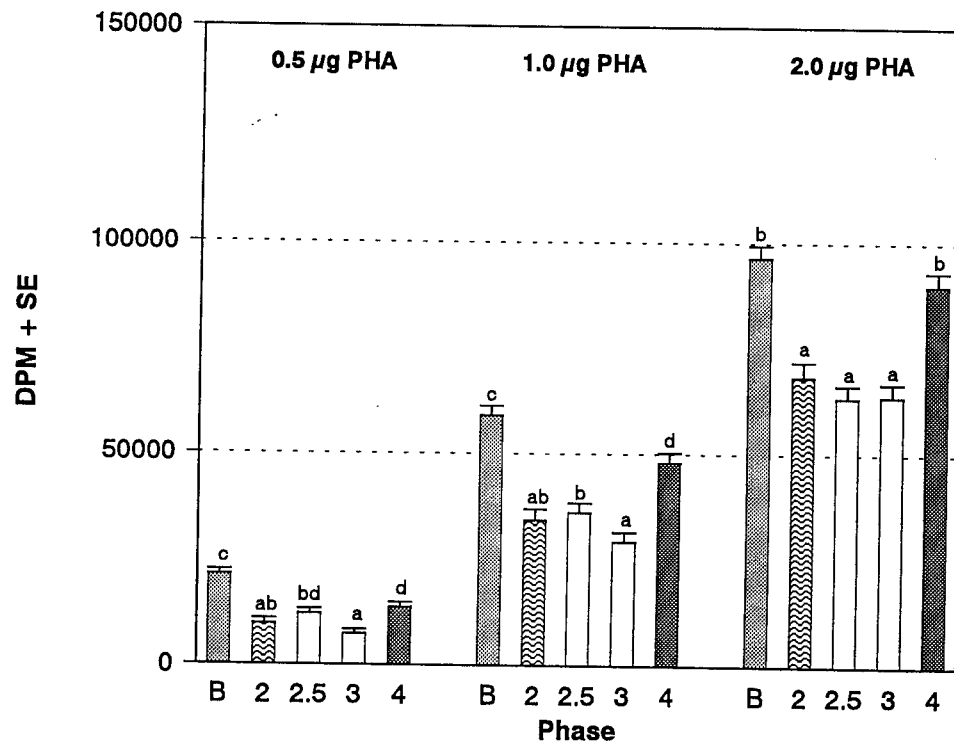
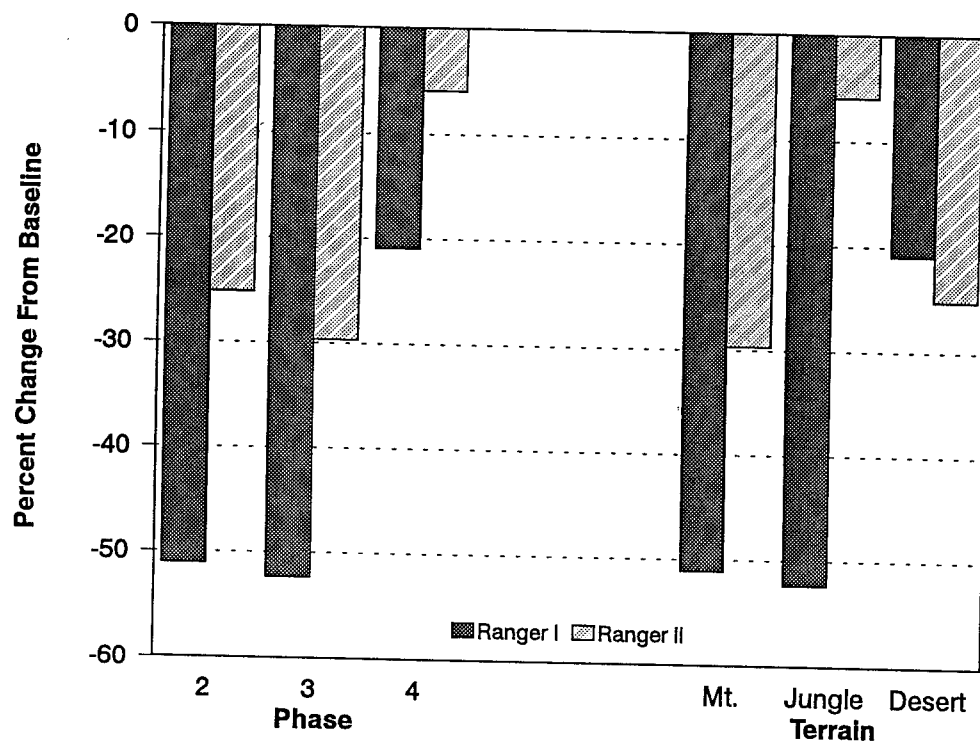
Statistical Analysis

A two factor general linear model with covariates were used to analyze the proliferation responses per volume, and activity per cell type. The unstimulated proliferation counts were used as covariates. Least Significant Differences (LSD) were performed to compare differences between phases. Descriptive statistics are expressed as mean and standard deviation.

RESULTS

To determine the effects of the Ranger training course on proliferative responsiveness of T-lymphocytes from trainees to suboptimal and optimal doses of PHA-M for maximum proliferation *in vitro*, whole blood cultures from volunteers of Class 11-92 (RGR- II) were stimulated with varying concentrations of PHA-M (Figure 9.1). When the cultures were stimulated with the optimal concentrations of PHA-M (4.0 ug/culture), the trainees showed equivalent T-lymphocyte proliferation at baseline and at the end of the course, phase 4 (jungle phase), but significantly ($p < 0.05$) lower activity at the end of phases 2 (desert phase) and 3 (mountain phase), and at the middle of phase 3 (phase 2.5; Figure 9.1). When the cultures were stimulated with the suboptimal concentrations of PHA-M (0.5 and 1.0 ug/culture), they also showed significantly ($p < 0.05$) reduced T-lymphocyte proliferation at the end of phase 4 (Figure 9.2).

An objective of the present study was to determine the effect of increased energy intake on reported suppressed T-lymphocyte proliferative responsiveness to PHA-M in trainees during the Ranger training course (Moore et al., 1992.) The mean percent suppressed T-lymphocyte proliferation from baseline for trainees of the RGR-II

Figure 9.1 Blood Lymphocyte Response to Phytohemagglutinin-M**Figure 9.2** Lymphocyte Proliferation Response in 4.0 µg PHA, RGR I & II

study to 4.0 ug PHA-M per cell culture was less at the end of phases 2, 3 and 4 than for trainees of RGR- I (Figure 9.2).

To reexamine the observation made in RGR-I trainees of quicker return to baseline values of the early (IL-2R and IL-2) versus late (DNA-synthesis) responses of T-lymphocyte activation/function during Ranger training, soluble IL-2R release and IL-2 production were measured in supernatants of whole blood cultures stimulated with PHA-M from RGR-II study trainees. Compared to baseline, IL-2R and IL-2 levels (Figure 9.3 & 9.4) were reduced in supernatants of PHA-M stimulated cell cultures from RGR-II trainees at the end of phases 2 and 3, and at the middle of phase 3 (phase 2.5) of training (Figure 9.4). In contrast to the RGR-I study, IL-2R and IL-2 levels for trainees of the RGR-II study did not return to baseline value at the end of phase 3. The IL-2R, but not IL-2, levels returned to baseline value by the end of the Ranger training course, phase 4. The earlier observation made in the RGR-I study of quicker return to baseline values of the early (IL-2R and IL-2) versus late (DNA-synthesis) responses of T-lymphocyte activation in Ranger trainees is not consistent.

To determine the effects of the Ranger training course on blood leukocyte subsets of Ranger trainees, blood samples from Ranger trainees of the RGR- II study were measured for leukocytes with subset markers CD3, CD4, CD8 and CD19. Compared to baseline, the percent CD3 positive T-lymphocytes and CD19 positive B-lymphocytes were decreased and increased, respectively, in the trainees at the end of phases 2 and 4 of training (Figure 9.5). The percent CD4 and CD8 positive T-lymphocytes were increased and decreased, respectively, in the trainees at phases 2.5, 3 and 4 of training, thus showing an increased ratio of CD4 to CD8 positive T-lymphocytes in the trainees at these times (Figure 9.6). Compared to baseline, the absolute numbers of CD3, CD4 and CD8 positive T-lymphocytes per volume of blood were decreased in the trainees at the end of training phases 2, 3 and 4 (Figure 9.7). While, at the middle of phase 3 (phase 2.5), following a short period of increased caloric intake, the absolute number of CD3 and CD8 positive T-lymphocytes per volume of blood were equivalent to baseline values, and the absolute number of CD4 positive T-lymphocytes was increased above baseline. The absolute number of CD19

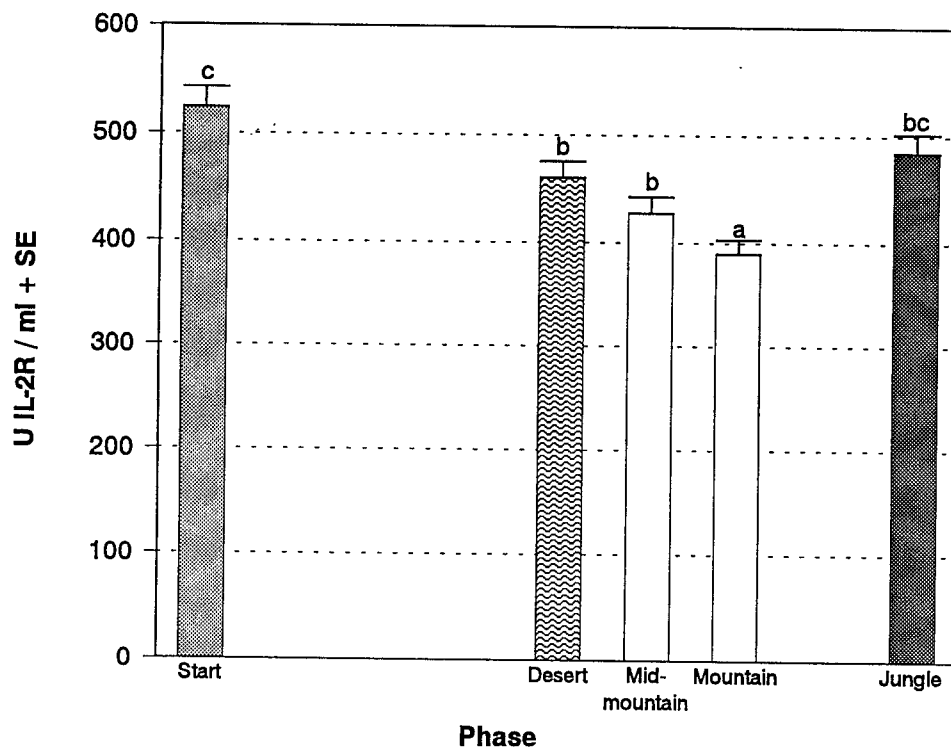
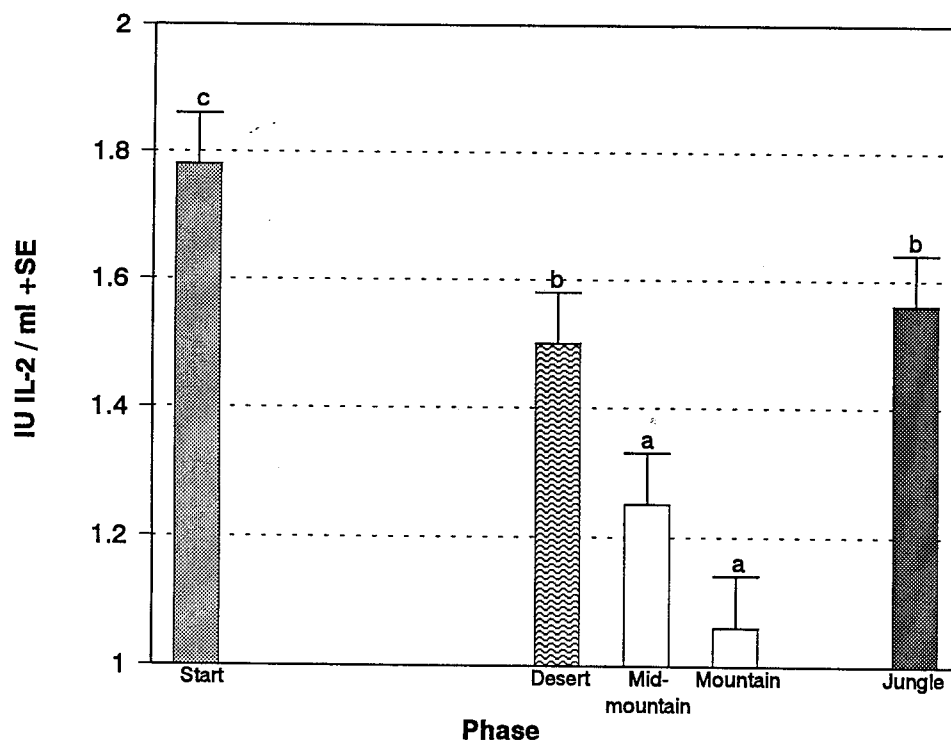
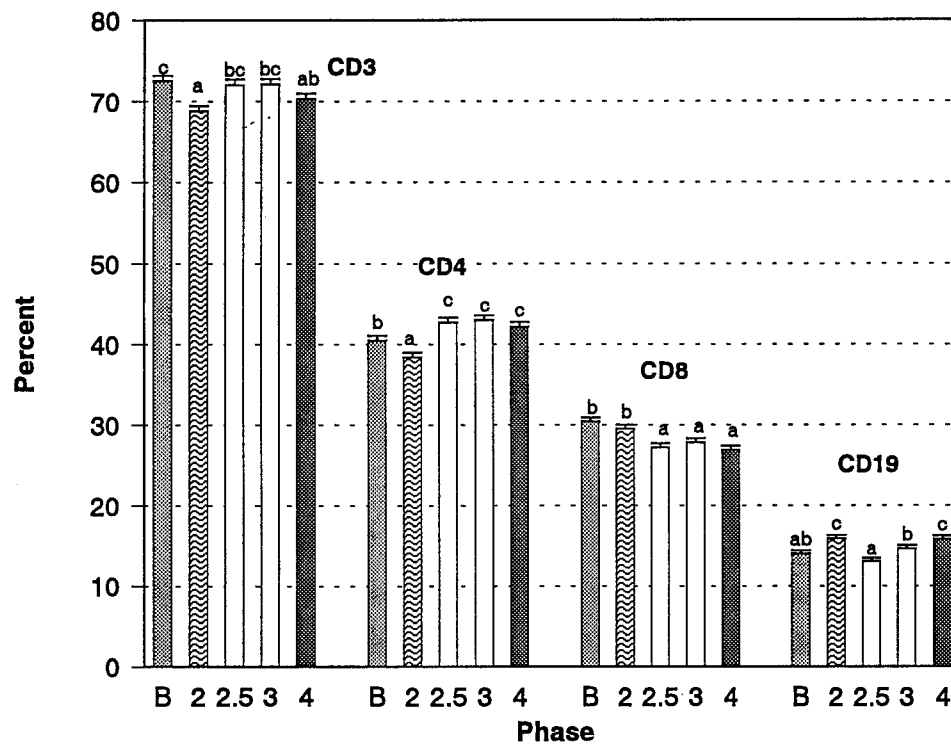
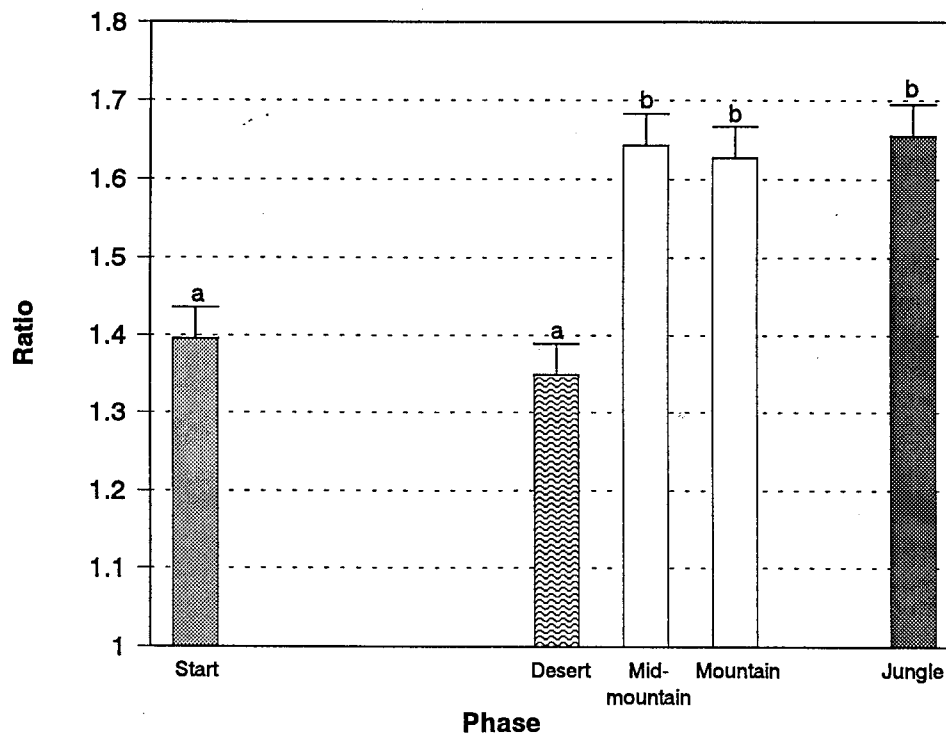
Figure 9.3 In Vitro Production IL-2 Soluble Receptors**Figure 9.4** In Vitro Production IL-2

Figure 9.5 Changes in Leukocyte Phenotypes as a % of Total Leukocytes**Figure 9.6** T-Helper to T-Suppression Ratio

positive B-lymphocytes per volume of blood was temporarily decreased in the trainees at the end of phase 3 of training.

An additional objective of the present study was to determine the effect of increased energy intake on suppressed plasma IL-6 in Ranger trainees. Trainees of RGR-II study showed a significant ($p < 0.05$) increase from baseline in plasma IL-6 at the end of phase 2 of the Ranger training course, followed by a return to baseline level by the middle of phase 3 (phase 2.5), with continued significant decreases at the end of phases 3 and 4 (Figure 9.8). Changes in plasma IL-6 for trainees of the RGR-II study were similar to those of trainees in RGR-I study. Thus, while increased energy intake by trainees of RGR-II study lessened the degree of suppressed T-lymphocyte proliferation in the trainees to PHA-M, it did not reduce the degree of suppressed plasma IL-6.

Similar to observations made in RGR-I study, it appears that the change in plasma IL-6 during the Ranger training course for trainees of the RGR-II study were not due solely to the production of IL-6 by blood cells. Plasma IL-6 levels in trainees of the RGR-II study at phases 2.5 and 3 were equivalent to, and decreased from baseline, respectively, while the IL-6 levels in supernatants from unstimulated whole blood cultures at the same times were increased and equivalent to baseline, respectively (Figure 9.9). Based on results from RGR-I and II, it is apparent that plasma IL-6 is a more accurate indicator of the effects of the Ranger training course on IL-6 status in trainees than IL-6 levels in the supernatant of whole blood cultures.

CONCLUSIONS

When interpreting the results discussed above, a number of issues must be addressed. The methodology we used to assess lymphocyte proliferation and the phenotyping involves the use of diluted whole blood. Alternatively, many investigators use isolated blood lymphocytes by gradient centrifugation before placing the lymphocytes in culture for subsequent stimulation with appropriated mitogens. Shinkai, et al., 1992 have shown that using the isolation techniques on blood from

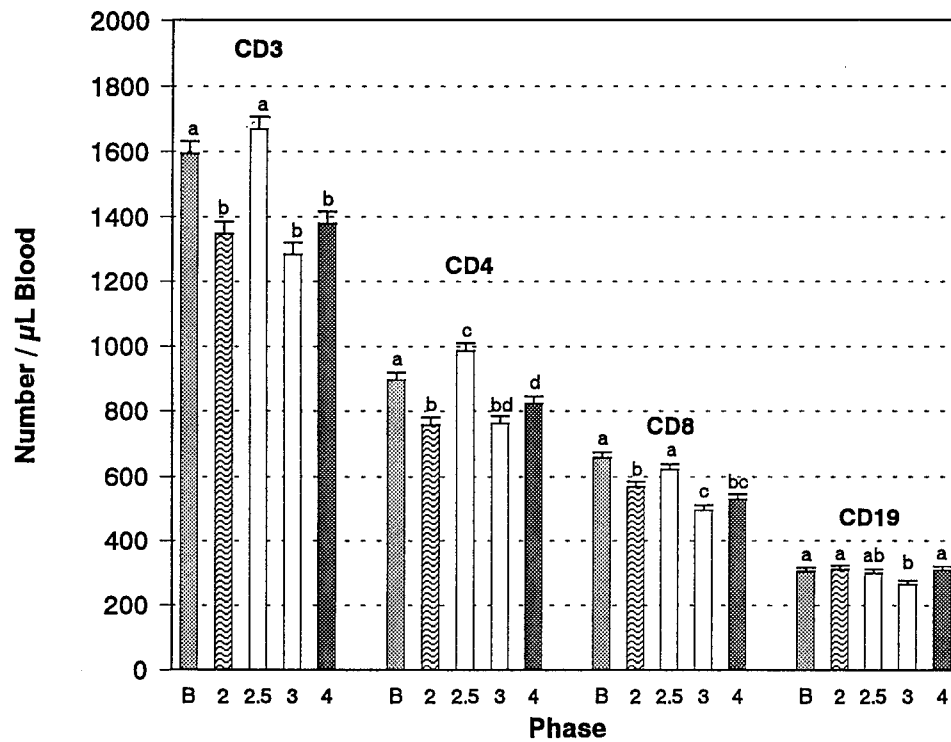
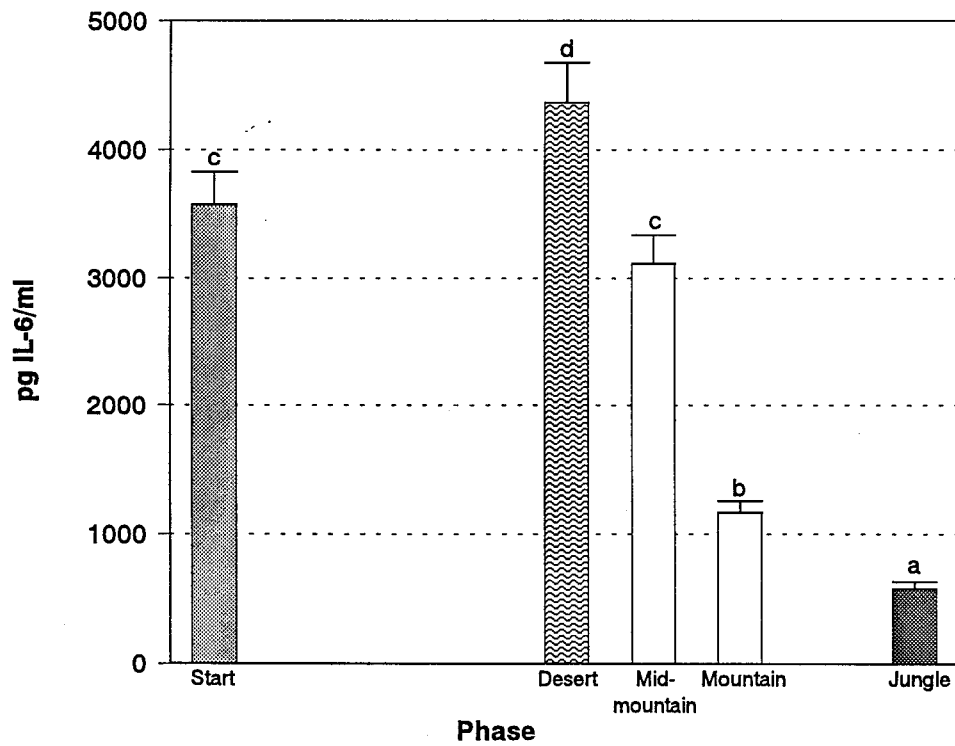
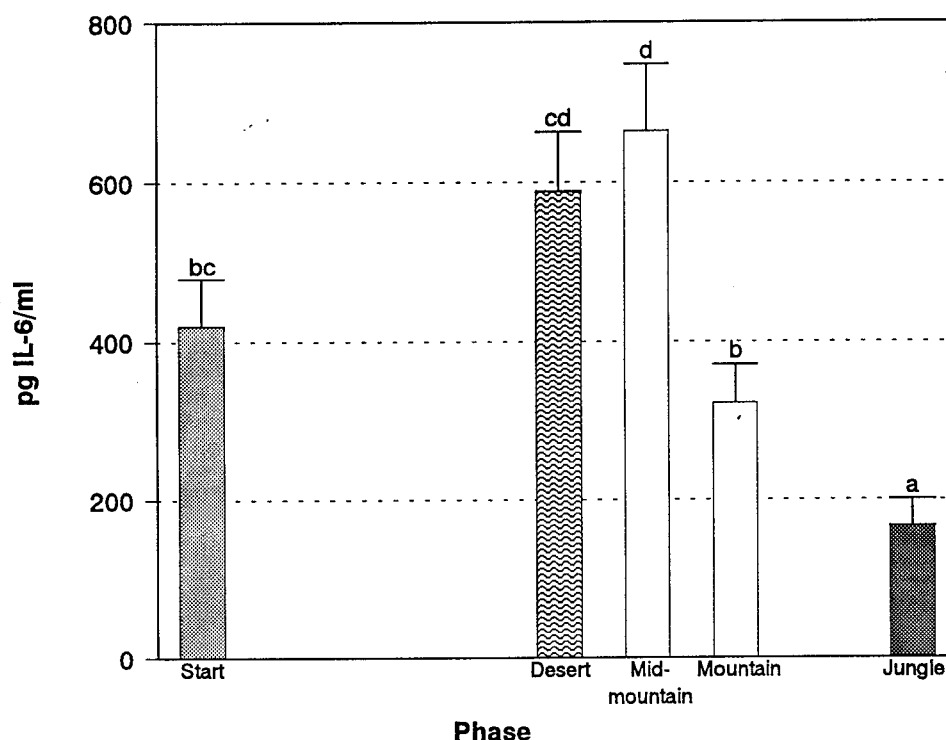
Figure 9.7 Changes in Leukocyte Phenotypes as Absolute Number of Cells**Figure 9.8** Plasma IL-6

Figure 9.9 Supernatant IL-6 Concentration

exercising individuals can bias the quality and/or quantity of the results.

Having a Coulter Counter on site to perform timely CBC counts is very important to the quality of the immunological data. Performing an accurate total leukocyte count and three part differential allows for adjusting results for changes in whole blood leukocytes. It is a well established fact that exercise causes a temporary leukocytosis. Table 8.11 shows that there was a significant increase in leukocytes as the training progressed in RGR-II. The impact of using the total leukocyte count to adjust the results is dramatically demonstrated when comparing the flow cytometry data shown in Figure 9.5 to Figure 9.7.

The *in vitro* test of lymphocyte proliferation is a commonly accepted test of immunocompetence. Clinically, the method is considered a sensitive indicator of acquired immunological deficient states (Oppenheim and Schechter, 1980). The assay showed a severe suppression in immunological response in RGR-I. Although it appears that the caloric intervention during RGR-II had a beneficial effect on the

proliferative response, the training stressors continued to suppress the response, particularly during the period of highest negative energy balance, the mountain phase.

The proliferative response involves the interaction of many cell types. This technique does not provide any information concerning which particular cell type(s) are affected by the training stressors. The flow cytometry techniques offer the potential to determine the effects on specific cell types.

The CD3 and CD19 surface markers identified primarily T cells and B cells, respectively. The B cells are the antibody-producing cells of the body. It was shown in Chapter 8 that the serum antibody concentration was not affected significantly by the training (Figure 8.10). Correspondingly, the total number of circulating B cells was not significantly changed except at the end of the mountain phase.

There have been many subsets of T cells identified in the literature. In this study, we used the CD4 and CD8 surface markers to identify T-helper and T-suppressor cells, respectively. The T-helper cells react to foreign molecules (antigens) and produce proteins (interleukins) that signal other T cells and B cells to respond. Actually, the term "suppressor" cell covers a broad range of cell types. Some of these cells act to suppress other cells of the immune system to keep the response from over-reacting. The responses of the immune system are often antithetical, i.e., beneficial to the host under some circumstances while causing catastrophic damage when the response is exaggerated.

While there was relatively little change in the B cell populations over time, there were dramatic changes in total T cells and the two subsets of T cells. The decrease in total T cells suggests a negative effect of the training and supports the suppressed proliferation data. It is of interest that all the T cell surface markers responded favorably to the change in energy balance during the early mid-mountain phase (phase 2.5).

The various cell types of the immune system exert their effect over other immune cells and other cell types by the production and excretion of

proteins called interleukins. To date, there have been over a dozen different interleukins identified that have many diverse and overlapping effects. The *in vitro* suppression of IL-2 and IL-2 soluble receptor as, well as the suppressed plasma IL-6, reinforces the immunosuppressive effect of the training suggested by the proliferation and the flow cytometry data.

CHAPTER 10

IMMUNE FUNCTION II: Monocyte & Granulocyte Function

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Walter Reed Army Institute of Research

INTRODUCTION

Over the years, significant morbidity from infections has been observed in Ranger trainees. This includes cases of cellulitis, severe streptococcal infection, various upper respiratory infections, and one epidemic of pneumococcal pneumonia. Polymorphonuclear phagocytes, also known as neutrophils are the body's first line of defense against many bacterial infections. Neutrophil response to infection can be divided into three stages. The initial response is "chemotaxis", as neutrophils respond to chemical signals to leave the bloodstream and accumulate at the site of inflammation. The second is "phagocytosis", where neutrophils engulf and internalize bacteria. The final is "bacteriocidal activation" wherein neutrophils secrete a variety of enzymes and other toxic substances which kill phagocytized organisms. One of the most important bacteriocidal mechanisms is the generation, by metabolically "activated" neutrophils, of reactive superoxide anions. A defect in this pathway causes an inability to kill phagocytized bacteria, and thus to limit infection. On the other hand, inappropriate or excessive activation of neutrophils can also be harmful, causing damage to surrounding tissues by release of toxic substances, as in the case of Adult Respiratory Distress syndrome, which can cause respiratory failure in patients with infections, shock, or trauma. In this study, a standard test of neutrophil activation, which measures the production of superoxide following stimulation of cells, was modified for use on a large number of subjects in a field study.

Another key portion of immune host defenses is the activation of monocytes and tissue macrophages by bacteria or bacterial products to secrete potent immunologic activators known as monokines. These substances orchestrate many

aspects of the immune response, causing fever, induction of infection-specific proteins by the liver (acute phase response), increasing the efficiency of lymphocyte responses to antigens, altering insulin secretion and tissue metabolism, and "priming" neutrophils for increased activation by bacteria. Interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor-necrosis-factor alpha (TNF- α) are three important monokines known to be released by monocytes or macrophages following activation. The outer membrane of gram-negative bacteria contains lipopolysaccharides (LPS) which are particularly potent activators of macrophages and of monokine secretion. Monocytes can also be desensitized or "tolerized" to LPS by exposure to very small "tolerizing" amounts of LPS. This can occur in vivo in the intact organism or in vitro. We measured the production of TNF, IL-6, and TNF- α by monocytes isolated from blood of Ranger trainees after three hours of incubation either without or with the addition of 100ng/ml of LPS purified from E. coli bacteria.

Both measurements of neutrophil superoxide production and of monocyte monokine secretion were performed at approximately 2-4 week intervals on Ranger trainees: immediately prior to Ranger school, at the end of Phase II (desert), the end of Phase III (mountain), and the end of Phase IV (jungle). Differences between time points were evaluated using ANOVA for repeated measures, where each individual's test values were compared across time to their own baseline value.

METHODS

Sedimentation of RBC's

Blood was drawn from subjects in Vacutainer-Plus plastic tubes containing lithium heparin as anticoagulant. Assessment of endotoxin-contamination performed on commercial blood-drawing tubes in our laboratory demonstrated that pyrogen free water incubated 1 h in these tubes contained significantly less endotoxin (< 50 pg/ml) than washes of other commercial glass sterile tubes. Blood was kept at room temperature, and within 120 minutes after phlebotomy, 7 ml were added to a sterile 15 ml polystyrene tube. Seven milliliters of cold (10°C), 2% dextran (T-500, Pharmacia,

Inc.) dissolved in sterile Hanks Buffered Salt Solution (HBSS, Bio-Whittaker, Inc.) was added to sediment erythrocytes. Tubes were mixed by inversion and allowed to sediment at room temperature for 20 to 30 minutes. The leukocyte-containing supernatant was then removed, and used for both superoxide and monokine secretion assays. Analysis of automated WBC counts with manual differentials of 20 heparinized blood samples and their subsequent dextran-sedimented supernatants revealed a mean WBC count of 7.5 with a SD of 1.26, and CV of 16.8 for whole blood, and 3.52 with a SD of 0.8, and CV of 22.8 for the sedimented supernatants. Absolute granulocyte counts were 4.96 with a SD of 0.96, and CV of 19.3, and 2.46 with a SD of 0.69, and CV of 28.7 for whole blood and dextran supernatants, respectively. The mean ratio of absolute granulocyte count between dextran supernatant and the corresponding whole blood was 0.51 with a SD of 0.13, and CV of 25.4. Dextran sedimentation also resulted in a mean 65% depletion of monocytes when absolute monocyte counts of whole blood and sedimented supernatants were compared. Thus, a mean error of approximately 20 to 30% could be conservatively attributed to differences between subjects in the WBC of freshly drawn blood and the leukocyte content of subsequently processed leukocytes.

Measurement of Superoxide Production

The leukocyte-supernatant from dextran-sedimented blood was added to 96 well plates, 0.1 ml per well, 12 wells per subject. Neutrophils were allowed to adhere for 30 minutes at 37°C. Plates were flicked empty, and 0.2 ml of HBSS (phenol red free, buffered to pH 7.4) was added to each well. A baseline OD was then obtained for each plate. Plates were then flicked empty and 0.1 ml HBSS containing 160 μ M cytochrome C, and either 1 micromolar chemotactic peptide f-met-leu-phe (FMLP), 10 ng/ml of phorbol myristate, FMLP and 2 μ g/ml indomethacin, or nothing (control) were each added to triplicate wells. Plates were immediately read at 550 nm with off-peak absorption at 490 nm subtracted out, using a Vmax kinetic ELISA reader (Molecular Devices Corp., Menlo Pk., CA.). Readings were performed at time 0, 10, 15, and 30 minutes, with incubation at 37°C between readings. Data was collected real-time and stored on a MacIntosh Powerbook 170 computer using SoftMax software (Molecular Devices Corp.). After subsequent analysis, the stimulation of superoxide production

was expressed as either the ratio or the difference between FMLP-stimulated and unstimulated wells. This microplate assay was adapted from the procedure previously described by Mayo and Curnutte, 1990.

In preliminary experiments, leukocytes from dextran-sedimented blood were further purified into neutrophil and mononuclear populations over Ficoll-Hypaque gradients and then recombined with 10% autologous human serum. FMLP-stimulated superoxide production was found to be entirely attributable to the neutrophil population over the first 15 minutes of this assay. Mononuclear cells did not produce measurable superoxide, nor did they alter the kinetics of release when added back to purified neutrophils. The fact that neutrophils are allowed to adhere to wells during this procedure does result in measurable background production of superoxide without stimulation, as neutrophil adherence to plastic can itself provide activation signals. This assay, unlike the classic superoxide assay, is performed after adherence of neutrophils in the presence of plasma, and thus may reflect any inhibitory or stimulatory factors present in the subject's plasma.

Stimulation and Measurement of Monokine Secretion

Each subject's leukocyte-containing supernatant from dextran-sedimented blood was immediately divided in 0.5 ml aliquots between 3 sterile Falcon tubes, to which was added either a.) 0.5 ml of HBSS containing 10 ug/ml of Polymyxin B to neutralize any contaminating endotoxin, b.) 0.5 ml HBSS containing 200 ng/ml of phenol-extracted LPS from *E. coli*, bort strain, or c.) 0.5 ml HBSS containing LPS and 2 ug/ml of indomethacin. Tubes were vortexed and incubated for 3.5 h in a 37°C water bath and agitated once each hour. At the end of 1 h, the supernatant was carefully decanted, frozen on dry ice, and kept at -30°C for subsequent assays.

Monokine measurements utilized ELISA kits obtained commercially from R&D Systems (Minneapolis, MN) for measurement of Interleukin-1-beta, Tumor Necrosis Factor-alpha, and Interleukin-6. The assay sensitivity ranges were 15 to 1250 pg/ml, 25 to 2500 pg/ml, and 10 to 1500 pg/ml, respectively.

In control experiments, this procedure was performed on whole blood, dextran-sedimented leukocyte supernatant, and Ficoll-Hypaque-gradient-purified mononuclear cells from four donors. Gradient purified cells were adjusted to approximately equal concentrations of mononuclear cells as enumerated in whole blood. After addition of Polymyxin B or of LPS, supernatants were examined after 2.5, 3.5, or 4.0 h for determination of TNF-alpha. TNF levels were increased from a mean of 32 pg/ml to a mean of 890 pg/ml with addition of 100 ng/ml of LPS. Increasing the incubation time from 2.5 to 4 h only increased the TNF levels by a mean of 22%. No significant differences were noted between levels from whole blood, dextran-sedimented leukocytes, or gradient-purified cells for each donor. Thus, the partial depletion of monocytes, the presence of dextran, and the presence of plasma did not significantly alter TNF-production by monocytes isolated by dextran sedimentation.

RESULTS

Superoxide Production

Figure 10.1 shows the mean optical density (OD) reading at 550 nanometers of triplicate wells of leukocytes from each subject 15 minutes after stimulation with the chemotactic peptide FMLP, with background OD (no FMLP, spontaneous superoxide release) subtracted. Using ANOVA for repeated measures, each student's response was compared to the baseline value (time point I). Responses were significantly elevated at time point II (end-desert) and time point III (end-mountain), but not at time point IV (end-jungle). At time points II and III, student superoxide responses to FMLP were also significantly greater ($p < .05$, Students Unpaired T-test) compared to simultaneous controls (leukocytes from study personnel).

Monokine Secretion

Figure 10.2 shows the mean and standard error of TNF secretion by LPS stimulated leukocytes at each of the four study time points. When evaluated by ANOVA for repeated measures, Ranger students showed a mean 75% reduction from baseline values at time point III (end-mountain).

Figure 10.1 Superoxide Response to N-Formyl-Met-Phe

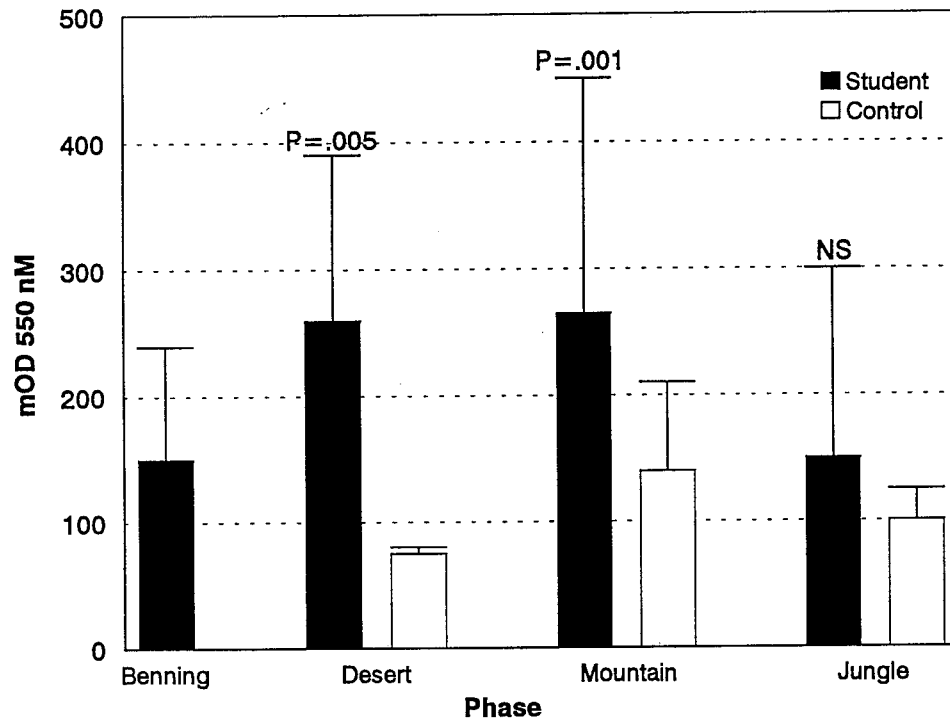


Figure 10.2 Leukocyte TNF Secretion after LPS Challenge

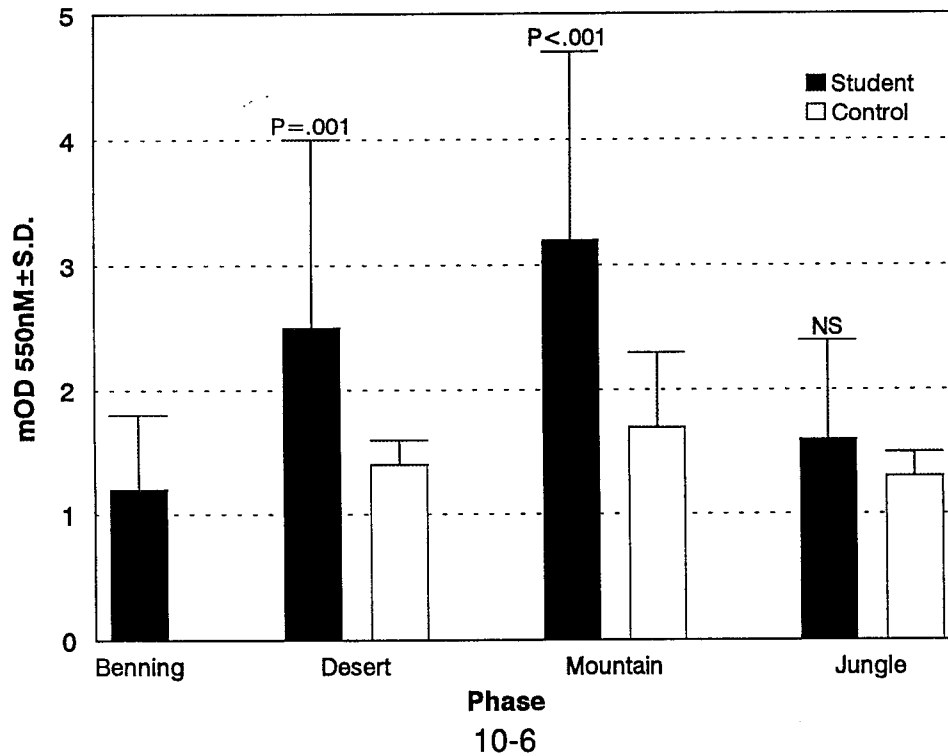


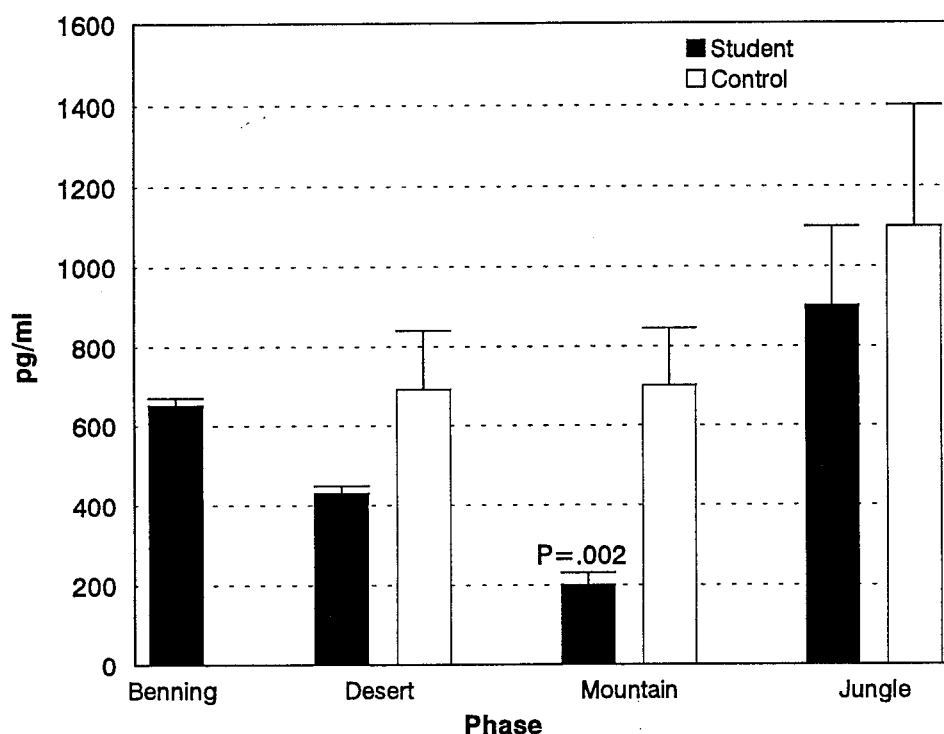
Figure 10.3 Leukocyte IL-6 Secretion after LPS Challenge

Figure 10.3 shows the mean IL-6 values at each time point, and also demonstrates suppressed secretion at time point III. Student leukocyte secretion of TNF and IL-6 at time point III was significantly suppressed relative to simultaneous controls at this time point ($p < .05$ by Students Unpaired T-test). At this and other time points, the presence of indomethacin, an inhibitor of cyclooxygenase (prostaglandin synthetase), did not affect the quantity of TNF or IL-6 produced by monocytes.

CONCLUSIONS

Military trainees, deployed and combatant soldiers, and refugee populations have historically suffered from a high incidence of infectious diseases. While deficiencies in personal hygiene and public sanitation may account for some of this increase in infections, it has been postulated that chronic stress may impair immune host defenses. Certainly, in medically-stressed patients following burn, multiple trauma, or surgery, alterations in both cell-mediated immunity and phagocyte function

can be documented, and correlate with increased infectious morbidity and mortality (Christou, Meakins, et al., 1981; Christou, Rode, et al., 1984; Rode et al., 1988; Christou, Tellado-Rodriguez, et al., 1989). In animal models, chronic behavioral stressors have been shown to alter cell-mediated immunity, and to decrease resistance to infectious diseases, including Japanese encephalitis virus, toxoplasmosis, and listeriosis. Such alterations of immune function are not well-documented in chronically-stressed (but not medically-ill) humans. In two prior classes of Ranger school trainees, significant decrements occurred in delayed-type hypersensitivity responses to a panel of seven intradermal antigens (Bernton, unpublished results.) This is summarized in Table 10.1.

Table 10.1 Delayed-type Hypersensitivity Response

Week	# Subjects	% Anergic (0 Positive)	%1 or Less Positive	Mean MM Induration Tetanus & Diphtheria
0	31	0	6	8.6
4 (end- mountain)	31	16	42	3.8
0	39	0	8	11.5
6 (end- mountain)	39	5	20	4.9
8	28	18	39	4.5

Non-specific mechanisms and phagocytes such as neutrophils provide the most rapid response to pathogens. Defective neutrophil number or function is associated with increased susceptibility to pyrogenic infections and to disseminated infection with fungal or encapsulated bacterial pathogens. While on one hand, insufficient or poorly functional neutrophils may increase susceptibility to infection, neutrophils can also play a role in pathologic inflammatory responses such as ARDS, secreting toxic reactive oxygen products and enzymes. Prior exposure to cytokines such as TNF, or to bacterial lipopolysaccharide can prime neutrophils for an augmented oxidative burst response.

Complement activation is an important mechanism facilitating rapid phagocytosis and clearance of many pathogens, independent of antibody. Several factors in the complement cascade are known to fall drastically in subjects immunosuppressed due to protein/calorie deficit. This is rarely seen in the presence of normal albumin levels, as were documented in the Ranger trainees.

Production of monokines (in particular tumor-necrosis factor) play a central role in recruitment and activation of effector cells in the T-lymphocyte dependent delayed-type hypersensitivity response. Suppression of this response in stressed Ranger trainees could result either from a) decreased proliferative responses of memory T-lymphocytes to recall antigens; b) impaired secretion of IL-2 and gamma-interferon by T-lymphocytes; c) impaired antigen presentation by macrophages or other antigen-presenting cells; d) impaired effector response of recruited macrophages, particularly secretion of IL-1 and TNF-alpha, or finally, e) increased production of anti-inflammatory mediators such as prostaglandin-E2 by monocytes (Faist, et al., 1990) . These data suggest that monocyte production of IL-1, TNF-alpha, and IL-6 is decreased in stressed Ranger students relative to their baseline values, and this decrease is not altered by addition of prostaglandin synthetase inhibitor. While this could reflect a general impairment of monocyte activation, it could also be a specific defect in the response to bacterial LPS. This impaired response to LPS results following prior exposure to low doses of LPS, and is called "endotoxin tolerance". Alternatively, the high levels of circulating cortisol (50-150% elevation from baseline) documented in stressed Ranger trainees may contribute to inhibition of monokine

secretion, as these hormones are known to do, in vitro.

In general, burn injury and trauma in both humans and in animal models have been associated with impaired neutrophil phagocytic and oxidative burst functions. In contrast, the stressed Ranger students had an increased neutrophil superoxide secretory response to the chemotactic peptide FMLP. However, superoxide production by neutrophils from both trauma patients (Tanaka, et al., 1991) and septic patients (Trautinger, et al., 1991) have also been reported to be increased relative to controls. In vitro, this is a well-described "priming" effect of bacterial LPS on neutrophils (Guthrie, et al., 1984). Priming of neutrophils for an augmented oxidative response to FMLP can also occur due to tumor-necrosis factor (Bajaj, et al., 1992) or to granulocyte or granulocyte-monocyte colony-stimulating factor (G-CSF or GM-CSF) (Yuo, et al., 1993), which are produced in vivo by myeloid and other cells exposed to LPS. It has been speculated that priming of neutrophils by LPS may contribute to the acute respiratory distress syndrome frequently seen in septic patients, by increasing the destructive capabilities of neutrophils adherent to the pulmonary endothelium (Carey, et al., 1991).

These data suggest that the combination of chronic stress, food restriction, and extreme exertion could lead to altered permeability of the small bowel, and/or altered clearance of LPS from the portal circulation by the reticulo-endothelial system. Episodic low-level endotoxemia has been documented in long distance runners (Brock-Utne, et al., 1985; Bosenberg, et al., 1988). In animal models, stress of thermal injury results in altered macromolecular permeability of the gut (Carter, et al., 1990). This hypothesis could be further explored by examining measures of gut permeability to macromolecules, serum endotoxin, and anti-LPS antibody levels in Ranger students at the end of the mountain phase.

Regardless of their etiology, this study provides descriptive data on changes in function of circulating monocytes and neutrophils during the course of stressful military training. This supplements the previous description of impaired lymphocyte blastogenic responses and the delayed-type hypersensitivity skin test responses in this population. These data suggest that impairment of cell-mediated immunity may occur

in stressed military trainees, possibly due to alterations in both monocyte and lymphocyte function. Increased neutrophil oxidative responses also may occur. This could increase tissue inflammation at sites of infection or trauma such as the lungs and skin.

CHAPTER 11

LONG RANGE PATROL RATION ACCEPTANCE

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INTRODUCTION

During Ranger course 11-92, the Meal, Ready-to-Eat (MRE) was issued to trainees for the first two phases (Benning and desert), and the Long Range Patrol Ration (LRP) was issued for phases 3 and 4 (mountain and jungle). Substitution of the MRE with the LRP plus one pouch bread had a number of purposes. The first was to provide additional calories so that an assessment could be made to determine whether an increase in the test volunteers' energy intake would lessen the decrease in immunological responses found in RGR-I. It was also hoped that average weight loss would be reduced from 15.6% to approximately 10%. The final purpose was to provide data for an assessment of the performance of the LRP when consumed as a restricted ration. This section discusses one aspect of this - "Acceptability."

Ration acceptability was assessed at the end of each phase. Ranger trainees were asked to complete a short, simple questionnaire for both rations, in which they rated the acceptability of each food item consumed once or more during the most recently completed phase of the course. An example of the Acceptability

Questionnaire is shown in Appendix D. A 9-point hedonic scale (1= "dislike extremely", 5= "neither like nor dislike", and 9= "like extremely") was used for this purpose. The trainees also indicated what they usually did with each ration item. Possible responses to this question ranged from "ate entire item" to "threw away." When completing the survey, volunteers were instructed to record only their personal views. Once completed, the questionnaires were checked by the trained data collectors who subsequently interviewed student volunteers to correct any errors or omissions.

RATION RATINGS

Meal Ready-To-Eat

Ratings for individual items of the Meal, Ready-to-Eat (MRE) are presented in Table 11.1a. Overall, the MRE components received ratings of "like slightly" or better except for Chicken ala King, the lowest rated item (5.1), which was followed closely by Potato au Gratin (5.2). M&M's scored highest (8.8), and the dessert bars, Chocolate Covered Cookie and Chocolate Nut Cake also scored well. Other items which received high ratings were Cocoa (8.2), Peanut Butter (8.0) and Crackers, Oatmeal Cookie Bar, and Tootsie Roll which each received ratings of 7.9.

Long Range Patrol Ration

Ratings for individual food items in the Long Range Patrol Ration (LRP) are presented in Table 11.1b. Overall, the LRP received ratings of "like moderately" or higher, except for Charms (6.6). M&M's and the Desert Bar (both 8.9) received the highest rating, while the Oatmeal Cookie Bar, Fig Bar and Cocoa (all with ratings of 8.7) also scored well.

Table 11.1a Mean Individual Food Item and Acceptability Ratings for the MRE

Food Item	Phase I	n	Phase II	n	Mean
<u>Entree</u>	6.4 (1.1)	91	6.8 (1.0)	91	6.6 (.92)*
Pork with Rice in BBQ Sauce	6.6 (2.3)	78	7.6 (1.5)	80	7.0 (1.9)*
Corned Beef Hash	5.4 (2.0)	57	6.5 (1.9)	80	6.0 (2.0)*
Chicken Stew	6.1 (2.0)	74	6.3 (1.8)	56	6.1 (1.9)*
Omelet with Ham	6.0 (2.3)	74	6.8 (2.0)	78	6.3 (2.1)*
Spaghetti with Meat Sauce	7.6 (1.5)	55	7.7 (1.4)	83	7.6 (1.3)
Chicken ala King	5.0 (2.4)	67	5.2 (2.4)	78	5.1 (2.2)
Beef Stew	6.2 (1.9)	84	6.2 (2.0)	82	6.2 (1.7)
Ham Slice	6.7 (1.7)	75	6.8 (1.7)	81	6.7 (1.7)
Meatballs with Rice	7.2 (1.8)	74	7.3 (1.8)	81	7.1 (1.7)
Tuna with Noodles	6.3 (2.3)	70	6.8 (2.0)	82	6.5 (2.1)
Chicken and Rice	7.0 (2.0)	50	7.5 (1.7)	84	7.4 (1.7)
Escalloped Potatoes with Ham	6.0 (2.2)	69	6.5 (1.9)	85	6.3 (1.8)
<u>Starches</u>	6.6 (1.7)	91	6.8 (1.6)	91	6.7 (1.4)
Crackers	7.9 (1.4)	91	8.0 (1.4)	91	7.9 (1.2)
Potato au Gratin	5.0 (2.7)	79	5.5 (2.4)	85	5.2 (2.4)
<u>Spreads</u>	7.3 (1.3)	91	7.3 (1.3)	91	7.3 (1.2)
Cheese Spread	7.4 (2.0)	89	7.2 (2.1)	91	7.3 (2.0)
Jelly	6.7 (2.1)	90	6.6 (2.1)	91	6.6 (1.8)
Peanut Butter	8.0 (1.5)	90	8.1 (1.6)	91	8.0 (1.4)
<u>Fruit</u>	7.3 (1.5)	87	7.1 (1.6)	91	7.2 (1.4)
Applesauce	7.4 (1.7)	85	7.3 (1.7)	91	7.4 (1.5)
Fruit Mix	6.6 (2.0)	52	6.9 (2.0)	85	6.8 (1.9)
Peaches (wet pack)	7.8 (1.7)	45	7.2 (2.4)	38	7.5 (2.0)
Pears	6.8 (2.1)	44	6.7 (2.2)	40	6.7 (2.1)
Strawberries	7.8 (1.8)	31	7.5 (2.1)	27	7.8 (1.6)
<u>Dessert</u>	7.4 (1.5)	91	7.9 (1.0)	91	7.7 (1.1)*
Chocolate Covered Brownie	6.7 (2.2)	88	7.2 (1.9)	86	6.9 (1.9)
Cherry Nut Cake	6.7 (2.6)	81	7.2 (2.0)	81	7.0 (2.1)
Chocolate Covered Cookie	8.2 (1.4)	85	8.3 (1.1)	87	8.3 (1.0)
Maple Nut Cake	6.8 (2.4)	56	7.5 (1.9)	81	7.3 (2.0)
Oatmeal Cookie Bar	7.3 (2.4)	61	8.3 (1.3)	86	7.9 (1.6)*
Chocolate Nut Cake	8.2 (1.7)	73	8.5 (1.3)	87	8.3 (1.4)
<u>Cold Beverages</u>	7.1 (1.3)	90	7.6 (1.2)	90	7.3 (1.1)
Grape Beverage	7.5 (1.4)	60	7.7 (1.5)	89	7.6 (1.4)
Orange Beverage	6.9 (1.8)	56	7.2 (2.1)	81	7.1 (1.8)
Lemon-Lime Beverage	6.8 (1.8)	90	7.2 (1.8)	85	6.9 (1.7)
Cherry Beverage	7.6 (1.6)	81	8.1 (1.2)	90	7.9 (1.2)*
<u>Hot Beverages</u>	7.3 (1.4)	89	7.6 (1.5)	91	7.5 (1.3)*
Coffee	6.3 (2.4)	77	6.9 (2.2)	83	6.5 (2.1)
Cocoa	8.2 (1.2)	89	8.2 (1.4)	90	8.2 (1.1)
<u>Candy</u>	7.7 (1.1)	91	7.7 (1.3)	91	7.7 (1.1)
Tootsie Roll	8.1 (1.2)	78	7.8 (1.9)	83	7.9 (1.6)
Charms	6.6 (2.3)	58	7.0 (2.4)	82	6.8 (2.2)
M&M's	8.8 (0.5)	84	8.8 (0.7)	84	8.8 (0.5)
Caramels	7.4 (1.6)	60	7.6 (1.8)	87	7.5 (1.6)
Gum	7.2 (1.8)	89	7.5 (1.9)	91	7.3 (1.7)
<u>Creamer/Sugar</u>	7.3 (1.5)	85	7.4 (1.7)	90	7.5 (1.3)
Creamer	7.1 (1.7)	84	7.4 (1.8)	90	7.2 (1.6)
Sugar	7.8 (1.3)	88	7.7 (1.7)	91	7.7 (1.3)
<u>Seasonings</u>	7.2 (1.5)	86	7.0 (1.8)	89	7.0 (1.5)
Hot Sauce	7.4 (2.3)	81	7.2 (2.2)	86	7.2 (2.1)
Salt	7.0 (1.7)	84	6.7 (2.2)	85	6.7 (1.8)

Table 11.1b Mean Individual Food Item and Acceptability Ratings for the LRP

<u>Food Item</u>	<u>Phase 3</u>	<u>n</u>	<u>Phase 4</u>	<u>n</u>	<u>Mean</u>
<u>Entrees</u>	7.3 (1.6)	91	7.9 (1.0)	91	7.6 (1.1)*
Beef Stew	7.4 (1.8)	80	8.4 (1.3)	88	7.9 (1.4)*
Escalloped Potatoes with Pork	7.3 (2.0)	80	7.9 (1.6)	86	7.6 (1.7)*
Chicken Stew	7.1 (2.2)	79	8.1 (1.4)	84	7.6 (1.7)*
Chicken ala King	6.7 (2.2)	82	7.5 (1.9)	88	7.2 (1.8)*
Chicken with Rice	7.4 (2.1)	83	7.9 (1.6)	88	7.7 (1.5)
Chili con Carne	7.2 (2.3)	84	7.2 (2.2)	84	7.2 (2.1)
Beef and Rice	7.4 (1.9)	79	8.0 (1.4)	85	7.7 (1.3)*
Spaghetti with Meat Sauce	7.6 (1.9)	84	8.0 (1.6)	88	7.8 (1.5)
<u>Starches</u>	8.6 (0.9)	89	8.6 (0.8)	91	8.6 (0.8)
White Pouch Bread	8.6 (0.9)	89	8.6 (0.8)	91	8.6 (0.8)
<u>Cereal Bars</u>	8.6 (0.6)	91	8.6 (0.7)	91	8.6 (0.6)
Cornflake Bar	8.5 (0.7)	91	8.6 (0.9)	90	8.5 (0.7)
Cornflake and Rice Bar	8.6 (0.7)	87	8.5 (0.8)	90	8.5 (0.6)
Granola Bar	8.6 (1.0)	90	8.6 (0.9)	91	8.6 (0.8)
<u>Dessert</u>	8.6 (0.6)	91	8.7 (0.6)	91	8.6 (0.6)
Oatmeal Cookie Bar	8.7 (0.7)	88	8.9 (0.5)	90	8.7 (0.5)
Fig Bar	8.7 (0.8)	88	8.7 (1.0)	88	8.7 (0.8)
Chocolate Covered Cookie	8.7 (0.6)	91	8.9 (0.3)	90	8.8 (0.4)*
Chocolate Covered Brownie	8.1 (1.3)	91	8.2 (1.3)	90	8.2 (1.2)
<u>Cold Beverages</u>	7.8 (1.1)	91	7.9 (0.9)	91	7.8 (0.8)
Apple Cider	8.2 (1.1)	91	8.5 (1.3)	91	8.3 (1.0)
Lemon Tea	7.2 (2.0)	89	7.3 (2.0)	90	7.3 (1.7)
Orange Beverage	7.8 (1.6)	90	8.1 (1.4)	91	8.0 (1.4)
Beverage Base	7.7 (1.7)	73	7.8 (1.5)	87	7.7 (1.4)
<u>Hot Beverages</u>	8.2 (1.2)	91	8.4 (1.0)	91	8.3 (0.9)
Cocoa	8.6 (1.0)	81	8.8 (0.7)	88	8.7 (0.8)
Coffee	7.9 (1.7)	87	8.0 (1.7)	90	7.9 (1.5)
<u>Candy</u>	8.0 (0.9)	91	7.8 (1.1)	91	7.9 (0.9)
Tootsie Roll	8.4 (1.0)	91	8.5 (1.0)	90	8.4 (0.9)
M&M's	8.9 (0.3)	83	8.9 (0.3)	87	8.9 (0.3)
Desert Bar	8.9 (0.4)	78	8.9 (0.6)	83	8.9 (0.4)
Chuckles	7.5 (2.1)	91	7.1 (2.2)	90	7.3 (2.0)
Charms	6.9 (2.1)	85	6.3 (2.6)	88	6.6 (2.2)
Caramels	7.7 (1.4)	84	7.3 (1.9)	91	7.5 (1.4)
Gum	7.6 (1.7)	91	7.8 (1.7)	91	7.7 (1.6)
<u>Creamer/Sugar</u>	8.0 (1.3)	91	8.3 (1.1)	91	8.1 (1.1)*
Creamer	7.7 (1.5)	89	8.1 (1.4)	91	7.9 (1.3)
Sugar	8.1 (1.3)	91	8.5 (1.0)	91	8.3 (1.0)
<u>Seasoning</u>	7.3 (2.0)	88	7.7 (1.8)	91	7.5 (1.6)*
Salt	7.3 (2.0)	88	7.7 (1.8)	91	7.5 (1.6)

* Significant Difference of $<.05$.

() Denotes Standard Deviation.

n = number of different subjects consuming the item.

RATION COMPARISONS

Meal, Ready-To-Eat Versus Long Range Patrol Ration

For comparison, the contents of the MRE and LRP were divided into seven food groups: Entrees, Desserts, Cold Beverages, Hot Beverages, Candy, Creamer/Sugar, and Seasonings. The mean acceptability ratings are summarized in Table 11.2 below and are taken from Tables 11.1a and 11.1b. The LRP received ratings that were significantly better ($p < .05$) in all food groups except Candy.

Table 11.2 Comparison of Acceptability Ratings for Food Groups in the MRE and LRP

	MRE	n	LRP	n	Significance
Entrees	6.6 (.92)	91	7.6 (1.1)	91	$p < .01$
Dessert	7.7 (1.1)	91	8.6 (0.6)	91	$p < .01$
Cold Beverages	7.3 (1.1)	91	7.8 (0.8)	91	$p < .01$
Hot Beverages	7.5 (1.3)	91	8.3 (0.9)	91	$p < .01$
Candy	7.7 (1.1)	91	7.9 (0.9)	91	n.s.
Creamer/Sugar	7.5 (1.3)	91	8.1 (1.1)	91	$p < .01$
Seasonings	7.0 (1.5)	89	7.5 (1.6)	91	$p < .05$

() Denotes Standard Deviation; n = number of different subjects consuming the item.

A further comparison was made between items in the two rations which either shared a common name or were identical. The results are summarized respectively in Tables 11.3 and 11.4. Six entrees items shared the same name but differed in that the MRE items were wet-pack and the LRP items were dehydrated. Beef Stew, Escalloped Potatoes, Chicken Stew, and Chicken ala King were all rated significantly

($p < 0.1$) higher in the LRP. Of the 14 items that were identical in the two rations, 11 were rated significantly higher by trainees when they consumed the LRP during the last two phases.

Table 11.3 Comparison of Acceptability Ratings for Similar Items in the MRE and LRP

	MRE	n	LRP	n	Significance
Beef Stew	6.2 (1.7)	89	7.9 (1.4)	90	$p < .01$
Escalloped Potatoes with Ham (Pork)	6.3 (1.8)	88	7.6 (1.7)	90	$p < .01$
Chicken Stew	6.1 (1.9)	81	7.7 (1.7)	88	$p < .01$
Chicken ala King	5.1 (2.2)	85	7.2 (1.8)	91	$p < .01$
Chicken and Rice	7.4 (1.6)	87	7.7 (1.6)	91	n.s.
Spaghetti with Meat Sauce	7.6 (1.3)	86	7.8 (1.5)	91	n.s.

() Denotes Standard Deviation; n = number of different subjects consuming the item.

Rating Differences Between Phases

The ratings for the majority of food items in the MRE (Table 11.1a) showed a slight improvement between phases 1 and 2 (31 out of 43). There was a statistically significant improvement in five items, three of which were entrees. In similar fashion to the MRE, the majority of ratings for individual items in the LRP (Table 11.1b) improved slightly between phases (22 out of 32). Seven of these were statistically significant, with five of the items located in the "Entree" Food Group.

An evaluation of changes within food groups found significant increased acceptability from the first to the second rating of each ration for entrees and desserts. Significant positive changes were also found for cold and hot beverages in the MRE and the creamer/sugar and seasoning for both the MRE and the LRP. For the rations

overall, the change over time was significant for both the MRE and the LRP.

Table 11.4 Acceptability Ratings for Identical Items in the MRE and LRP

	MRE	n	LRP	n	Significance
Oatmeal Cookie Bar	7.9 (1.6)	90	8.8 (0.5)	91	p<.01
Chocolate Covered Cookie	8.3 (1.0)	90	8.8 (0.4)	91	p<.01
Chocolate Covered Brownie	6.9 (1.9)	89	8.2 (1.2)	91	p<.01
Tootsie Roll	7.9 (1.6)	89	8.5 (0.9)	91	p<.01
M&M's	8.8 (0.5)	89	8.9 (0.3)	91	p<.05
Charms	6.8 (2.2)	88	6.6 (2.2)	90	n.s.
Caramels	7.5 (1.6)	88	7.5 (1.4)	91	n.s.
Cocoa	8.2 (1.1)	90	8.7 (0.8)	90	p<.01
Coffee	6.5 (2.1)	87	7.9 (1.5)	90	p<.01
Beverage Base	7.3 (1.1)	91	7.7 (1.4)	89	p<.05
Gum	7.3 (1.7)	91	7.7 (1.6)	91	n.s.
Creamer	7.2 (1.6)	91	7.9 (1.3)	91	p<.01
Sugar	7.7 (1.3)	91	8.3 (1.0)	91	p<.01
Salt	6.7 (1.9)	88	7.5 (1.6)	91	p<.01

() Denotes Standard Deviation; n = number of different subjects consuming the item.

Ration Consumption

As might be expected in a course where food deprivation is a major stressor, soldiers reported eating the entire ration in most instances. Soldiers reported consuming 80% of the items received during the first phase of training and this percentage increased over time to 93% for the final phase. Moreover, ration items were rarely either thrown or given away, with the percentage of such instances decreasing from 4.4% during the first training phase to 0.3% in the last.

Although fewer than 10% of instances of trading were reported by soldiers as what they typically did with a ration item, it is interesting to note that even in the final phase of training, trading was highest in the lesser liked items. For instance, Chicken ala King was the lowest or second lowest rated entree during each of the phases and was also the most traded with the exception of the instance where it was rated second lowest (and traded second most often).

CONCLUSIONS

A number of caveats are appropriate when evaluating the current results. First, while it is apparent that soldiers rated the LRP as more acceptable than the MRE, one cannot reliably conclude that the LRP is markedly more popular. Acceptability ratings of the LRP are likely to be somewhat higher due to the relative novelty of the ration. In addition, it is clear that the acceptability of both rations increased over time. Whether this is a function of the psychological and physiological consequences of the soldiers' food deprivation or other unevaluated variables cannot be determined with the available information. If some soldiers had consumed the rations in the reverse order (i.e., LRP during the first two phases and MRE during the latter two phases), it would be easier to determine the relative importance of ration type as opposed to whether the ratings for each ration were obtained early or late in the course. Nevertheless, ration items which are given a rating of "6" or better are regarded as acceptable, so regardless of these concerns, Ranger trainees endorsed both the MRE and LRP as acceptable products.

CHAPTER 12

RECOVERY FOLLOWING RANGER SCHOOL

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INTRODUCTION

The Minnesota starvation study was one of the first concerted efforts to examine recovery from severe weight loss. Keys et al. (1950) reported that normal physiologic function had not yet been fully restored even after 12 weeks of recovery from semi-starvation. Studies from World War II have also shown that long recovery periods from prolonged periods of food deprivation are necessary, especially when large amounts of fat-free mass are lost. Ranger training provides a unique model in which to observe recovery and differs from previous studies in that Rangers are young, healthy, fit males and that *ad libitum* eating is allowed in recovery (compared to controlled refeeding in earlier studies).

Physical performance and physiologic function can be adversely affected during Ranger training, Special Forces training, and other military operations of several weeks duration involving high daily energy expenditure and continuous caloric deprivation (Frykman et al., 1993; Friedl et al., 1994; Johnson et al., 1994). Knowing the time course to recovery of normal function from these situations has important military applications with respect to planning appropriate recovery periods between repeated deployments of combat units.

Limited assessment of long term recovery from the RGR-I study did show that several physiological parameters were restored by 6 months post-Ranger training, suggesting that no long lasting ill-effects result from Ranger training (Moore et al., 1992). Anecdotal reports from RGR-I indicate possible metabolic disturbances and severely hampered physical capacity for the first few weeks after Ranger training. With the exception of a Ranger study done in the early 70's which reported that aerobic capacity was still depressed 3 days after Ranger school, little data are available on short-term recovery from Ranger school (Johnson et al., 1976). Understanding the short-term problems experienced by soldiers may provide valuable data to support beneficial therapeutic strategies.

METHODS

One week measurements

Morning fasted blood samples were obtained one week after the end of Ranger training from nine study volunteers who remained at Fort Benning. Serum hormones were determined via radioimmunoassay as described previously: IGF-1, testosterone, SHBG, LH, T3, T4, TBG, TSH.

Five week measurements

Ten study volunteers traveled to USARIEM for extensive interviewing and questioning concerning diet habits and physical well-being, blood sampling, and body composition and physical performance testing. Dietary intakes from before and after the course were analyzed for macronutrient content using the Food Intake Analysis System (version 2.1, 1993, Univ. of Texas Health Science Center, Houston, TX). An eight page questionnaire (Appendix F) was administered to identify problems experienced after Ranger school. Serum hormones (IGF-1, testosterone, SHBG, LH, T3, T4, TBG, TSH), indices of vitamin status (vitamins B₁, B₂, B₆, B₁₂, C, and D), metabolic markers (ferritin, glycerol, cholesterol, HDL cholesterol, lactate, nonesterified FA, prealbumin, transferrin) were measured as described previously. Body mass components (body mass, fat-free mass, and fat mass) were determined by DEXA, and maximal lift capacity, vertical jump, and explosive power were tested as described in Chapter 4.

RESULTS

Dietary intake

Estimated caloric intake and macronutrient distributions of diets are listed in Table 12.1. Energy intake during the month after Ranger school was approximately 68% higher than before Ranger training. Overall, the percentage of calories from

Table 12.1 Comparison of caloric intake and macronutrient distribution (carbohydrate/protein/fat) as percentage of calories before and during the month after Ranger School.

Subject	Before	After	Comments
122	*	*	<First day after coming in from final phase of course "ate until felt sick but 2 hrs. later ate again" - reported intake this day of ~4780 kcal (45% fat) <Intense snacking lasted ~3 days, still higher than normal
127	3664 kcal (60/12/28)	5057 kcal (46/14/40)	<"Not a sweet eater" before the course, craved sweets after
150	1918 kcal (52/18/30)	3283 kcal (46/15/38)	<The biggest meal eaten after school was a few days after while at a restaurant on leave consisting of ~2100 kcal (49% fat) <During the first two weeks after would "eat anything in sight, would see a Taco Bell and stop and eat even if not hungry, the sight of food would trigger a desire for it" <Four weeks after in one sitting ate 1/2 roll of raw cookie dough, "would never have done that before"
170	1962 kcal (45/23/27)	4891 kcal (48/17/34)	<Driving by a fast food restaurant, would get the urge to eat <Food cravings started to lessen after 4 weeks
203	2950 kcal (48/13/38)	5045 kcal (54/13/32)	<Ate more breads, drank soda (didn't before the course)
221	2674 kcal (50/16/34)	3333 kcal (52/16/32)	<Normally skips lunch, couldn't after course, starting to feel like doesn't need lunch again (5 weeks after) <First chance to eat outside Ranger School, ate chocolate and fried foods
235	2427 kcal (52/14/33)	4506 kcal (58/8/33)	<Tried to control eating after course to keep weight off for athletic competition
238	*	*	<Ate double portions in mess hall and many fast food meals after the course
250	2738 kcal (68/17/14)	5836 kcal ^a (53/12/34)	< ^a Reflects 2nd to 4th week after while eating in mess hall <During the first week after (while home on leave) gave a sample day's intake of 8673 kcal (42% fat) <Stated that during Ranger School while eating in mess hall between phases ate so much had to vomit
266	2975 kcal (52/16/32)	3953 kcal (53/12/35)	<The first evening outside Ranger school went to two restaurants and consumed ~3425 kcal (34% fat) <Cravings subsided after 4 weeks - initially after craved food in general but now considers cravings of specific foods at certain times to be his normal habit
Mean	2664 (53/16/30)	4488 (51/13/35)	

*unable to quantify intake for nutrient analysis

carbohydrate and protein decreased, and the calories from fat increased from values recorded before Ranger school (30% vs. 35%).

Body composition

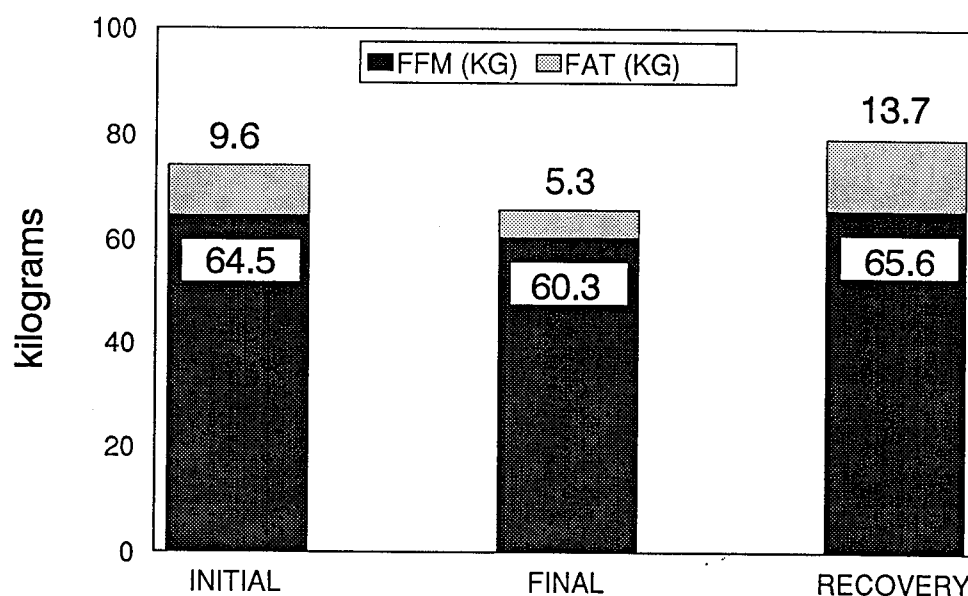
In recovery, fat-free mass returned to initial levels by the fifth week. Total body mass increased to significantly ($p < .05$) greater than initial levels (107%). This reflected primarily large gains in fat mass (~162% of initial levels). In recovery, fat mass (13.7 kg) nearly tripled from the post-value (5.3 kg) (Tables 12.2 and 12.3 and Figure 12.1). In recovery, all subjects possessed greater than initial levels of fat mass. By comparison, eight and six subjects had greater than initial amounts of body mass and fat-free mass, respectively.

Physical performance

Maximal lift capacity and explosive power returned to pre-levels, as did vertical jump (Tables 12.2 and 12.3). However, while vertical jump at the start of the course (48 cm) and in recovery (45 cm) were statistically similar ($p > .05$), the marginal difference can most likely be attributed to the large gain in fat mass. In comparison to the body composition components, the physical performance measures exhibited less of a return when viewed in the context of individual values. When the number of individuals returning to or exceeding initial values in strength (6), power (4), and vertical jump (3) was observed in recovery, it was clear that, for some individuals, components of body mass were restored before full recovery in physical performance was achieved. Because of greater than initial amounts of body mass and fat mass, the strength-to-mass ratio for subjects was less in recovery than initially. Figure 12.2 illustrates initial, final, and recovery values for all subjects.

Serum hormones

Serum hormones were restored to normal levels within one week after the end of the training except for T3 which was markedly elevated over start values (Table

Figure 12.1 Body Composition Changes**Table 12.2** Initial, final, and recovery means $\pm$ SD of body composition and physical performance variables.

Variable	Initial	Final	Recovery
Body Composition			
% BODY FAT	12 $\pm$ 5 ^a	8 $\pm$ 4 ^b	17 $\pm$ 3 ^c
FAT-FREE MASS (kg)	65 $\pm$ 5 ^a	60 $\pm$ 6 ^b	66 $\pm$ 7 ^b
FAT MASS (kg)	10 $\pm$ 5 ^a	5 $\pm$ 3 ^b	14 $\pm$ 4 ^c
Physical Performance			
STRENGTH (kg)	77 $\pm$ 9 ^a	61 $\pm$ 9 ^b	77 $\pm$ 8 ^a
POWER (watts)	3816 $\pm$ 407 ^a	2949 $\pm$ 269 ^b	3820 $\pm$ 274 ^a
VERTICAL JUMP (cm)	48 $\pm$ 7 ^a	39 $\pm$ 3 ^b	45 $\pm$ 5 ^a

* different letters denote statistical significance ($p < .05$)

Table 12.3 Relative body composition and physical performance (mean (range)).

Variable	% loss from initial	% gain from final	% of initial value 5 wks recovery
Body Composition			
Body mass	-11.3 (-8.1 - -14.3)	20.8 (11.4 - 30.2)	107.1 (97.9 - 115.3)
Fat-free mass	-6.6 (-2.6 - -8.8)	8.8 (2.8 - 15.9)	101.6 (96.6 - 108.3)
Fat mass	-42.6 (-22.5 - -63.4)	190.3 (81.4 - 337.4)	161.7 (102.4 - 238.5)
Physical Performance			
Strength	-21.2 (-10.5 - -26.7)	27.9 (11.8 - 45.5)	99.7 (93.3 - 106.7)
Power	-22.3 (-13.0 - -31.7)	29.5 (24.8 - 37.7)	100.4 (90.8 - 113.2)
Vertical jump	-17.5 (-2.5 - -29.9)	16.5 (2.9 - 26.8)	94.6 (82.5 - 109.7)

Figure 12.2 Initial, Final and Recovery Data for (a) Maximal Lift Capacity (n=9), (b) Explosive Power (n=8) and (c) Vertical Jump (n=8).

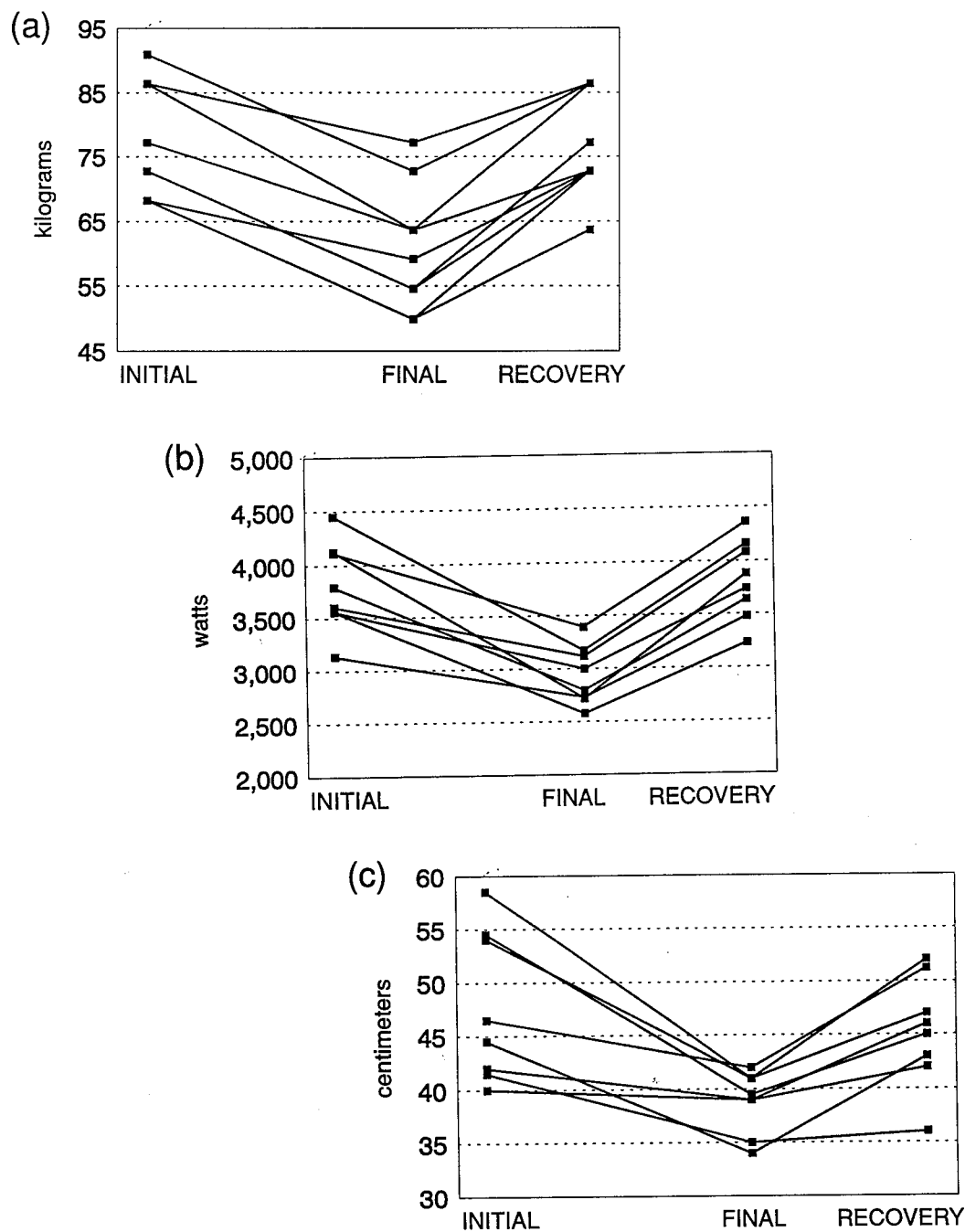


Figure 12.3 Relative values for serum hormones at the end of the course, and at 1 and 5 weeks after the end compared to start values (% of start).

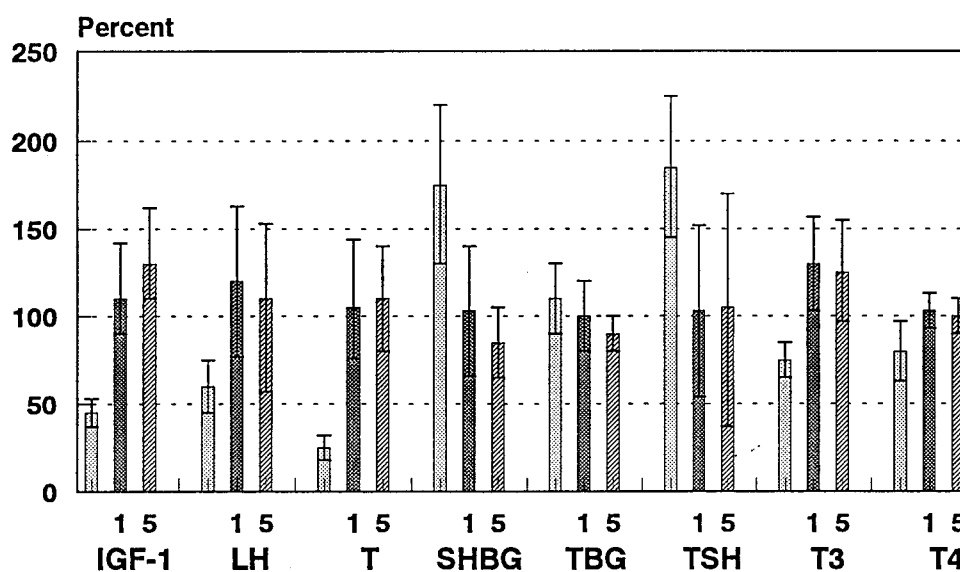


Table 12.4 Relative values for serum hormones at the end of the course, and at 1 and 5 weeks after the end compared to start values (% of start).

Parameter	End (n=19)	+1 wk (n=9)	+5 wks (n=10)
Serum Hormones			
IGF 1	45±12*	111±29	129±30*
Testosterone	18±9*	111±34	116±26
SHBG	191±35*	109±29	82±21*
LH	68±27*	124±37	117±38
T3	76±10*	135±18*	130±21*
T4	86±12*	104±13	100±14
TBG	120±12*	102±15	88±11*
TSH	217±73*	114±39	118±53

★ p<0.01, single sample t-test

12.4 and Figure 12.3). T3 levels were higher than starting values for each of the nine individuals assessed, including a large rise from below normal range starting value for an ovolactovegetarian Ranger. These changes are comparable to the changes observed at the mid-mountain sampling period which also represented approximately one week of refeeding. At five weeks, T3 was still elevated above starting values and the binding proteins (TBG & SHBG) were significantly reduced from initial values. The increase in thyroid hormone and the reduction in insulin-sensitive binding proteins supports the self-reported hyperphagia of these soldiers at five weeks post-training. T3 and metabolic rate are specifically stimulated by carbohydrate overfeeding (Welle & Campbell, 1983), but with a more gradual refeeding, T3 levels and metabolic rate do not necessarily return to baseline by five weeks (Barrows & Snook, 1987).

Vitamin indices and metabolic markers

Tables 12.5 and 12.6 lists all vitamin indices and metabolic markers. All the vitamin values were within the normal reference ranges at the five week follow-up. With the exception of lactate, all of the metabolic markers were within normal reference values and close to the baseline values. The blood draw was scheduled for the second morning the subjects were at USARIEM. The subjects were in a 12-hour fasted state and had not exercised prior to the blood draw.

Follow-up questionnaires

Gastrointestinal symptoms Table 12.7 gives the breakdown of gastrointestinal symptoms including the number of subjects reporting each symptom and the frequency and duration of the symptoms. Immediately after Ranger school, most volunteers reported experiencing some form of gastrointestinal distress. This is common during refeeding after a period of calorie deprivation (one of the lowest periods of energy intake during Ranger school was at the end of the final phase).

Table 12.5 Indices of Vitamin Status at 5 Week Follow-up

(n=10)	Reference values	Units	Baseline	End	5 week follow-Up
Vit B ₁	<76	%	18±7	14±5	11±6
Vit B ₂	<23	%	5±2	9±6	6±4
Vit B ₆	<130	%	81±17	72±26	71±12
Vit B ₁₂	171.2-839.6	pmol/L	376.2±103.8	541.9±115.0	346.0±90.0
Vit C	25.2-126.1	µmol/L	83.9±24.5	162.3±12.5	55.2±6.4
Vit D	25.2-126.1	nmol/L	98.7±21.5	111.9±17.3	81.7±29.5

Table 12.6 Metabolic Markers at 5 Week Follow-Up

(n=10)	Reference values	Units	Baseline	End	5 week follow-Up
Ferritin	0.49-10.04	pmol/L	1.9±0.9	3.6±1.2	1.1±0.6
Glycerol	61.0-232	µmol/L	90.3±21.9	131.1±45.2	132±46.0
HDL Cholesterol	0.70-1.70	mmol/L	1.2±0.4	2.3±0.5	1.2±0.2
Lactate	0.30-1.30	mmol/L	2.4±0.7	2.6±0.5	4.7±0.7
Nonesterified FA	0.10-0.60	mmol/L	0.4±0.2	0.9±0.3	0.1±0.1
Prealbumin	17.0-42.0	mg/dL	26.8±4.0	21.3±2.7	26.8±3.5
Transferrin	32.1-54.6	µmol/L	37.8±3.0	36.7±2.3	37.6±2.8

Table 12.7 Gastrointestinal symptoms experienced after Ranger School

Symptom	n	Freq/day	Duration in days
Nausea	3	3.7	7.7
Vomiting	2	2.0	2.0
Diarrhea	6	4.1	8.2
Cramping	2	2.0	12.0

One subject who had reported nausea, diarrhea and cramping commented that he felt light-headed after eating. Another attributed stomach upset to eating many "Mexican and Italian" foods, but that it did not stop him from eating those foods. A third attributed gastrointestinal problems to eating a lot more "real" food as opposed to dehydrated rations, while a fourth attributed problems to eating many "unhealthy foods" after the course. One subject said that there were certain foods that upset his stomach that would not have before Ranger school.

When asked whether they reached a "full" feeling faster than they had prior to Ranger school, 50% answered "yes." One approximated that he reached fullness after eating 3/4 of his regular portion sizes of foods. Two who answered "no" reported that it took more food than usual to reach fullness. One reported never feeling full in the three weeks following the course.

Food frequency and food cravings

Table 12.8 depicts the results of the frequencies of food groups eaten before and after Ranger school. Subjects were asked to state whether they consumed more, less or the same amount of groups of foods during the one month following Ranger school than before the course. Subjects were unanimously consuming more sweet

and high-fat foods during the month following the course. Most, in fact, craved these foods after leaving the course.

The most frequently craved foods and the percentage of volunteers reporting them were: chocolate - 80%, doughnuts - 40%, ice cream - 40%, pizza - 30%, and peanut butter - 30%. Two subjects reported craving beer. Other foods reported included: meat, bread, cake, eggs/bacon, chili, Pop Tarts®, french fries, lasagna, cookie dough, pancakes, waffles, brownies, pasta, salami sandwiches, fritos/bean dip, Twix®, M&M's®, Reeses Pieces®, rice/beans, salads, cheeseburgers, Taco Bell®, Arby's®, barbecue, Chinese, Mexican, milk, and soy products. Only one subject reported avoiding foods - sweets, fast foods, candy bars and "junk food." He stated this was due to not wanting to gain weight back.

Table 12.8 Frequency of food consumption by food groups before and after Ranger School

Foods	More	Less	Same
Beef	6	1	3
Fried foods	5	1	4
Breads	7	0	3
Doughnuts, cookies, cakes, pastries	10	0	0
Fruits/vegetables	5	2	3
Salty snacks (chips, peanuts)	5	0	5
Ice cream	7	2	1
Chocolate candy	8	2	0
Beverages *	5	1	4
Other*			

* see discussion

Eating environment

Volunteers were asked how often in a two-week period they ate in fast food restaurants before Ranger school, and in the two weeks after the course. On average, they frequented fast food restaurants three times (range zero to 12) as often immediately following compared to prior to the course. Sixty-percent were now eating their meals primarily in the military dining facility, 20% in restaurants and 20% at home.

Physical training

Only four of the subjects took a PT test within the first month after graduation from Ranger school. Three reported scores lower than their usual with the run being the most affected event. One reported being able to complete only half his usual number of pushups. The other six subjects expressed concern about being able to pass the PT test at Battalion standards. Three of these six felt their endurance was affected most, and two believed that they had lost a lot of arm strength. One reported his score was the same as usual but felt weaker all over (this subject was the one that entered Ranger school 15-20 pounds over ideal body weight).

In terms of physical training pre- and post-Ranger school, nine reported a decrease in the intensity and the amount of work they could accomplish. Two had not established a workout routine. The one subject that had completed Air Assault school after Ranger school did PT daily at the school, but said it was not a very difficult PT program. One of the subjects was an avid bicyclist and reported dropping from his pre-Ranger routine of 175 miles per week to 75 miles per week.

Sleep Prior to Ranger school, ten subjects reported they slept for a period of 5-10 hours per night (average = 6.5 hours) with no sleep problems. All the subjects reported changes in their sleep patterns at the five week follow-up. The majority appeared to sleep more total hours in a day but in shorter increments. Three reported an inability to stay asleep, and two also reported an inability to stay awake. Four said they added naps to increase the amount of sleep in a day. One said that he basically

only woke to eat during the 5-10 days following Ranger school.

Mood Eight claimed that they were not back to normal motivation levels. Particularly, they felt lazy, unable to push themselves physically, and unmotivated at work. The two that were back to normal levels reported different recovery methods. One did "strong" PT and regulated his diet and reported that it took 20 days to get back to normal. The other did low intensity exercise until his weight increased and was back to normal in two weeks.

When asked what five things (in order of priority) were important to do on their first day post-graduation, five chose eating as being the first priority and four picked eating again up to a week after graduation. Eating was the most commonly chosen priority ranked between first and third for both sets of rankings. Sleep was only chosen once as an immediate priority and normally ranked between third and fifth priority for both sets of rankings.

Return to normal eating When asked how long it took for eating habits, i.e. amount and types of foods, to return to normal, 50% reported that they had not yet returned (~five weeks). One reported that it took 15 days to begin to return to normal, three reported it took 20-21 days, and one reported 30 days.

CONCLUSIONS

Following Ranger school, Rangers experienced full restoration of all measured parameters (body composition, hormones and metabolic makers, physical performance) by the fifth week of recovery. A hierarchy of recovery did appear to exist as variables seemed to recover at varying rates. The accretion of fat mass occurred at an accelerated rate when compared to other body mass components. Also, the tissue components of the body appeared to recover more rapidly than physical performance measures. Thus, caution should be exercised when relying solely on the monitoring of tissue mass for assessment of malnutrition and/or recovery (Keys et al., 1946; Lopes et al., 1982; and Russel et al., 1984). Evaluation should be

done on a individualized basis because, for some individuals, body mass and fat-free mass had been restored before full recovery in physical performance.

The hormone markers indicated full recovery. Based on the vitamin analyses coming back down to baseline levels, the LRP appears to be very well fortified and provides more than usual amounts of Vitamin B12, C, D, and thiamin. The drop in ferritin due to the stressors of Ranger training quickly returned to normal. Elevated HDL promptly returns after a substantial rise in response to the deficit. Prealbumin decreases and returns to normal, indicating the possibility of its merit as a marker of early protein-energy malnutrition (PEM) during starvation. Thus, the adequacy of protein intake with a restricted ration during intense exercise is a legitimate concern that should be addressed in future studies.

The recovery process, however, was not without complications as Rangers exhibited a rebound in fat mass over baseline levels as well as gastrointestinal and sleep irregularities. Rangers were allowed to eat *ad libitum* after Ranger school, and dietary recall data revealed that food intake was approximately 68% greater in recovery than before Ranger training. Interviews revealed that the eating habits after the course were characterized by overconsumption of all foods but especially by cravings for fatty foods from fast food restaurants and for foods tasting "sweet". In the context of this large energy surplus, other factors such as 1) a possible depressed resting metabolic rate that sustains during recovery, 2) an increased caloric contribution from fats, and 3) a "gorging" pattern of eating allowed a propensity to weight gain, and most notably, fat mass gain (Grande et al., 1958; Heshka et al., 1990; James et al., 1990; and Munch et al., 1993). It should be pointed out that two of these factors are behavior characteristics, and can therefore be changed. The elevated IGF-1 and T3 values in recovery reflect this overnutrition, and the SHBG and TBG suppression is directly traceable to high insulin. Rangers also reported reduced physical activity after Ranger training associated with feelings of fatigue and loss of motivation. In recovery, Ranger students seemed to possess many symptoms that mimic diabetes. The elevated lactate values and reports of diarrhea among the Rangers are also commonly seen in patients with short bowel syndrome in which there is an inadequate absorptive surface. This suggests that during recovery, a

deficiency with respect to absorption may exist for Rangers (Karton et al., 1987).

In summary, large decrements in body mass components, hormones, metabolic markers, and physical performance attributed to an energy deficit are reversible by 5 weeks after *ad libitum* eating. Despite these restorations, during recovery Rangers can be expected to experience a rebound in fat mass, sleep irregularities, gastrointestinal problems, and voracious appetites.

CHAPTER 13

RECOMMENDATIONS

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DISCUSSION

The main objective of this study was to increase caloric intake by at least 15% above the estimated intake of the RGR-I study, thereby decreasing the impact of the training on weight loss and suppressed immune function while still providing a stressful level of food deprivation acceptable to the Ranger cadre.

Body weight losses for RGR-I and RGR-II were 12.1 and 10.0 kg, respectively. Although the caloric intervention had a relatively small effect on weight loss, it had a marked effect on metabolic status. It was sufficient to keep the men from entering the next phase of starvation involving increased catabolism of lean mass. At the end of training, the soldiers in the RGR-II study still had some fat stores, even though they were significantly reduced. In RGR-I, most men had depleted their fat stores. This difference between studies was also reflected in expedient indicators of body composition such as skinfold thicknesses and abdominal circumference.

Thyroid hormones, primarily T3, remained within the low normal range, indicating that the regulation of metabolic rate was closer to normal than in RGR-I where T3 was reduced below normal.

There was only a slight attenuation of the decline in FFM (-6.1% of initial, compared to -6.9% in RGR-I), probably representing an inevitable FFM loss which accompanies a large reduction in fat weight. This similarity to RGR-I reiterates how small the nutritional intervention really was, even though it had a significant physiological benefit. The benefit stems from keeping the soldiers just below the threshold for marked muscle catabolism which occurs subsequent to depletion of fat stores. In RGR-I, soldiers finished the course just over this threshold and were metabolically poised for cachexia. During RGR-II, there was a delayed rise in cortisol in response to the declining fat stores. This indicates that the feeding intervention extended these soldiers' energy stores by roughly two weeks (one phase of training); the same estimate is obtained from the change in body energy stores.

Dynamic muscular strength assessed by maximal lift capacity and by power from jump performance demonstrated significant decrements which are only moderately associated with declines in muscle mass. The decrement in maximal lift capacity was nearly identical to the decrement observed in RGR-I, again indicating how small the intervention was, or reflecting an all-or-none decline in performance associated with restricted feeding.

With the exception of rebound changes in body fat and several other metabolic markers, all measures had returned to normal within approximately one month (five weeks) following the end of Ranger training. In the ten soldiers assessed, fat had increased by 40% above normal levels, and binding proteins (SHBG and TBG) were suppressed in response to hypercaloric intakes. Data obtained in RGR-I suggests that hyperphagia peaks at approximately one month, and all parameters are normal by six months post-training (and probably sooner). As body composition and metabolic rate normalizes, sleep quality is also expected to be restored (Shapiro et al. 1990).

The fact that the sleep deprivation data from RGR-II is remarkably similar to the data collected during RGR-I is important for two reasons. First, given the severity of the sleep deprivation, there is little doubt that this stressor continues to be a major variable that may affect many of the physiological responses. Second, the fact that the sleep deprivation patterns are similar between the studies demonstrates strict standardization of the Ranger training plan of instruction (POI). This adds to the validity of comparing data from RGR-II to RGR-I.

Cognitive performance showed a substantial impairment during RGR-II. While the volunteers showed a brief period of recovery during the dining hall feeding portion of the mountain phase, deficits appeared to be cumulative over the course.

It was concluded from the RGR-I study that the results suggested a condition of uncomplicated energy deficiency. There was no evidence of a vitamin or mineral deficiency despite the extreme level of energy deficit. This same conclusion can be made concerning RGR-II.

The pattern of changes in the various metabolic markers measured during the 8 weeks of training is consistent with changes in body composition and the semi-starvation regimen. Although a number of the markers were shown to be outside of the normal reference ranges, there is no reason to consider any of these observations would produce long term health risks. Even the hyperlipidemia corrects within five weeks of the end of training. Many of the markers returned to normal by the end of the dining hall feeding of the mountain phase. Despite the caloric restriction, there was no indication from our data that the subjects would have benefitted from increased levels of, vitamins, and minerals.

Some descriptive comparisons can be made between the two studies. The mean values for serum nonesterified fatty acids, β -hydroxybutyrate, and lactate at the end of the mountain phase for both studies are shown in Figure 8.4. The mean values for all three of these metabolic markers were decreased in the RGR-II study, indicating a benefit of the caloric intervention. This trend is in agreement with those discussed above concerning body composition.

The most important finding from the RGR-I study was the decreased *in vitro* lymphocyte proliferative response to mitogen stimulation. With respect to the effect of the increased caloric intake, the immunological results from RGR-II are in agreement with the conclusions discussed above. When the proliferative data are compared by phase, the increased caloric intake showed a marked benefit in both the mountain and the desert phases of training. Despite the improved proliferative response shown in this caloric intervention study, the immune response is still suppressed below baseline levels and may continue to increase susceptibility to infection.

While the proliferative response provides information concerning cells involved with a specific immune response, the oxidative burst assay reflects the functioning of the non-specific immune response. Given the enhanced oxidative response as the training progressed, the first conclusion would be that this would suggest a beneficial response. However, as discussed in Chapter 10, these findings may suggest impaired gut function leading to translocation of gut bacteria and subsequent 'priming' of neutrophils.

In summary, the results of this study exploring the metabolic, physiological, and cognitive effects of a 15% increase in calories provided during Ranger training confirm the general conclusions of an earlier assessment (RGR-I). The present results provide some evidence that the decrease in immune function may be at least partially a consequence of an imbalance between energy expenditure and energy intake. Weight loss, body composition changes, and immune function responded positively, as anticipated, to the provision of 400 additional kcal/day. This was a relatively small diminution of the total negative caloric debt but did bring weight loss down. Immune function tests seemed to reflect a positive response to the increased calories, but it was impossible to separate combined stressors (such as physical, mental, and sleep deprivation) from the caloric deprivation. Cognitive function tests showed a significant impairment, probably due to the combination of food and sleep restriction.

RECOMMENDATIONS

The following recommendations are presented with the understanding that the Ranger cadre considers some level of energy deficit a critical stressor during Ranger training.

◆ During the FTX periods, students should be offered a caloric intake between 1600 to 2000 kcal/d. This could be accomplished by offering one MRE/d supplemented with pouch bread or by offering three MRE's every two days. Alternatively, once the LRP is available, the cadre should offer one LRP and one pouch bread per day.

◆ If any changes are made to the current POI such as lengthening the FTX phases or adding activities that increase energy expenditure, the Cadre should consider an additional caloric supplementation regimen.

◆ Additional research studies should be planned and directed at ameliorating the documented immunological perturbations. These studies could involve single nutrient supplementation that do not affect the caloric restriction programmed in the Ranger training.

◆ The effect of Ranger training on cognitive function should be further characterized. The effect on cognitive function from interventions such as nutrition supplements or changes in sleep patterns should be evaluated.

◆ Additional research investigations should be planned to study the effects of Ranger training during the winter months. These studies should attempt to better characterize perturbations at the cellular and molecular level as they would allow inferences to be made about metabolic consequences and the risk of infection, and they would allow provisions to be made to protect against environmental injury.

◆ An intensive, short-term (2-3 weeks) study on recovery from Ranger school would yield valuable information. The hierarchy of recovery needs to be firmly established, as this will allow for practical markers of recovery.

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VOLUNTEER AGREEMENT AFFIDAVIT

PART B — TO BE COMPLETED BY INVESTIGATOR

INSTRUCTIONS FOR ELEMENTS OF INFORMED CONSENT: *(Provide a detailed explanation in accordance with Appendix C, AR 40-38 or AR 70-25.)*

You are being asked to participate in a study to determine what effect Ranger training has on your body composition, your resistance to infection, and your strength. The data collected from this study will be useful in the continued improvement of the effectiveness and safety of Ranger Training and it will contribute to our understanding of the effects of stress on the nutritional status and infection resistance in soldiers.

If you participate in the study you will be asked to submit to a set of measurements before you begin the course, at the end of the Benning Phase, end of the Desert Phase, middle of the Mountain Phase, end of the Mountain Phase, and end of the Jungle Phase. This study has been fully coordinated with the Ranger Cadre and will not interfere with the training you must be able to complete to earn your Ranger tab.

We will ask you to complete a brief questionnaire which asks a series of general questions about you (such as name, age, rank, ethnic background) and your normal lifestyle (tobacco/alcohol use, exercise habits, weight status).

Before you begin the course or within the first 4 days and at the end of the course, we will take a number of measurements on you. These measurements are described as follows:

1. We record your height and body weight (shorts only). We will assess your body composition using several methods:

a. We will obtain tape circumference measurements at 5 sites (neck, abdomen, hips, thigh and calf). A caliper device will be used to determine skinfolds measurements at your biceps, triceps, thigh and at two areas on your back. These measurements will be used as an estimate of your body fat. These measurements will not cause any pain or discomfort.

b. We will measure your body composition using a dual energy x-ray absorptiometry (DEXA) device. This machine is a low-energy x-ray scanner which enables us to measure your body fat, lean body mass (muscle) and your bone density accurately. You will lie on your back and the machine will automatically scan the length of your body, and a computerized detector will record the x-ray transmissions. The entire test will take 10 minutes. The device will not cause any pain or discomfort and only exposes you to 1/30 the radiation dose of a conventional chest x-ray.

(continued on following page)

I do ☐ do not ☐ (check one & initial) consent to the inclusion of this form in my outpatient medical treatment record.

SIGNATURE OF VOLUNTEER

DATE

PERMANENT ADDRESS OF VOLUNTEER

TYPED NAME OF WITNESS

SIGNATURE OF WITNESS

DATE

Volunteer's Initials _____

Witness's Initials _____

c. We will measure your body water concentration using a device called a bio-impedance analyzer. This device will be attached to your hands and feet with wires held in place by velcro straps. The measurement will take less than 5 minutes and will cause you no pain or discomfort. The device will perform an electrical safety check to guard against the danger of shock.

2. We will collect 5 blood samples in order to measure the levels of certain substances which will tell us the level of particular nutrients in your body and also about how stress and physical activity are affecting your muscles. Your blood will be analyzed for the concentrations of albumin, blood urea nitrogen, calcium (total), cholesterol, CO₂, gamma glutamyl transferase, glucose, B-hydroxybutyrate, iron, lactate dehydrogenase, magnesium, phosphorus, potassium, sodium, total bilirubin, total iron binding capacity, total protein, triglycerides, uric acid, alanine aminotransferase, aspartate aminotransferase, glycerol, high density lipoprotein, lactate, non-esterfied fatty acids, ascorbic acid, copper, folacin, glutathion peroxidase, metallothionein, riboflavin, selenium, thiamin, vitamin A, vitamin B₆, vitamin B₁₂, vitamin D, zinc, serum transferrin, ferritin, prealbumin, retinol binding protein, gamma globulins, testosterone, estradiol-17b, cortisol, thyroxine, thriiodothyronine, thyroid binding globulin, thyroid stimulating hormone, growth hormone, sex hormone binding globin, insulin like growth factor-1, and malatonin. We will also isolate cells from two of the blood samples and use these to determine how well specialized white blood cells, which are important in disease resistance, are working. We will take blood samples by needle puncture of a vein in your arm. There is a small risk of a bruise forming at the puncture site, but this will gradually disappear. This procedure will be performed using a sterile technique by a skilled technician. At each blood draw we will collect 5 tubes of blood or about 52 milliliters of blood which is the equivalent of about 1/10 of a pint. The total blood volume that will be taken over the whole study period is less than the amount given in a one time normal blood donation.

3. We will give you a skin sensitivity test to determine how well your immune system is working. After the blood samples are taken, a trained technician will administer a group of eight (8) tests called "tine tests". The tine test consists of a plastic applicator with 8 round disks, the diameter about the size of a pencil eraser. Each little disk has 6 pins that are coated with a chemical that has been isolated from a different bacteria. The test is given by pressing the applicator on the forearm. The application causes minor discomfort which disappears usually in one to two minutes. There is no chance of becoming infected from the bacteria because the substance that is placed on your skin is an extract from the bacteria, not the bacteria itself. If you have been exposed to this infectious organism before, you will develop a small red area around the tine stick site. The size of the spot depends on if you have ever been exposed to the organism before and how well your immune system is working. The procedure is performed using a sterile technique by a skilled technician and there is a slight risk of infection at the stick site by bacteria in the air, but by using aseptic techniques the chance of infection is slight. If you react positive to any of the seven tests, you will get a reddening, itching and mild discomfort at the stick site.

There is a risk that you may have a severe local or systemic allergic reaction to the chemical compounds used in the skin test kit. If you have a history of allergies or allergic reactions to medications or insect stings, you will not be tested with the skin test kit until you have been carefully examined and cleared by a physician. Medical personnel and equipment will be present when you are administered the skin test, so that emergency treatment can be provided if you have a severe reaction to the test materials. In addition, medical personnel will be closely observing soldiers who receive the test kit in case any delayed reactions occur.

4. You will be asked to perform two tests which provide estimates of your muscle strength. First, you will be asked to perform a maximum incremental lift test. The device used in this test has a set of weights enclosed in a rack. The rack is attached to handles which when lifted move the rack up a vertical track (similar to a fixed weight Nautilus machine). In this test you first lift a

Volunteer's Initials _____

Witness's Initials _____

moderate weight (40 pounds) to 72 inches. Additional weight will be added in 10 or 20 pound increments until you cannot lift the rack to a full 72 inches. The second test is a jump test for which you will be asked to jump up in the air from a special metal pad which measures the force of your liftoff. Both of these tests will produce information about muscular explosive power and how that changes through Ranger training. Although you could over exert yourself during this test which would result in musculoskeletal injury, our staff will monitor you to prevent this from occurring.

5. You will be asked to complete questionnaires which test several aspects of mental performance. The tests will take no more than 10 minutes to complete. The purpose of these tests is to show the effects of Ranger training on your ability to analyze and remember information. You will be asked to complete a variety of pencil and paper tests of memory, reasoning, mood, personality, motivation and personal history. Your answers on these tests will be treated as confidential information within the limits prescribed by military regulations. As is true of any element of this experiment, you may decline to participate, if you feel that these tests are an imposition or an invasion of privacy.

In addition to taking measurements at the beginning and end of the training, we will make a few of the same measurements at 4 intermediate time points: the end of the Benning Phase, end of the Desert Phase, middle of the Mountain Phase, end of the Mountain Phase. These repeated measurements, as described above, are:

1. body weight, abdominal circumference, 1 skinfold measurement, and bio-impedance,
2. a skin test,
3. the test of memory, performance and reason, and
4. at each blood draw we will collect 5 tubes of blood or about 52 milliliters of blood which is the equivalent of about 1/10 of a pint. The total blood volume that will be taken over the whole study period is less than half the amount given in a one time normal blood donation.

Ten volunteers will be asked to have their energy expenditure determined using a non-radioactive isotope technique. You will be asked not to eat or drink anything for 6 hours before this test. We will give you about 1/2 glass of modified water to drink. This water contains a non-radioactive marker which is safe to drink. We will allow 3-4 hours for the modified water you drink to mix with your body water. During this time, you will be asked not to do any strenuous exercise or work, and not to eat, drink, smoke or chew tobacco. A saliva sample (1 teaspoon) will be collected for chemical analysis three times during this procedure. On the first day and on several days after you drink the water, you will also be asked to provide a urine sample from the first time you urinate in the morning. This sample will be only tested for the modified water we administered to you. The results of this test will give an accurate estimate of the calories which you burned up each day. One possible side effect has been shown to occur with this procedure. Some subjects that have been given solution containing the non-radioactive isotope have experienced dizziness. However, this was at concentrations much higher than those used in this study.

Forty soldiers will be asked to wear a wrist activity monitor. This is a small battery driven device about the size of a wrist watch which is worn all the time for the duration of a training phase. This device will measure your sleep and activity patterns. There is no risk of electrical shock wearing this device, even when wet.

All the students that are from the Fort Benning Ranger Brigade will be asked to participate in a follow-up study after the graduation of this class. The measurements taken at the follow-up periods of 1, 2, 4 and 8 weeks post-graduation will be the same as described above for the beginning and end of the training.

1. DEXA, body weight, circumference measurements, skinfold measurements, and bio-impedance,

Volunteer's Initials _____

Witness's Initials _____

2. the test of memory, performance and reason, and

3. at each blood draw we will collect 5 tubes of blood or about 52 milliliters of blood which is the equivalent of about 1/10 of a pint. The total blood volume that will be taken over the whole study period is less than the amount given in a one time normal blood donation.

Participation in this study is on a voluntary basis. However, if you choose not to take part or if you choose to withdraw from the study, it will not change the course schedule for you. You may withdraw your participation from the study at any time with no penalty or adverse action taken against you.

The information you give, together with the other information that we will collect, will be treated in the strictest confidence. Only information bearing on your health may be revealed to appropriate medical or Command authorities if your data reveals something important to your health and well being. Information about you in your records may be inspected by officials of the US Army Medical Research and Development Command.

You will not receive any direct benefit from participating in this study, except the knowledge of your own strength, nutritional status, and body composition. You may request a copy of your results and the results of this study.

Before you sign this document, be sure that you have read it and fully understand it. If you have any questions concerning this study please ask so that you have a complete understanding of the nature and details of the study. You will be provided with a copy of this consent document for your information and personal record.

Personal History Survey

page 1

Army Research Institute of Environmental Medicine
Research for the Soldier

Rank

E ☐
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O ☐

1 ☐
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2 ☐
3 ☐
4 ☐
5 ☐
6 ☐
7 ☐
8 ☐
9 ☐

ETS

1992 ☐
1993 ☐
1994 ☐
1995 ☐
1996 ☐
1997 ☐
1998 ☐
1999 ☐
2000 ☐
>2000 ☐

Age

0 ☐
1 ☐
2 ☐
3 ☐
4 ☐
5 ☐
6 ☐
7 ☐
8 ☐
9 ☐

Race

☐ Asian origin
☐ African origin
☐ Hispanic origin
☐ Caucasian origin
☐ Other

Marital Status

☐ single
☐ divorced
☐ separated
☐ married

None Older Younger Twin

Brothers
Sisters

☐ ☐ ☐ ☐

Highest Educational Level

Unknown High School College Graduate School

Yours
Father's
Mother's

☐ ☐ ☐ ☐

Service in the Military

Yes No Unknown Not Applicable

Father
Mother
Brother
Sister

☐ ☐ ☐ ☐

I have been planning to come to Ranger School for:

☐ less than 3 months
☐ 4 to 6 months
☐ 7 to 9 months
☐ 10 to 12 months
☐ 1 year
☐ 2 years
☐ more than 2 years

APPENDIX C. Climatic Data

Phase I		Aug																		Climate Data	
Temp (F)		3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19			
High		93	93	92	91	89	91	96	95	95	92	81	83	84	85	86	88	78			
	Low	71	68	67	69	72	70	68	72	67	68	72	68	66	67	68	67	69			
Relative Humidity (%)																					
High		98	99	100	100	98	100	100	100	100	100	100	100	100	100	100	100	100			
	Low	41	53	50	58	57	58	43	49	49	53	79	63	57	58	51	55	86			
Rain Fall (in)		T	T	0.03	T	0	0	0	T	0.54	1.57	0.01	0	0.4	0	0	0.4	0			
Phase II		Sept																			
Temp (F)		20	22	23	24	25	26	27	28	29	30	31	1	2	3	4	5	6	7	8	
High		94	95	90	86	90	93	90	93	91	90	93	95	93	96	97	97	97	99	99	
	Low	69	73	73	70	63	66	68	67	74	70	64	68	68	68	68	69	71	64	76	
Relative Humidity (%)																					
High		70	50	61	91	83	84	64	72	41	55	71	59	41	41	39	44	29	45	40	
	Low	25	25	32	39	27	24	30	26	30	27	20	18	18	15	16	17	15	16	16	
Rain Fall (in)		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Phase III		Sept																			
Temp (F)		9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	
High		83	84	83	82	75	75	78	79	79	80	80	81	80	74	80	73	60	71	65	
	Low	61	61	59	60	60	57	54	50	51	63	64	63	64	68	64	53	53	53	59	
Relative Humidity (%)																					
High		No Relative Humidity Data Available																			
	Low																				
Rain Fall (in)		0.01	0	0	0	0	0	0	0	0	0	1.36	0.17	0.02	2.02	0.32	T	0	0	0.23	
Phase IV		Oct																			
Temp (F)		28	29	30	1	2	3	4	5	6	7	8	9	10	11	12	13				
High		73	70	69	73	70	69	81	76	75	76	79	83	75	78	75	77				
	Low	60	62	65	60	62	65	62	59	58	59	66	65	59	56	48	51				
Relative Humidity (%)																					
High		66	65	98	66	73	97	82	79	89	75	88	66	86	49	70	88				
	Low	44	58	94	48	61	94	46	51	33	65	71	51	68	28	24	48				
Rain Fall (in)		0	T	3.59	0	T	3.59	T	0	0	T	0	0	T	0.23	0	0				

(T) = Trace

CLINICAL CHEMISTRIES, NORMAL LIMITS

<u>Parameter</u>	<u>Reporting Units</u>	<u>Reference</u>
GENERAL CHEMISTRY PANEL		
<u>ELECTROLYTE PANEL</u>		
Blood Urea Nitrogen (BUN)	7-18 mg/dL	Pennington Labs
Total Carbon Dioxide (TCO ₂)	21-31 mmol/L	Pennington Labs
Chloride (Cl)	101-111 mmol/L	Pennington Labs
Creatinine (Crea)	0.6-1.3 mg/dL	Pennington Labs
Glucose (Glu)	70-105 mg/dL	Pennington Labs
Potassium (K)	3.6-5.0 mmol/L	Pennington Labs
Sodium (Na)	135-145 mmol/L	Pennington Labs
<u>CHEMISTRY PANEL</u>		
Albumin (Alb)	3.2-5.5 g/dL	Pennington Labs
Alanine Aminotransferase (ALT)	10-60 IU/L	Pennington Labs
Aspartate Aminotransferase (AST)	10-42 IU/L	Pennington Labs
Cholesterol (Chol)	140-200 mg/dL	Pennington Labs
Glutamyl Transferase (GGT)	7-64 IU/L	Pennington Labs
HDL Cholesterol (HDL)	27-67 mg/dL	Pennington Labs
Lactate Dehydrogenase (LDH)	91-180 IU/L	Pennington Labs
Magnesium (Mg)	1.8-2.5 mg/dL	Pennington Labs
Phosphorus (P)	2.5-4.6 mg/dL	Pennington Labs
Total Bilirubin (TBil)	0.2-1.0 mg/dL	Pennington Labs
Total Iron Binding Capacity (TIBC)	300-420 ug/dL	Pennington Labs
Total Protein (TP)	6.7-8.2 g/dL	Pennington Labs
Triglycerides (Trig)	35-160 mg/dL	Pennington Labs
Uric Acid (Uric)		
MARKERS OF METABOLISM		
β-hydroxybutyrate (BHBA)	0.0-0.42 mmol/L	Pennington Labs
Glycerol (Gly)	61-232 umol/L	Pennington Labs

APPENDIX D

2A-D

<u>Parameter</u>	<u>Reporting Units</u>	<u>Reference</u>
Lactate (Lac)	0.3-1.3 mmol/L	Pennington Labs
Nonesterified Fatty Acids (NEFA)	0.10-0.60 mmol/L	Pennington Labs
MARKERS OF PROTEIN METABOLISM		
Ferritin	22-447 ng/ml	Pennington Labs
Ceruloplasmin	21-23 mg/dL	Pennington Labs
Immunoglobulins [IgA]	69-382 mg/dL	Pennington Labs
Immunoglobulins [IgG]	723-1685 mg/dL	Pennington Labs
Immunoglobulins [IgM]	63-277 mg/dL	Pennington Labs
Immunoglobulins [IgE]	0-120 IU/ml	Pennington Labs
Prealbumin	17-42 mg/dL	Pennington Labs
Retinol Binding Protein	3-6 mg/dL	Pennington Labs
Transferrin	252-429 mg/dL	Pennington Labs
VITAMINS		
Retinol	30-80 mg/dL	Pennington Labs
Vitamin B ₆ [Glutamate Oxaloacetate transaminase (B ₆)]	>130%	Pennington Labs
Vitamin B ₁₂	232-1138 pmol	Pennington Labs
Vitamin C	5-15 mg/L	Pennington Labs
Vitamin D	10-50 ng/ml	Pennington Labs
Folate	2.2-17.3 ng/ml	Pennington Labs
Riboflavin [Glutathione Reductase (GR)]	>76%	Pennington Labs
Thiamin [Transketolase] (TK)	>23%	Pennington Labs
TRACE ELEMENTS		
Copper (Cu)	70-165 ug/dL	Brook Army Hospital
Iron (Fe)	50-160 ug/dL	Pennington Labs
Zinc (Zn)	80-165 ug/dL	Brook Army Hospital

APPENDIX D

3A-D

<u>Parameter</u>	<u>Reporting Units</u>	<u>Reference</u>
COMPLETE BLOOD COUNT (CBC)		
White Blood Cells (WBC)	3.6-9.6 $10^9/L$	Coulter
Lymphocyte Percent(LY%)	20.5-51.1 %	Coulter
Monocyte (MO)	1.7-9.3 %	Coulter
Granulocyte (GR)	42.2-75.2 %	Coulter
Red Blood Cells (RBC)	3.9-5.7 $10^{12}/L$	Coulter
Hemoglobin (Hgb)	12.1-17.2 g/dL	Coulter
Hematocrit (Hct)	36.1-50.3 %	Coulter
Mean Corpuscular Volume (MCV)	82.2-97.4 fL	Coulter
Mean Corpuscular Hemoglobin (MCH)	27.6-33.3 pg	Coulter
Red Cell Distribution Width (RDW)	11.6-13.7 g/dL	Coulter
Platelet (Plt)	202-386 $10^9/L$	Coulter
Mean Platelet Volume (MPV)	7.8 -11.0 fL	Coulter

APPENDIX E
LLRP ACCEPTABILITY

1A-E

FULL NAME: _____
RANGER NUMBER: _____ COMPANY: _____

Please use the following scale to indicate how much you like or dislike each of the items in the Long Life Ration Packet by filling in the oval below the number that best describes your opinion of each item. For example, if you did not try an item, fill in the oval under "0" or, if you liked it very much, fill in the oval under "8".

NEVER TRIED	DISLIKE EXTREMELY	DISLIKE VERY MUCH	DISLIKE MODERATELY	DISLIKE SLIGHTLY	NEITHER LIKE NOR DISLIKE	LIKE SLIGHTLY	LIKE MODERATELY	LIKE VERY MUCH	LIKE EXTREMELY
0	1	2	3	4	5	6	7	8	9

	0	1	2	3	4	5	6	7	8	9
Beef Stew	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Escaloped Potatoes with Pork	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Chicken Stew	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Chicken ala King	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Chicken and Rice	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Chili con Carne	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Beef and Rice	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Spaghetti with Meat Sauce	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Cornflake Bar	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Cornflake and Rice Bar	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Oatmeal Cookie Bar	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Granola Bar	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Fig Bar	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Chocolate Covered Cookie	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Chocolate Covered Brownie	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
White Pouch Bread	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Tootsie Roll	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
M&M's	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Desert Bar	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Jelly Candy (Chuckles)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Hard Candy (Charms)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Caramels	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Apple Cider Drink Mix	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Lemon Tea	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Cocoa	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Coffee	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Orange Beverage (White Package)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Powdered Beverage Base (MRE)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Gum	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Creamer	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Sugar	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Salt	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

Do Not Write Below This Line

0 1 2 3 4 5 6 7 8 9	0 1 2 3 4 5 6 7 8 9	0 1 2 3 4 5 6 7 8 9	0 1 2 3 4 5 6 7 8 9

Please indicate what you USUALLY did with each item by filling in the appropriate circles.

	Ate Entire Item	Ate Some	Traded	Gave Away	Threw Away
Beef Stew	☐	☐	☐	☐	☐
Escalloped Potatoes with Pork	☐	☐	☐	☐	☐
Chicken Stew	☐	☐	☐	☐	☐
Chicken ala King	☐	☐	☐	☐	☐
Chicken and Rice	☐	☐	☐	☐	☐
Chili con Carne	☐	☐	☐	☐	☐
Beef and Rice	☐	☐	☐	☐	☐
Spaghetti with Meat Sauce	☐	☐	☐	☐	☐
Cornflake Bar	☐	☐	☐	☐	☐
Cornflake and Rice Bar	☐	☐	☐	☐	☐
Oatmeal Cookie Bar	☐	☐	☐	☐	☐
Granola Bar	☐	☐	☐	☐	☐
Fig Bar	☐	☐	☐	☐	☐
Chocolate Covered Cookie	☐	☐	☐	☐	☐
Chocolate Covered Brownie	☐	☐	☐	☐	☐
White Pouch Bread	☐	☐	☐	☐	☐
Tootsie Roll	☐	☐	☐	☐	☐
M&M's	☐	☐	☐	☐	☐
Desert Bar	☐	☐	☐	☐	☐
Jelly Candy (Chuckles)	☐	☐	☐	☐	☐
Hard Candy (Charms)	☐	☐	☐	☐	☐
Caramels	☐	☐	☐	☐	☐
Apple Cider Drink Mix	☐	☐	☐	☐	☐
Lemon Tea	☐	☐	☐	☐	☐
Cocoa	☐	☐	☐	☐	☐
Coffee	☐	☐	☐	☐	☐
Orange Beverage (White Package)	☐	☐	☐	☐	☐
Powdered Beverage Base (MRE)	☐	☐	☐	☐	☐
Gum	☐	☐	☐	☐	☐
Creamer	☐	☐	☐	☐	☐
Sugar	☐	☐	☐	☐	☐
Salt	☐	☐	☐	☐	☐

1. What were your reasons for trading, giving, and throwing away

2. Other comments:

MRE ACCEPTABILITY



NAME: _____

RANGER NUMBER: _____ COMPANY: _____

Please use the following scale to indicate how much you like or dislike each of the items in the MRE by filling in the oval below the number that best describes your opinion of each item. For example, if you did not try an item, fill in the oval under "0" or, if you liked it very much, fill in the oval under "8".

NEVER TRIED	DISLIKE EXTREMELY	DISLIKE VERY MUCH	DISLIKE MODERATELY	DISLIKE SLIGHTLY	NEITHER LIKE NOR DISLIKE	LIKE SLIGHTLY	LIKE MODERATELY	LIKE VERY MUCH	LIKE EXTREMELY
0	1	2	3	4	5	6	7	8	9

MRE ITEMS

	0	1	2	3	4	5	6	7	8	9
Pork w/Rice and BBQ Sauce	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Corned Beef Hash	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Chicken Stew	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Omelet with Ham	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Spaghetti with Meat Sauce	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Chicken a la King	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Beef Stew	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Ham Slice	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Meatballs with Rice and Sauce	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Tuna with Noodles	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Chicken and Rice	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Escalloped Potatoes with Ham	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Crackers	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Potato au Gratin	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Cheese Spread	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Jelly	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Peanut Butter	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Applesauce	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Fruit Mix	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Peaches (Wet Pack)	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Pears	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Strawberries	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Brownie	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Cherry Nut Cake	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Chocolate Covered Cookie	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Maple Nut Cake	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Oatmeal Cookie Bar	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

Do Not Write below This Line

0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9	0	1	2	3	4	5	6	7	8	9



NEVER TRIED	DISLIKE EXTREMELY	DISLIKE VERY MUCH	DISLIKE MODERATELY	DISLIKE SLIGHTLY	NEITHER LIKE NOR DISLIKE	LIKE SLIGHTLY	LIKE MODERATELY	LIKE VERY MUCH	LIKE EXTREMELY
0	1	2	3	4	5	6	7	8	9

MRE ITEMS	0	1	2	3	4	5	6	7	8	9
Chocolate Nut Cake	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Grape Beverage	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Orange Beverage	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Lemon-Lime Beverage	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Cherry Beverage	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Coffee	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Cocoa	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Tootsie Roll	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Charms	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
M&M'S	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Caramel	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Gum	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Hot Sauce	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Cream Substitute	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Sugar	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Salt	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

Please indicate what you USUALLY did with each item by filling in the appropriate circle.

	ATE ENTIRE ITEM	ATE SOME	TRADED	GAVE AWAY	THREW AWAY
Pork w/Rice and BBQ Sauce	☐	☐	☐	☐	☐
Corned Beef Hash	☐	☐	☐	☐	☐
Chicken Stew	☐	☐	☐	☐	☐
Omelet with Ham	☐	☐	☐	☐	☐
Spaghetti with Meat Sauce	☐	☐	☐	☐	☐
Chicken a la King	☐	☐	☐	☐	☐
Beef Stew	☐	☐	☐	☐	☐
Ham Slice	☐	☐	☐	☐	☐
Meatballs with Rice and Sauce	☐	☐	☐	☐	☐
Tuna with Noodles	☐	☐	☐	☐	☐
Chicken and Rice	☐	☐	☐	☐	☐
Escaloped Potatoes with Ham	☐	☐	☐	☐	☐
	☐	☐	☐	☐	☐
Crackers	☐	☐	☐	☐	☐
Potato au Gratin	☐	☐	☐	☐	☐
	☐	☐	☐	☐	☐
Cheese Spread	☐	☐	☐	☐	☐
Jelly	☐	☐	☐	☐	☐
Peanut Butter	☐	☐	☐	☐	☐

Please indicate what you USUALLY did with each item (cont.).

	ATE ENTIRE ITEM	ATE SOME	TRADED	GAVE AWAY	THREW AWAY
Applesauce	☐	☐	☐	☐	☐
Fruit Mix	☐	☐	☐	☐	☐
Peaches (Wet Pack)	☐	☐	☐	☐	☐
Pears	☐	☐	☐	☐	☐
Strawberries	☐	☐	☐	☐	☐
Brownie	☐	☐	☐	☐	☐
Cherry Nut Cake	☐	☐	☐	☐	☐
Chocolate Covered Cookie	☐	☐	☐	☐	☐
Maple Nut Cake	☐	☐	☐	☐	☐
Oatmeal Cookie Bar	☐	☐	☐	☐	☐
Chocolate Nut Cake	☐	☐	☐	☐	☐
Grape Beverage	☐	☐	☐	☐	☐
Orange Beverage	☐	☐	☐	☐	☐
Lemon-Lime Beverage	☐	☐	☐	☐	☐
Cherry Beverage	☐	☐	☐	☐	☐
Coffee	☐	☐	☐	☐	☐
Cocoa	☐	☐	☐	☐	☐
Tootsie Roll	☐	☐	☐	☐	☐
Charms	☐	☐	☐	☐	☐
M&M'S	☐	☐	☐	☐	☐
Caramel	☐	☐	☐	☐	☐
Gum	☐	☐	☐	☐	☐
Hot Sauce	☐	☐	☐	☐	☐
Cream Substitute	☐	☐	☐	☐	☐
Sugar	☐	☐	☐	☐	☐
Salt	☐	☐	☐	☐	☐

What were your reasons for trading, giving, and throwing away.

Other comments?

Ranger 2.1 Questionnaire - 4 Weeks

Volunteer Number _____

1. Had you ever attended Ranger training before class 11-92?

Yes No

a.) If yes, please list approximate date(s), phase where you dropped or recycled, and if medical, the reason.

2. How much do you think you weighed when you entered Ranger school?

_____ pounds

a.) Do you consider this your ideal weight?

Yes No

b.) If no, what do you consider your ideal weight to be?

_____ pounds

c.) Was this your usual weight or did you gain or lose weight just before Ranger School?

Usual Gained Lost

d.) If you gained or lost, what is your usual weight?

_____ pounds

e.) If you circled either gained or lost above, did you change your weight specifically for Ranger School?

Yes No

f.) If you changed weight specifically for Ranger School, why did you feel this was necessary?

3. If you recycled during Ranger School, how much weight do you think you lost by the beginning of gulag? (If these questions apply to you, answer them, then go to

question 5).

_____ pounds

a.) How much weight did you regain by the end of gulag?

_____ pounds

b.) How much weight did you lose by the end of Ranger School?

_____ pounds

4. If you did not recycle during Ranger School, how much weight do you think you lost by the end of the school?

_____ pounds

5. Have you regained all the weight you lost at Ranger School? (How long until your clothes fit normally?)

Yes No

6. Did you gain more than you lost after Ranger School?

Yes _____ pounds No

7. If you have not gained back all lost weight, are you currently trying to gain?

Yes No

a.) How much do you still want to gain?

_____ pounds

8. After leaving Ranger School, did you experience any nausea and/or vomiting after eating a meal?

Nausea Vomiting Both None

a.) If so, roughly how often?

Nausea _____ times/day Vomiting _____ times/day

b.) Are you still experiencing these problems and, if not, how long did it take

APPENDIX F

3A-F

for them to subside?

Yes _____ days No

9. Did you experience any bouts of diarrhea and/or cramping after leaving Ranger School? Yes No

a.) If so, roughly how often?

Diarrhea _____ times/day Cramping _____ times/day

b.) Are you still experiencing these problems and, if not, how long did it take for them to subside?

Yes _____ days No

10. After leaving Ranger School, did you get a "full" feeling faster when eating a meal than you did prior to Ranger School?

Yes No

11. Did you notice any unusual change(s) in your dietary patterns (for example, types of food and amounts) in the first month following Ranger training? Please mark any changes or choose "same" if frequency and amount of that type of food did not change.

Beef (hamburger, steak, pork, etc)	more	less	same
Fried foods	more	less	same
Breads	more	less	same
Doughnuts, cookies, cakes, pastries	more	less	same
Fruits/vegetables	more	less	same
Salty snacks (chips, peanuts, etc)	more	less	same
Ice cream	more	less	same
Chocolate candy	more	less	same
Beverages	more	less	same
Other _____	more	less	same
Other _____	more	less	same

12. Approximately how many days after leaving Ranger training until the types and amounts eaten returned to normal?

_____ days

13. Currently, where do you eat most of your meals?

Home

Messhall

Restaurant

14. Prior to entering Ranger School, approximately how many meals did you eat from a fast food restaurant during an average two-week period?

_____ meals

15. Within the first two weeks after leaving Ranger School, approximately how many meals did you eat from a fast food restaurant?

_____ meals

16. Were there any foods that you "craved" for the first month after Ranger School? Please list.

17. Did you avoid eating any foods after Ranger training that you normally ate prior to Ranger training? Please list.

18. Did you take vitamin supplements on a regular basis during Ranger training?

Yes

No

19. How was your score on the first PT test you took after leaving Ranger School?

Worse than usual

Same as usual

Better than usual

a.) If worse than usual, did your second PT test scores (if you have had a second test) reach the usual score you got prior to Ranger training?

Yes

No

20. After leaving Ranger School, did you feel weaker, about the same, or stronger than before Ranger training?

Weaker

Same

Stronger

21. Was it difficult to exercise in the first month after leaving Ranger School?

Yes

No

a.) If it was difficult to exercise, please explain.

22. What did you do differently after Ranger training to get back in shape (e.g, different training regimen, vitamin/nutritional supplements, protein supplements, etc.)? Please explain.

23. Did you use training aids (for example, protein powders) prior to Ranger training?

Yes _____ No

24. How much sleep per night did you get on the average prior to Ranger training?

_____ hours

25. Did you normally have any problems falling asleep prior to Ranger training? Please describe.

26. How much sleep per night on the average did you get during the classroom phases of Ranger training?

_____ hours

27. How much sleep per night on the average did you get during the field phases of Ranger training?

_____ hours

28. After leaving the school, did you sleep more, the same, or fewer hours at night than prior to Ranger training?

More Same Fewer

a.) If more or fewer, how many hours?

_____ hours

b.) Approximately how many days did this change last?

_____ days

29. Immediately after leaving Ranger School, did you have any problems falling asleep that you did not have prior to Ranger training?

Yes No

a.) If yes, please describe.

b.) Do you currently have problems with sleep? Please describe.

30. Do you now sleep the same amount on the average as you did prior to Ranger training?

Yes _____ hours No _____ hours

31. Based on your usual levels prior to Ranger training, how energetic/motivated were you immediately upon leaving Ranger School? Please explain.

a.) Are you back to your normal levels and, if so, how long did it take?

Yes _____ days No

b.) Please describe what you did to recover or what you are currently doing to recover.

32. Prior to attending Ranger School, did you plan to take leave immediately after graduation?

Yes No

a.) If yes, was it in anticipation of needing to recover from the demands of Ranger training?

Yes No

b.) How much leave did you plan to take?

_____ days

c.) How much leave did you actually take?

_____ days

d.) Upon returning to duty, did you feel you needed more leave time? If so, how many more days?

Yes _____ days No

33. If you did not plan in advance to take leave after Ranger training, did you ask for leave when you returned to your unit?

Yes No

a.) If yes, how much leave did you request?

_____ days

b.) Were you granted the leave?

Yes No

c.) If yes, why did you feel you needed some leave time?

d.) Did you take all the leave you requested?

Yes No

e.) Did you feel you needed more leave upon return to duty?

If yes, please explain why.

Yes _____ days No

34. Did you continue on immediately to another school (e.g. airborne, sharpshooter, LRSD)?

Yes _____ school No

35. If you returned to your unit, did you go to the field upon return from Ranger training? If yes, how many days were you in the field?

Yes _____ days No

36. With 1=highest priority and 5=lowest priority, please list what your most pressing concerns were on the first free day after Ranger training (for example: eating, sleeping, going for a 2 mile run, seeing a movie, drinking (alcohol), etc). Please be honest but not too crude!

1.

2.

3.

4.

5.

37. Using the same rating scale, what were your priorities a week later?

1.

2.

3.

4.

5.

38. And finally, did you notice any other changes (positive or negative) in your life (performance, attitudes, etc.) that occurred between leaving for Ranger School and returning home? Please explain the situation, whether it has returned to normal, how

long it lasted, if you did anything different to affect the return to normalcy and how it affected you.

Thank you for taking the time to answer these questions. Since your answers are important, please make sure all questions are answered.

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