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14. ABSTRACT
The Collaborative Research to Optimize Warfighter Nutrition (CROWN) II grant funded research in nutrition, metabolism and human physiology to address objectives relevant to the nutritional health of military personnel. Scientists at Pennington Biomedical Research Center and the Military Nutrition Division at the U.S. Army Research Institute of Environmental Medicine collaborated on the design, execution and analysis of 13 research projects in four focus areas:

1. Metabolism and Performance
2. Stress and Inflammation
3. Nutrients and Resilience
4. Healthy Eating and Behavior

The overarching objective was to discover novel strategies that promote Warfighter resilience, improve Warfighter combat readiness, and assure optimal Warfighter performance. Specifically, this project provided for the most efficient and cost-effective execution of the Department of Defense (DoD) objectives to ensure a healthy and fit fighting troop base, ready for deployment and resilient to the stressors of duty.

15. SUBJECT TERMS
Metabolism, nutrition, energy expenditure, readiness, performance, warfighter, resilience

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INTRODUCTION

The modern Warfighter faces numerous physiological challenges during training and combat. The Collaborative Research to Optimize Warfighter Nutrition II (CROWN II) program consisted of a series of research projects in nutrition, metabolism and human physiology designed to discover novel strategies that promote Warfighter health and resilience, improve Warfighter combat readiness and sustain optimal Warfighter health and performance during all phases of the deployment cycle. Our hypothesis was that through the use of state-of-the-art technologies and metabolic profiling, we would be able to develop better models to assess resilience or performance capacity and that these might allow us to better predict dietary requirements of military units. The two collaborating institutions involved in the CROWN II program are Pennington Biomedical Research Center (PBRC) and the Military Nutrition Division (MND) at the United States Army Research Institute of Environmental Medicine (USARIEM). The CROWN II project enabled USARIEM and PBRC scientists to work together on the design, execution, analysis and translation of 13 research projects in four thematic areas:

1. Metabolism and Performance (2 projects)
2. Stress and Inflammation (1 project)
3. Nutrients and Resilience (7 projects)
4. Healthy Eating and Behavior (3 projects)

Projects led by Pennington Biomedical (3)

Project 1 (Metabolism and Performance)
PBRC IRB # 2015-063
HRPO log #A-18080.2a
Title: Physiological and psychological effects of testosterone during severe energy deficit and recovery: a randomized, placebo controlled trial
PI: Jennifer Rood

Project 10 (Nutrients and Resilience)
PBRC IRB # 2017-027
HRPO log # A-18080.12a
Title: Supplementation Trial on Arginine with Metabolic Profiling (STAMP)
PI: John Apolzan/Jennifer Rood

Project 13 (Healthy Eating and Behavior)
PBRC IRB # 2015-013
HRPO log # A-18080.a
Title: H.E.A.L.T.H. II. Healthy Eating, Activity, & Lifestyle Training Headquarters
PI: Tiffany Stewart

Projects led by USARIEM (10)

Project 2 (Metabolism and Performance)
PBRC IRB # 2016-015
Title: H16-02: Substrate utilization, exercise performance, and skeletal muscle response to energy deficit and altitude acclimatization
PI: Stefan M. Pasiakos

Project 3 (Stress and Inflammation)
Title: H14-33: Effects of carbohydrate and protein supplementation on whole-body protein balance and skeletal muscle mass during winter military training: a randomized controlled trial
PI: Stefan M. Pasiakos

Project 4 (Nutrients and Resilience)
PBRC IRB # 2016-003
Title: H14-02: Effect of protein supplementation on lean body mass recovery and physiological resilience following Survive, Evade, Resist, Escape (SERE) School
PI: Stefan M. Pasiakos

Project 5 (Nutrients and Resilience)
PBRC IRB # 2016-004
Title: H15-07: Efficacy of a once daily calcium and vitamin D fortified food product to improve bone microarchitecture in response to Army basic combat training
PI: Erin Gaffney-Stomberg

Project 6 (Nutrients and Resilience)
PBRC IRB # 2016-005
Title: H15-10: A Prospective Study of Factors associated with Career Success among Soldiers in Special Forces Training: The Army Career Resiliency and Injury Cohort Study
PI: Emily Farina

Project 7 (Nutrients and Resilience)
PBRC IRB # 2016-006
Title: H15-12: Effects of Meal, Ready-to-Eat (MRE) consumption on gut health
PI: J. Philip Karl

Project 8 (Nutrients and Resilience)
PBRC IRB # 2016-007
HRPO log # A-19344
Title: H15-21: Evaluation of calcium and vitamin D supplementation for optimizing bone health during Marine Corps recruit training
PI: Erin Gaffney-Stomberg

Project 9 (Nutrients and Resilience)
PBRC IRB # 2016-016
Title: H15-23: Energy balance and physiological responses to the U.S. Marine Corps Forces Special Operations Command (MARSOC) Individual Training Course (ITC)
PI: Stefan M. Pasiakos

Project 11 (Healthy Eating and Behavior)
PBRC IRB # 2016-013
Title: H14-14: Nutritional Practices of Soldiers Consuming Meals in Military Dining Facilities (DFAC-Nutrition Study)
PI: Asma Bukhari

Project 12 (Healthy Eating and Behavior)
PBRC IRB # 2014-061
HRPO log # A-17113
Title: H15-04: Dining satisfaction and diet quality of soldiers eating at two Fort Bragg DFACS
PI: Renee Cole

KEYWORDS

Metabolism, nutrition, energy expenditure, readiness, performance, warfighter, resilience

ACCOMPLISHMENTS

The major goals as defined in the statement of work involve 13 individual projects. Each of these projects is described below along with milestones and dates of completion.

Thematic Area 1. Metabolism and Performance

Projects in this area focused on metabolically regulated physiological functions that affect performance and susceptibility to illness, infection and injury. These findings will be used to support simulation modeling and will be incorporated into dietary policy and military ration development to promote Warfighter health, resilience and recovery.

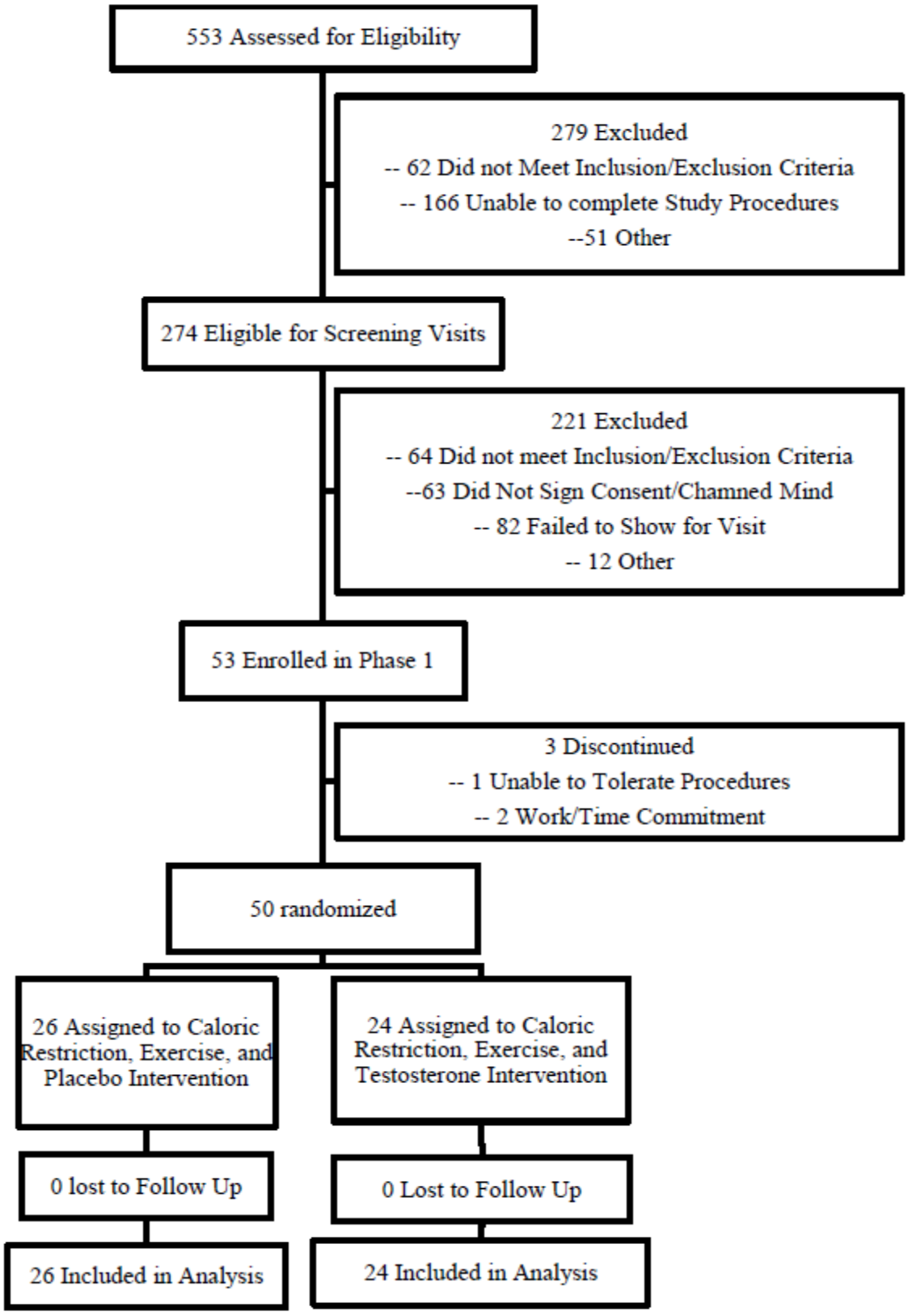
Project 1: Determine the physiological and psychological effects of testosterone during severe energy deficit and recovery.

Specific Aim: To conduct a study to assess the effects of maintaining normal levels of testosterone during severe energy deficit and recovery.

Dr. Jennifer C. Rood, Ph.D. (PI), and Dr. Cathy Champagne, Ph.D. (co-investigator), from PBRC collaborated with USARIEM scientists Stefan M. Pasiakos, Ph.D., James P. McClung, Ph.D., and Harris R. Lieberman, Ph.D., on the development and execution of this clinical trial conducted at PBRC from April 2016 to September 2017 **(Major Task 1)**. The study was titled "Physiological and psychological effects of testosterone during severe energy deficit and recovery: a randomized, placebo-controlled trial for Optimizing Performance for Soldiers (OPS)."

The objective of this study was to determine whether maintaining a eugonadal state during severe, sustained energy deficit attenuates physiological decrements, particularly the loss of lean body mass, and maintains mental performance. To address these objectives and more (e.g., gut health, appetite regulation, physiological and psychological recovery), we enrolled 53 physically active men to have 50 complete a 3-phase, randomized, placebo-controlled study (see Figure 1 for consort diagram). After completing a 14-day (free-living, phase 1), energy-adequate, diet acclimation phase (protein, 1.6 g·kg-1·d-1; fat, 30% total energy intake; with remaining energy derived from carbohydrate), participants were randomized to one of two experimental groups and underwent a 28-day (live-in, phase 2), 55% energy deficit phase: energy deficit alone (DEF) or energy deficit + exogenous testosterone (DEF+TEST). Recovery (free-living, phase 3) was assessed after completing phase 2 to determine when body mass was recovered within ± 2.5% of initial body mass (duration varied; 42-day maximum for phase 3). Body composition, state-of-the-art stable isotope methodologies, proteomics, metabolomics, muscle biopsies, whole-room calorimetry, molecular biology, activity/sleep monitoring, personality and cognitive function assessments, functional MRI (fMRI), biochemistries, and rigorously controlled diet and physical activity were used to assess physiological and psychological responses to energy restriction and recovery feeding while in a hypogonadal versus eugonadal state.

Figure 1. OPS Consort Diagram



The study was conducted at PBRC. PBRC provided the following: regulatory (IRB) approval, subject recruitment, dietary menu design, food preparation and distribution to subjects, physical activity monitoring, blood and urine collection, saliva and fecal collection, biomarker analyses, measures of body composition and energy expenditure, and muscle biopsy collection and analysis.

An initial manuscript detailing the methods used in this study was published in 2017 **(Milestone #1)**. A copy of the manuscript is included in the Appendix.

> Physiological and psychological effects of testosterone during severe energy deficit and recovery: A study protocol for a randomized, placebo-controlled trial for Optimizing Performance for Soldiers (OPS). Pasiakos SM, Berryman CE, Karl JP, Lieberman HR, Orr JS, Margolis LM, Caldwell JA, Young AJ, Montano MA, Evans WJ, Vartanian O, Carmichael OT, Gadde KM, Harris M, Rood JC. Contemp Clin Trials. 2017 Jul;58:47-57. doi: 10.1016/j.cct.2017.05.001. Epub 2017 May 4. PMID: 28479217

Data analysis **(Major Task 2)** began upon completion of the clinical trial in September 2017. Collaborators drafted a manuscript of initial results from the project in 2018 that has been submitted for publication. Ongoing data analysis and publication efforts are being funded by a subsequent award.

Major findings from this study indicate that testosterone supplementation during sustained periods of severe underfeeding resulted in increased fat-free mass with no effects on lower-body muscular strength and endurance, insulin, cortisol, lipids, lipoproteins, ALT, PSA or blood pressure. The primary findings from this study provide rationale for further exploration of more practical pharmacologic interventions to mitigate losses of fat-free mass experienced by military personnel during periods of unavoidable, severe energy deficit, in particular those that may enhance muscular performance.

Project 2: H16-02. Substrate utilization, exercise performance, and skeletal muscle response to energy deficit and altitude acclimatization.

Specific Aim: To conduct a study to evaluate nutrition requirements for missions at high altitude.

This project was conducted from March to September 2016 and was led by Stefan M. Pasiakos, Ph.D. (USARIEM) **(Major Task 1)**. The purpose of this research was to obtain new information that could be used to optimize nutrient content specifications for the Modular Operational Ration Enhancement, High Altitude (MORE-HA) to better protect Soldiers from muscle wasting during HA sojourns and sustain their performance during physically challenging missions. We specifically evaluated the effects of dietary protein intake (1.0 ± 0.2 versus 2.0 ± 0.2 g protein/kg) during 22 days of energy deficit (ED) at HA on lean body mass (LBM) and the regulation of skeletal muscle mass. This study also evaluated the ergogenic effects of a carbohydrate beverage (0.8 fructose to glucose ratio) on total, exogenous and endogenous carbohydrate oxidation during steady-state exercise and subsequent exercise performance (2-mile run) at sea level, acute HA and after 22 days of ED at HA. Sea level testing took place at the USARIEM research labs in Natick,

MA (50 m), and the acute and chronic HA portions were conducted at the summit of Pikes Peak, CO (USARIEM John Maher Altitude Laboratory, 4300 m).

During the 22-d sea level phase, volunteers received dietary counseling to maintain baseline weight and consumed protein at levels consistent with recommendations for periods of low physical activity (1.0 g/kg/d). During the 22-d ED at HA, all meals and beverages (water ad libitum) were prepared and provided to volunteers by research staff. Changes in total body, lean body and fat mass were assessed using dual-energy X-ray absorptiometry (DXA) at sea level and after a 22-d ED at HA. In addition, regulation of muscle mass was assessed using stable isotope methodology, muscle biopsies and various molecular techniques to directly measure muscle protein synthesis, whole body protein balance and the cellular mechanisms that regulate these processes. Furthermore, exercise testing, substrate oxidation rates and expired CO2 labeled with the stable isotope 13C were used to assess total, exogenous and endogenous carbohydrate oxidation and performance capacity. Sub-aims of the study included evaluation of cognitive function, sleep patterns, eating behavior and appetite, gut microbiome composition and activity, and gut permeability at sea level, acute HA and chronic HA.

This study was conducted by USARIEM investigators. PBRC provided specimen collection materials and biomarker analysis. Additionally, PBRC provided guidance on which biomarkers should be measured to address the hypotheses and guidance on scientific interpretation of data.

The biomarker collection scheme is shown below:

Study Day Analyte, volume (mL)	0	6	7	12	HA0	HA2	HA7	HA13	HA19	HA21
Priority: USARIEM										
Hemoglobin	X	X	4	X	4	X	X	X	X	4
Hematocrit	X	X	4	X	4	X	X	X	X	4
Creatinine	X	X		X	X	X	X	X	X	
Erythroferrone	X				X				X	
Messenger RNA/micro-RNA	X									
Priority: PBRC										
Osmolality	X	X		X		X	X	X	X	
Plasma protein	X	X		X		X	X	X	X	
Lactate	X		16	X	16	X	X	X	X	16
Free fatty acids	X		16	X	16	X	X	X	X	16
Insulin	X		16	X	16	X	X	X	X	16
Glucose	X		16	X	16	X	X	X	X	16
Glycerol	X		16	X	16	X	X	X	X	16
Glucagon			2		2					2
Total testosterone	X	X		X		X	X	X	X	
SHBG	X	X		X		X	X	X	X	
Leptin			3		3		X			3
Acylated ghrelin			6		6		X			6
Cholecystokinin			5		5		X			5
GLP-1			5		5		X			5
GLP-2			X		X		X			X
LBP			X		X		X			X
Claudin-3			X		X		X			X
I-FABP			X		X		X			X
Creatinine (urine)	X		X	X	X	X	X	X	X	X
Nitrogen (urine)	X		X	X	X	X	X	X	X	X
Lactulose/mannitol (urine)				X		X			X	
Priority: Metabolic Solutions, Inc.										
Expired $^{13}CO_2$ (breath)			15		15					15
Priority: Center for Translational Research in Aging and Longevity										
Blood enrichment and EAA			14		14					14
Muscle enrichment			4		4					4
Archive: PBRC										
Hepcidin	X				X				X	
Interlukin-6	X				X				X	
sTfR	X				X				X	
Ferritin	X				X				X	
Iron (serum)	X				X				X	
TIBC	X				X				X	
Cortisol	X	X		X		X	X	X	X	
DHEA-S	X	X		X		X	X	X	X	
Hepcidin (urine)	X				X				X	
Archive serum	X	X	X	X	X	X	X	X	X	X
Archive EDTA plasma	X		X		X		X			X
Archive LiHep plasma	X	X		X	X	X	X	X	X	
Archive urine	X			X		X	X	X	X	
Total volume (mL)	20.6	5.7	143.1	7.3	147.6	7.3	19.0	7.3	11.4	143.1

PBRC prepared labeled vials for blood and urine collection and provided specimen analysis for this study. **(Major Task 2)** In total, 5,244 results were sent to the PI via the secure website https://safe.amrdec.army.mil/safe/. Biomarkers that were assayed include the following:

1. Urine nitrogen and creatinine to calculate nitrogen balance
2. Urine mannitol and lactulose as markers of gastrointestinal permeability
3. Biochemistry/metabolism markers including: free fatty acids, glycerol, glucose, glucagon, total protein, DHEA-s, testosterone, sex hormone binding globulin, cortisol, insulin, lactate, osmolality, hepcidin, interlukin-6, soluble transferrin receptor, ferritin, serum iron, total iron binding capacity, % iron saturation, leptin, acyl ghrelin, CCK, GLP-1, GLP-2, lipopolysaccharide binding protein, claudin-3,intestinal fatty acid-binding protein and hepcidin.

Six abstracts on findings from this study were presented in 2017:

1. Berryman CE, Karl JP, Cole RE, Kenefick RW, Margolis LM, Carbone JW, Ferrando AA, Lieberman HR, Young AJ, Pasiakos SM. Prolonged high altitude exposure exacerbates fat-free mass and fat mass loss during negative energy balance regardless of dietary protein intake. Experimental Biology, April 2017.
2. Derosier AN, Berryman CE, Karl JP, Wilson MA, Young AJ, Pasiakos SM. Higher-Protein Intake During Sustained Negative Energy Balance Attenuates Elevations in Resting Metabolic Rate at High Altitude (4300 m). Experimental Biology, April 2017.
3. Karl JP, Cole RE, Berryman CE, Kominsky MT, Radcliffe PN, Margolis LM, Young AJ, Pasiakos SM. Higher Protein Diet Suppresses Appetite at High Altitude. Experimental Biology, April 2017.
4. McClung JP, Hennigar SR, Berryman CE, Young AJ, Pasiakos SM. Prolonged High Altitude Exposure Results in Elevated Erythroferrone and Diminished Hepcidin Levels in Healthy Young Male Volunteers. Experimental Biology, April 2017.
5. Kenefick RW, Luippold AJ, Bradbury KE, Young AJ, Derosier AN, Wilson MA, Berryman CE, Pasiakos SM. No Impact of Carbohydrate Supplementation and Altitude Acclimatization on Aerobic Exercise Performance. American College of Sports Medicine, May 2017.
6. Young AJ, Berryman CE, Derosier AN, Kenefick RW, Wilson MA, Pasiakos SM. Effects of Acclimatization to High Altitude on Exogenous Carbohydrate Oxidation During Steady-State Exercise. American College of Sports Medicine, May 2017.

An initial manuscript was drafted but not published during Year 3 of this award. Ongoing analysis and reporting of results is continuing under funding from a subsequent award.

Initial findings from this project are that participants remained weight-stable during sea level (day 1: 82.1 ± 14.1 and day 20: 82.9 ± 14.5 kg, $P > 0.05$), while consuming 2394 ± 426 kcal/d and 1.1 ± 0.3 g protein/kg/d. During high altitude, participants lost 7.9 ± 1.9 kg (main effect of time, $P < 0.01$) regardless of protein group. Similarly, whole-body, leg, and trunk fat-free mass (aqueous and non-aqueous) and fat mass decreased at high altitude (main effect of time, $P < 0.01$), but were not statistically different between higher protein and standard protein. Higher protein oxidized 75% more protein than SP (2.21 ± 0.41 compared to 1.26 ± 0.21 g protein/kg/d, $P < 0.01$. However,

carbohydrate (2.63 ± 1.38 compared to 3.38 ± 1.34 g carbohydrate/kg/d) and fat (1.00 ± 0.64 compared to 1.37 ± 0.55 g fat/kg/d) oxidation were not statistically different between higher protein and standard protein. Postabsorptive whole-body protein synthesis was increased at acute high altitude compared to sea level and decreased at chronic high altitude compared to both sea level and acute high altitude (main effect of time, $P < 0.01$. Protein balance was lower at chronic high altitude compared to both sea level and acute high altitude (main effect of time, $P < 0.01$. Nitrogen balance did not differ by time ($P = 0.40$). To investigate diet-specific effects on whole-body protein turnover after chronic high altitude, protein synthesis, protein balance and nitrogen balance were examined with adjustment for acute high-altitude measures. Higher protein and standard protein synthesis (2.08 ± 0.23 compared to 2.17 ± 0.21 g/kg/d, $P = 0.17$) and protein balance (2.52 ± 0.24 compared to 2.43 ± 0.24 g/kg/d, $P = 0.79$) were not significantly different; however, negative balance was more negative for higher protein than standard protein (-0.44 ± 0.08 compared to -0.26 ± 0.06 g/kg/d, $P < 0.01$).

Glucose and insulin concentrations decreased during prolonged energy deficit at high altitude, while free-fatty acid concentrations increased (main effect of time, $P < 0.01$) regardless of protein group. Glycerol concentrations increased acutely but returned to sea level values by high-altitude day 20 (main effect of time, $P < 0.01$; no group differences). There was an interaction effect (diet x time, $P = 0.02$) for total testosterone concentrations; however, no post hoc comparisons were significant and the change from sea level to high altitude day 20 was not different by diet group. Free testosterone decreased during prolonged energy deficit at high altitude and sex hormone binding globulin and cortisol concentrations increased (main effect of time, $P < 0.01$.

This study demonstrated 1) within the context of severe energy deficit (~70%), a higher-protein diet did not spare fat-free mass due, in part, to the inducement of greater protein oxidation, 2) body composition changes were highly variable independent of diet group, participants who lost predominantly fat-free mass had lower aerobic fitness and testosterone concentrations and more fat mass prior to high altitude than individuals who lost predominantly fat mass (> 50% of total body mass loss), and 3) the total energy deficit, indicated by changes in body composition during the 21 days at high altitude, could not be entirely accounted for by measured energy intake, estimated energy expenditure of prescribed daily physical activity, or elevated resting metabolic rate.

In conclusion, consuming a higher-protein diet was ineffective at sparing fat-free mass during severe energy deficit at high altitude. However, individuals with greater aerobic fitness and testosterone concentrations prior to high-altitude ascent experienced attenuated fat-free mass loss. Therefore, incorporating increased aerobic training into pre-ascent preparations may provide added benefit (i.e., beyond the improved cardiovascular responses to exercise and physical activity) in terms of protection against fat-free mass loss during energy deficit at high altitude.

Thematic Area 2. Stress and Inflammation

The project in this area focused on the inflammatory and stress-related response to military operational stress. This project evaluated nutritional countermeasures to operational stress and

assessed whether functional (e.g., physical and psychological) performance can be maintained using anti-inflammatory directed nutrition.

Project 3: H14-33. Effects of carbohydrate and protein supplementation on whole-body protein balance and skeletal muscle mass during winter military training: a randomized controlled trial.

Specific Aim: Conduct a clinical study to determine if an energy-dense, anti-inflammatory nutrition supplement can attenuate the detrimental effects of short-term, severe operational stress in Norwegian Soldiers.

This project was led by Stefan M. Pasiakos, Ph.D. (USARIEM). Norwegian Armed Forces personnel participating in a 7-day winter training exercise were recruited to participate in a 30-day double-blind randomized controlled trial. Samples were collected at multiple timepoints (day 0 baseline, days 1-13 pre-training nutrition supplementation, days 14-17 pre-march winter survival training and nutrition supplementation, days 18-20 ski march and nutrition supplementation, days 21-30 recovery and nutrition supplementation). Volunteers were randomly assigned to receive either a combined anti-inflammatory supplement (ANTI-I, n = 30) or placebo (PLA, n = 30) daily throughout the intervention. 89 participants completed the study **(Major Task 1)**.

PBRC used advanced techniques to determine the efficacy of an energy-dense, anti-inflammatory nutrition supplement, including stable isotope methodologies to further characterize energy and nitrogen flux, metabolomics to assess novel metabolic biomarkers associated with inflammation and nutritional status in response to training, and clinical biochemistries. Samples were collected and shipped to Pennington Biomedical. All blood and urine assays were completed, and data was transmitted to investigators at USARIEM **(Major Task 2)**. A total of 2,044 blood assays were completed on pre and post samples and included the following tests: blood urea nitrogen, creatine kinase, interleukin6, lactate dehydrogenase, TNFa, ferritin, high sensitivity c reactive protein, insulin, myoglobin, hepcidin, osmolality, soluble transferrin receptor, lipopolysaccharide and zonulin. A total of 438 urine assays were completed and included the following tests: creatinine, sucralose and mannitol. In addition to the biomarkers, urine samples also were analyzed for the stable isotopes deuterium and oxygen 18 to determine energy expenditure. A total of 294 urine samples were analyzed to determine energy expenditure on 42 subjects.

Three abstracts on findings from this study were presented **(Milestone #2)**:

1. Karl JP, Margolis LM, Murphy NE, Martini S, Gundersen Y, Castellani JW, Carrigan CT, Teien HK, Madslien EH, Montain, SJ, Pasiakos SM. Increased Gastrointestinal Permeability During Prolonged Physical Stress is Associated with Lower Energy Intakes but not Dietary Macronutrient Composition. Experimental Biology, April 2016
2. Margolis LM, Murphy NE, Martini S, Gundersen Y, Castellani JW, Karl JP, Carrigan CT, Teien HK, Madslien EH, Montain SJ, Pasiakos SM. Energy not protein or carbohydrate intake attenuates whole-body protein loss during 4-d arctic military training. Medicine & Science in Sports & Exercise, June 2016
3. Karl JP, Margolis LM, Murphy NE, Martini S, Gundersen Y, Castellani JW, Carrigan CT, Teien HK, Madslien EH, Montain SJ, Pasiakos SM. High Energy Expenditure and

Negative Energy Balance Modulate Composition and Metabolism of the Gut Microbiota. Experimental Biology, April 2017

Three manuscripts on initial results from this study were published **(Milestone #3)** and are included in the appendix of this report:

1. Pasiakos Sm, Margolis LM, Murphy NE, McClung HL, Martini S, Gundersen Y, Castellani JW, Karl JP, Teien H, Madslien E, Stenberg PH, Young AJ, Montain SM, McClung JP. Effects of exercise mode, energy and macronutrient interventions on inflammation during military training. Physiol Rep. 2016 Jun;4(11). pii: e12820. doi: 10.14814/phy2.12820. PMID: 27273884 PMCID: PMC4908496
2. Margolis LM, Murphy NE, Martini S, Gundersen Y, Castellani HW, Karl JP, Carrigan CT, Teien H, Madslien E, Montain SJ, Pasiakos SM. Effects of supplemental energy on protein balance during 4-d arctic military training. Med Sci Sports Exerc. 2016 Aug;48(8):1604-12. doi: 10.1249/MSS.0000000000000944. PMID: 27054679
3. Karl JP, Margolis LM, Madslien EH, Murphy NE, Castellani JW, Gundersen Y, Hoke AV, Levangie MW, Kumar R, Chakraborty N, Gautam A, Hammamieh R, Martini S, Montain SJ, Pasiakos SM. Changes in intestinal microbiota composition and metabolism coincide with increased intestinal permeability in young adults under prolonged physiological stress. Am J Physiol Gastrointest Liver Physiol. 2017 Jun 1;312(6):G559-G571. doi: 10.1152/ajpgi.00066.2017. Epub 2017 Mar 23. PMID: 28336545

This study is the first to characterize the effects of load carriage exercise during a real-world military training exercise. During the exercise, total daily energy expenditure was very high (approximately 6,000 kcal). As such, the soldiers were in negative energy balance. Increasing energy intake through the use of protein and/or carbohydrate energy bars attenuated the negative energy balance and spared whole-body protein. However, the macronutrient source itself did not have an independent effect on attenuating the negative energy balance. Rather, it appears to be the increase in calories alone. This study reinforced the need to identify strategies to attenuate negative energy balance during military field operations. Additionally, this type of exercise is not by itself any more inflammatory than conventional endurance exercise. Supplementation with carbohydrate and protein did not attenuate the inflammatory response where there was substantial energy deficit. This study provides new information to improve military field feeding to promote energy balance and attenuate the inflammatory response to military training.

Thematic Area 3. Nutrients and Resilience

Projects in this area examined the role nutrition plays in recovery and resilience following military stress.

Project 4: H14-02. Effect of protein supplementation on lean body mass recovery and physiological resilience following Survive, Evade, Resist, Escape (SERE) School.

Specific Aim: Conduct a study to determine the role of protein supplementation on lean mass recovery and resilience following SERE training.

This study was designed to evaluate the efficacy of a prototype protein-optimized combat recovery ration item on psychological resilience, lean mass accretion, physical and cognitive performance, and biomarkers of physiological status during recovery from extreme operational stress at U.S. Marine (MARSOC) SERE training. Evidence from recent studies, including those conducted by PBRC and USARIEM, suggested consuming high-protein diets or supplemental tyrosine confers protection and promotes recovery from extreme physiological and psychological stress. Whether supplemental protein, high-quality protein with high levels of tyrosine, enhances skeletal muscle and physical and behavioral recovery from "real-world" military stress had not been determined. In a collaborative effort, Dr. Jennifer Rood (PBRC) and Drs. Stefan M. Pasiakos, Harris R. Lieberman and James P. McClung (USARIEM) evaluated the efficacy of a tyrosine-enriched whey protein recovery supplement on lean body mass recovery (i.e., accretion), and physiological and psychological recovery from extreme operational stress. Specimen collection materials were prepared and shipped by PBRC, specimen collection was completed by USARIEM and samples were shipped to PBRC for analysis **(Major Task 1)**.

PBRC used advanced analytical techniques to determine if there are novel biochemical markers of physiological and psychological resilience and targeted approaches for recovery-based nutrition **(Major Task 2)**. A table of the completed assays is provided below:

Analyte	Baseline		Post-SERE		Recovery		Sample Type	Draw Tube	Aliquot Vol. (µl)
	D 2	D 3	D 18	D 19	D 45	D 46			
Glucose	X		X		X		Serum		500
IGF-11	X		X		X		Serum		500
NPY1	X		X		X		Serum		500
Cytokines	X		X		X		Serum		500
Cortisol	X		X		X		Serum		
C-Reactive Protein	X		X		X		Serum		
DHEA-S1	X		X		X		Serum		
Growth hormone	X		X		X		Serum	10ml and 7ml Red	
Insulin	X		X		X		Serum		1000
Luteinizing hormone	X		X		X		Serum		
Prolactin	X		X		X		Serum		
SHBG1	X		X		X		Serum		
Testosterone	X		X		X		Serum		
Fatty acids	X		X		X		Serum		1000
Archived serum	X		X		X		Serum		3x 1000
Epinephrine	X		X		X		Treated Plasma	10ml Green +10% Na-metabisulfite	1x 2000
Nor-epinephrine	X		X		X		Treated Plasma		2x 1500
Amino Acids	X		X		X		NaHep Plasma	7ml Green	1000
Omega 3:62	X						RBC's - NaHep		1x1500

All data was sent to the principal investigator at USARIEM electronically via the secure website https://safe.amrdec.army.mil/safe/. Data was analyzed, and the initial results are listed below:

1. SERE training induced significant weight loss, including the loss of fat-free mass, and resulted in a negative net protein balance.
2. The majority of physiological decrements incurred during SERE training resolved and returned to baseline levels after 27 days, with one exception of net protein balance.
3. Protein supplementation, in addition to consuming an ad libitum higher-protein, energy-adequate diet, is not necessary to restore fat-free mass following periods of severe negative energy balance.

Two abstracts on initial findings were presented in 2016 **(Milestone #4)**:

1. Pasiakos SM, Berryman CE, Margolis LM, Sepowitz JJ, McClung HL, Lieberman HR, McClung JP, Ferrando AA. Changes in protein turnover, hormonal status, and body composition during physiologically demanding military training. Experimental Biology, April 2016
2. Sepowitz JJ, McClung HL, Berryman CE, Armstrong NH, Ferrando AA, Lieberman HR, McClung JP, Pasiakos SM. Supplementing an energy adequate high protein diet with additional protein is not necessary for recovery of lean body mass after short-term starvation. American College of Sports Medicine Annual Meeting, June 2016

Project 5: H15-07. Efficacy of a once-daily calcium and vitamin D fortified food product to improve bone microarchitecture in response to Army basic combat training.

Specific Aim: Conduct a study to determine the efficacy of a calcium and vitamin D fortified food product in improving bone microarchitecture in response to Army basic combat training.

This project was led by Erin Gaffney-Stomberg, Ph.D. (USARIEM) at Ft. Jackson, SC. The primary objective of the trial is to determine the efficacy of a once-per-day calcium and vitamin D fortified food product on bone turnover markers, including parathyroid hormone and microarchitecture, during Army basic combat training. Volunteers were randomized to receive a food product bar either fortified with calcium and vitamin D or a placebo food product bar. Food product bars were taken once per day. The dose of calcium (as calcium carbonate) and vitamin D (as vitamin D3) was 1,000 mg and 1,000 IU/day, respectively. Blood samples were collected at pre and post study timepoints. As part of this project, PBRC prepared and shipped barcoded specimen collection tubes and specimen aliquot cryovials, and Dr. Rood and members of her team traveled to Ft. Jackson to assist with blood collection, sample processing and testing at the site **(Major Task 1).**

Blood samples were shipped to PBRC for biomarker analysis **(Major Task 2):**

a. 6,816 biochemistry assays were completed and include the following: cortisol, DHEAs, growth hormone, glucose, hsCRP, IGF-1, IL-6, insulin, LH, prolactin, SHBG, testosterone, neuropeptide Y, alanine, arginine, asparagine, aspartate, cysteine, glutamine, glutamate,

glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, serine, threonine, tryptophan, tyrosine, and all fatty acid assays were performed (n= 1704 tests) and the following fatty acids were included in the profile: C14:0, C14:1, C16:0, C16:1, C18:0, C18:1c, C18:2c, C20:0, C18:3 n-6, C20:1, C18:3 n-3, C20:2, C22:0, C20:3 n-6, C22:1, C20:3 n-3, C20:4, C22:2, C24:0, C20:5, C24:1, and C22:6.

b. All data was sent to the principal investigator at USARIEM electronically via the secure website https://safe.amrdec.army.mil/safe/.

The data analysis is in progress and the PI is currently reviewing the data for publication.

Project 6: H15-10. A Prospective Study of Factors associated with Career Success among Soldiers in Special Forces Training: The Army Career Resiliency and Injury Cohort Study.

Specific Aim: To conduct a prospective study of factors associated with career success among soldiers in Special Forces training.

This study is led by Emily Farina, Ph.D. (USARIEM) at Camp Mackall, NC. This is an observational study to identify factors associated with short-term and long-term career success and injury/illness in soldiers enrolled in Special Forces training. The objective of the study is to determine whether biomarkers, body composition, genetic polymorphisms, usual dietary intake, mood and resiliency questionnaires, sleep quantity and quality, and potential stressors to life events predict career success and injury. We hypothesize that soldiers with desirable measures of biochemical markers of nutrient status will have lower levels of inflammatory markers, higher Army Physical Fitness Test scores, faster run and ruck times, lower body fat and higher lean mass, and will be more likely to complete training and retain active duty status, and will be less likely to experience injuries during their career. We also hypothesize that soldiers with higher levels of inflammatory markers will have higher fasting blood glucose, insulin and HbA1C.

PBRC prepared and shipped barcoded specimen collection tubes and specimen aliquot cryovials to the field site on a regular basis as the pre and post collections occurred for the multiple iterations of this long-term project. Fasted blood samples are being collected by USARIEM at three timepoints: at the start of Special Forces Assessment and Selection (SFAS), at the end of SFAS and at the end of Special Forces Qualification Course (SFQC). Multiple iterations of this study were completed **(Major Task 1)**.

Samples are being shipped to PBRC for analysis of basic biochemistry markers, markers of endocrine function, fatty acids and measures of metabolism. Several physiologic markers will be assessed, which may include (but not be limited to): cortisol, testosterone, sex hormone binding globulin (SHBG), albumin, neuropeptide Y (NPY), dehydroepiandrosterone-sulfate (DHEA-S), brain derived neurotrophic factor (BDNF), ghrelin, epinephrine, norepinephrine, high sensitivity C-reactive protein (hsCRP), interleukin-6 (IL-6), insulin like growth factor-1 (IGF-1), and IGF-1 binding proteins. Markers of nutritional status also will be assessed, which may include (but not be limited to): ferritin, transferrin receptor, transferrin saturation, hepcidin, 25-OH vitamin D, parathyroid hormone, insulin, HbA1c, a profile of red blood cell fatty acids, cholesterol, triglycerides and apolipoproteins. Fatty acids will be quantified from red blood cell membranes,

while all other markers will be assessed from serum and/or plasma. The procedures used for extraction and quantification will be specific for each marker and may include the use of high performance liquid chromatography and immunoassays. Additional nutritional or physiological markers may also be assessed, as deemed relevant by the current literature. PBRC has completed the following **(Major Task 2)**:

a. Testing has been completed for all iterations for the following analytes: HbA1C, cortisol, hsCRP, dhea-S, ferritin, insulin, prolactin, para thyroid hormone, sex hormone binding globulin, testosterone, BDNF, ghrelin, soluble transferrin receptor, 25 hydroxy vitamin D, IL-6 and neuropeptide Y.
b. All RBC fatty acid assays were completed for all iterations and the following fatty acids were included in the profile: C14:0, C14:1, C16:0, C16:1, C18:0, C18:1c, C18:2c, C20:0, C18:3 n-6, C20:1, C18:3 n-3, C20:2, C22:0, C20:3 n-6, C22:1, C20:3 n-3, C20:4, C22:2, C24:0, C20:5, C24:1 and C22:6.
c. Data was sent to the principal investigator at USARIEM electronically via the secure website https://safe.amrdec.army.mil/safe/.
d. A total of 46,600 assays were completed. Samples were collected pre and post training on 1,200 subjects.

This project is ongoing and new samples are being collected and analyzed under a different contract. Data analysis will begin at the completion of sample collection.

Project 7: H15-12. Effects of Meal, Ready-to-Eat (MRE) consumption on gut health.

Specific Aim: To conduct a study to determine the impact of consuming MREs as a sole source of subsistence for 21 days on gut barrier integrity.

This study was led by J. Philip Karl, Ph.D. (USARIEM) at the U.S. Army Natick Soldier Systems Center (NSSC) in Natick, MA. We hypothesize that:

1. Consuming MREs as the sole source of subsistence for 21 days will decrease gut barrier integrity.
2. Decreased gut barrier integrity will occur concomitant to and be associated with reduced gut microbiota diversity and increased inflammation.
3. Consuming MREs as the sole source of subsistence for 21 days will not adversely affect nutritional status.

The study was a randomized, parallel-arm, controlled trial. Participants were randomized to one of two study groups. The control group did not receive any intervention but was instructed to continue following their normal diet and activity patterns throughout the study. The intervention group was provided with and instructed to consume MREs and nothing else (except for water) for 21 consecutive days, and then eat their normal diet for 10 consecutive days.

Differential sugar absorption tests were used to provide a functional assessment of permeability within both the small intestine and large intestine. These tests were conducted by orally

administering multiple saccharide probes that differentially cross the intestinal barrier into circulation and are subsequently excreted in urine. A large molecular weight probe (e.g., sucralose, lactulose) was used to assess absorption via paracellular pathways. Absorption of these probes increases as intestinal permeability increases. A small molecular weight probe (e.g., mannitol), which freely crosses the intestinal barrier independent of barrier integrity, also was used and provided a control for confounding factors potentially influencing urinary excretion rates (e.g., differences in transit time, gastric emptying, surface area, etc.) The ratio of large to small molecular weight probe excretion in urine provides a functional assessment of gastrointestinal permeability.

To assess gastroduodenal, small and large intestinal permeability, volunteers consumed 5 g sucrose, 2 g sucralose, 5 g lactulose and 4 g mannitol dissolved in water on the morning of study days 0, 10, 21 and 31. Volunteers then collected all urine produced over the subsequent 24 hours. Aliquots were taken from 0-1 hr, 0-2 hr, 0-5 hr and 5-24 hr collections. Volunteers were then provided with urine collection containers, coolers and ice packs, and instructed to collect all urine produced during the 24 hr period. Blood samples also were collected on days 0, 10, 21 and 31 to measure markers of gut barrier integrity and inflammation.

PBRC prepared and shipped barcoded specimen collection tubes and specimen aliquot cryovials to the field site. USARIEM collected blood and urine samples. **(Major Task 1)**

Urine aliquots were frozen and shipped to PBRC for analysis of sucrose, sucralose, lactulose and mannitol concentrations. Blood samples also were analyzed for markers of gut barrier integrity and inflammation. Markers of metabolic health and nutritional status also were analyzed **(Major Task 2)**. Data was sent to the principal investigator at USARIEM electronically via the secure website (https://safe.amrdec.army.mil/safe/.

Results are being compiled and readied for publication by the PI.

Project 8: H15-21. Evaluation of calcium and vitamin D supplementation for optimizing bone health during Marine Corps recruit training.

Specific Aim: To conduct a study to evaluate calcium and vitamin D supplementation as a method to optimize bone health during Marine Corps recruit training.

The project was led by Erin Gaffney-Stomberg, Ph.D. (USARIEM) at Parris Island, SC. The primary objective and hypothesis of this study was to determine the efficacy and effectiveness of daily calcium and vitamin D supplementation (Ca+D) on maintenance of parathyroid hormone and indices of bone strength in Marine Corps recruits during training. We hypothesized that Ca+D would prevent elevations in parathyroid hormone and result in greater increases in indices of bone strength compared to placebo. Two methods of delivery (capsules vs. fortified food product) were evaluated. Effectiveness will be determined first by study attrition rates and compliance of the capsule groups compared with bar groups. We also examined the effects of Marine Corps recruit training on nutrient intake and biochemical indicators of nutrition status, to include iron status and explored the effects of training on energy intake/energy balance and bone health.

The completed study was a randomized, double-blind, placebo-controlled trial conducted by USARIEM and Uniformed Services University of the Health Sciences (USUHS) researchers. Calcium and vitamin D or placebo were provided daily to male and female Marine recruits undergoing Marine Corps recruit training as either supplement pills or a fortified food product (First Strike bar) during both summer/fall and winter/spring iterations in order to test the effect of season. The main methodologies included peripheral quantitative computed tomography scanning of the tibia, biochemistries, food frequency questionnaires and body composition measures. Data was collected at the start of training (during reception) and again at the end of the 12th week of training prior to graduation.

PBRC prepared and shipped barcoded specimen collection tubes and specimen aliquot cryovials to the field site, and a specimen processing team (n=3) from PBRC traveled to Parris Island to assist with blood and urine collection/processing and performing blood assays onsite **(Major Task 1)**.

Samples were shipped to PBRC for analysis of nutritional status markers, biomarkers of inflammation, immune function and stress, and bone health **(Major Task 2):**

a. 7,249 biochemical assays were completed to include the following: % iron saturation; 1,25 dihydroxy vitamin D; 25 hydroxy vitamin D; ferritin; folate; iron; soluble transferrin receptor; total iron binding capacity; whole blood folate; alpha carotene; and beta carotene.
b. Data was sent to the principal investigator at USARIEM electronically via the secure website https://safe.amrdec.army.mil/safe/.

Enrollment, compliance and volunteer demographics are shown below:

Participant Recruitment/Volunteer numbers:

	Enrolled	Dropped	Completed	Attrition (%)†
Iteration 1 (July-Sept 2015)	180 (108/72)	35 (19/16)	145 (89/56)	19.4
Iteration 2 (Feb-May 2016)	200 (104/96)	59 (41/18)	141 (63/78)	29.5
Combined	380 (212/168)	94 (60/34)	286 (152/134)	24.7

Data are total (male/female). †Total attrition of volunteers includes attrition from training in addition to attrition from the study.

Average Weekly Compliance (%)

	Treatment			
	Placebo Bar	Ca/D Bar	Placebo Pills	Ca/D Pills
Male (n=152)	97.3 ± 3.3	96.7 ± 5.5	69.8 ± 14.1	70.5 ± 14.3
Female (n=134)	98.6 ± 2.8	95.8 ± 9.3	63.3 ± 17.3	58.6 ± 17.4
Combined (n=286)	97.9 ± 3.1	96.3 ± 7.6	66.9 ± 15.9	65.2± 16.8
Bar (n=147)	97.1 ± 5.7			
Pill (n=139)			66.0 ± 16.3	

Only subjects that completed the study included. Data are means ± SD.

Demographics

		Total	Treatment			
			Placebo Bar	Ca/D Bar	Placebo Pill	Ca/D Pill
Sex		n	n	n	n	n
Male	Enrolled	212 (56%)	53 (14%)	54 (14%)	52 (14%)	53 (14%)
	Completed	152 (53%)	39 (14%)	36 (13%)	37 (13%)	40 (14%)
Female	Enrolled	168 (44%)	45 (12%)	45 (12%)	38 (10%)	40 (11%)
	Completed	134 (47%)	37 (13%)	35 (10%)	30 (10%)	32 (11%)
Race		n	n	n	n	N
White	Enrolled	286 (75%)	76 (20%)	71 (19%)	70 (18%)	69 (18%)
	Completed	218 (76%)	57 (20%)	51 (18%)	55 (19%)	55 (19%)
Black	Enrolled	62 (16%)	18 (5%)	19 (5%)	11 (3%)	14 (4%)
	Completed	44 (15%)	16 (6%)	14 (5%)	6 (2%)	8 (3%)
Native American/ Alaskan Native	Enrolled	5 (1%)	0	2 (1%)	2 (1%)	1 (<1%)
	Completed	2 (1%)	0	1 (<1%)	1 (<1%)	0
Asian	Enrolled	13 (3%)	1 (<1%)	4 (1%)	3 (1%)	5 (1%)
	Completed	11 (4%)	0	3 (1%)	3 (1%)	5 (2%)
Native Hawaiian/ Pacific Islander	Enrolled	1 (<1%)	0	0	1 (<1%)	0
	Completed	1 (<1%)	0	0	1 (<1%)	0
Other	Enrolled	13 (3%)	3 (1%)	3 (1%)	3 (1%)	4 (1%)
	Completed	10 (3%)	3 (1%)	2 (1%)	1 (<1%)	4 (1%)
Age (year)		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
	Enrolled	19.00 ± 1.66	19.07 ± 1.62	18.91 ± 1.49	19.04 ± 1.75	18.89 ± 1.65
	Completed	18.91 ± 1.60	18.93 ± 1.53	18.77 ± 1.45	19.01 ± 1.74	18.92 ± 1.70

Two abstracts on initial findings from the study were presented at scientific meetings (**Milestone #5):**

1. Scott JM, Gaffney-Stomberg E, Palmer JP, Daigle R, Kazman JB, McClung JP, Gasier HG. Vitamin D supplementation augment SIgA secretion rates in Marine Corps basic trainees. American College of Sports Medicine Annual Meeting, June 2016
2. Nakayama AT, Lutz U, McClung JP, Gaffney-Stomberg E. Calcium intake below the recommended dietary allowance is associated with lower tibia bone mineral content and strength in young adults entering initial military training. Experimental Biology, April 2017

Initial findings from this project are listed below:

1. Study attrition was higher and compliance was lower in pill groups compared to bar groups.
2. The Ca+D fortified bar improved vitamin D status compared to placebo bar; there were no group differences with pills.
3. Modest bone strength changes in the distal tibia suggest Ca+D fortified bars may result in greater improvement compared to other groups.

Project 9: H15-23. Energy balance and physiological responses to the US Marine Corps Forces Special Operations Command (MARSOC) Individual Training Course (ITC).

Specific Aim: To conduct a study to examine energy expenditure and response to MARSOC training.

This project was directed by Stefan M. Pasiakos, Ph.D. (USARIEM). The objectives of this study are listed below:

1. Determine energy balance by assessing energy expenditure and energy intake during multiple phases of ITC.
2. Determine changes in body composition and skeletal muscle mass.
3. Determine changes in androgen, immune and nutritional status and gut permeability.

We hypothesized that energy expenditure will exceed energy intake during rigorous training phases of ITC, particularly when subsisting on US combat rations. We also hypothesized that Survival, Evasion, Resistance and Escape (SERE); Raider Spirit; Close Quarter Battle (CQB); and Derna Bridge will cause decrements in total body and skeletal muscle mass. Total body mass will be recovered when in garrison and we expect body composition will be the same at the conclusion of ITC relative to baseline. Our final hypothesis is that Androgen (e.g., testosterone, follicle-stimulating hormone [FSH], luteinizing hormone [LH], insulin-like growth factor one [IGF-1], sex hormone binding globulin [SHBG]), immune (e.g., C-reactive protein [CRP] and immunoglobulins [Ig]), and nutritional (e.g., ferritin, soluble transferrin receptor, hemoglobin and hepcidin) status and gut permeability will be diminished after SERE, Raider Spirit, CQB and Derna Bridge. Status will improve with recovery, although we expect androgen, immune and nutritional status to be lower at the conclusion of ITC compared to baseline.

This longitudinal, observational study measured energy balance (intake – expenditure), dietary macronutrient composition and changes in diet quality in consenting male U.S. Marines participating in ITC (Stone Bay, Camp Lejeune, NC). Twenty-six Marines volunteered; however, six withdrew at various times during the study. Data were analyzed on the 20 Marines (mean ± SD; 25 ± 2 y, 86 ± 10 kg) who completed all testing. Data were collected before (PRE), during and after (POST) four predetermined, sequential field training exercises selected by MARSOC Training Command Staff: 1) Survival, Evasion, Resistance, Escape (SERE) School, 2) Raider Spirit (RS), 3) Close Quarter Battle (CQB) and 4) Derna Bridge (DB). Diet quality was assessed at the start and end of ITC.

As part of this protocol, Pennington Biomedical provided doubly labeled water for the measurement of total daily energy expenditure during the different phases of training. PBRC also provided specimen collection supplies and biochemical assays at multiple timepoints throughout the study. Blood samples were collected to assess androgen status, immune function, nutritional status and gut permeability.

Total daily energy expenditure (TDEE via doubly labeled water, DLW), energy intake (EI) and energy balance (EB = EI – TDEE) were assessed in a random subset of the original 20 volunteers during SERE (n = 10), RS (n = 12), CQB (n = 9) and DB (n = 13). Subset analyses were intended for 15 volunteers per phase; however, the sample sizes were smaller due to study attrition (SERE and RS) and because volunteers were tasked with other duties during CQB.

For TDEE assessments, volunteers provided a urine sample after an overnight fast to determine background abundance of 18O and 2H before ingesting the DLW (0.285 g H218O·kg total body

water [TBW]-1 and 0.15 g 2H2O·kg TBW-1; Sigma-Aldrich, St. Louis, MO). Volunteers remained fasted for the next 4 hours and provided urine samples at 4 hours and 6 hours to determine Total Body Water (TBW). Second-morning void urine samples were then collected daily during each training phase. Enrichments of 2H and 18O were measured using isotope ratio mass spectroscopy (Finnigan Mat 252, Thermo Fisher Scientific, Waltham, MA, USA). The 2H and 18O isotope elimination rates (kH and kO) were calculated by linear regression using the isotopic disappearance rates in the urine samples collected during each training phase **(Major Task 1)**.

Specimens were sent to PBRC for analysis of markers of basic biochemistry, energy expenditure, endocrine function and measures of metabolism **(Major Task 2):**

a. Urine specimens were analyzed for isotopic enrichment of deuterium and oxygen 18 to calculate total daily energy expenditure and total body water.
b. Urine data was sent to the principal investigator at USARIEM electronically via the secure website https://safe.amrdec.army.mil/safe/.
c. Blood sample analysis was completed for glucose, hepcidin, soluble transferrin receptor, igf-1, 25-hydroxy vitamin D, leptin, IgG, IgA, IgM, lipopolysaccharide, I-fatty acid binding protein, high sensitivity C reactive protein, FSH, ferritin, insulin, LH, sex hormone binding globulin, testosterone and claudin 3.
d. Completed blood testing results were sent to the principal investigator at USARIEM electronically via the secure website https://safe.amrdec.army.mil/safe/.

An abstract on initial findings from the study was presented in 2016 (**Milestone #6):**

> Sepowitz HH, Armstrong NJ and Pasiakos SM. Effects of intermittent periods of severe negative energy balance on weight maintenance during US Special Operations Forces training. American College of Sports Medicine. June 2016

The initial manuscript was published in the Journal of Special Operations Medicine:

> Sepowitz JJ, Armstrong NJ, Pasiakos SM. Energy balance and diet quality during the U.S. Marine Corps Forces Special Operations Command Individual Training Course. J Spec Oper Med. Winter 2017;17(4):109-113. PMID: 29256207

Results are summarized below:

Total daily energy expenditure (TDEE) was highest during Raider Spirit (RS) compared to Close Quarter Battle (CQB), Derna Bridge (DB) and the field phase of Survival, Evasion, Resistance, Escape (SERE) School ($P < 0.05$). Energy intake (EI) was the lowest during the field phase of SERE compared to CQB, DB and RS ($P < 0.05$). Energy balance (EB) was more negative during the field phase of SERE and RS than CQB and DB ($P < 0.05$). There were no differences in TDEE between the academic (3869 ± 233 kcal/d) and field (4011 ± 475 kcal/d) phases of SERE. EI during SERE was higher during the academic (3633 ± 662 kcal/d) versus field (346 ± 0.0 kcal/d) phase ($P < 0.05$). Energy balance during SERE was more negative during the field versus academic phase ($P < 0.05$). This data is summarized in the table below:

	SERE	RS	CQB	DB
TDEE (kcal/day)	4011 ± 475	6376 ± 712*	4189 ± 476	3754 ± 314
EI	346 ± 0*	2410 ± 488	2819 ± 488	2702 ± 738
EB	-3665 ± 475*	-3966 ± 776*	-1374 ± 683	-1027 ± 740

Note: Values are means ± SD; (SERE, n=10), RS (n= 12), CQB (n=9), DB (n=13). *Specific outcome measure different from other phases, $P < 0.05$.

The primary findings from this study are that the field training phases of Individual Training Course (ITC) resulted in negative energy balance due to high TDEE and low EI. Overall, TDEE during the four phases of ITC was approximately 4500 kcal/d; expenditure levels similar to those observed during U.S. Army Special Forces Small Unit Tactics (SUT, ~4400 kcal/d), pre-mission (~3900 kcal/d) and combat diver qualification (~4500 166 kcal/d) training. The highest TDEE measured during ITC was during RS (6376 ± 712 kcal/d), which was 22% higher than the highest TDEE measured during SUT (~5200 kcal/d), and comparable to the TDEE measured in Norwegian Infantry Soldiers participating in strenuous, short-term Arctic training (~6200-6900 kcal/d). The Marines were unable to balance TDEE with EI, particularly during SERE and RS because food availability was intentionally restricted, resulting in a 5-6% loss of body mass. These findings suggest that the magnitude of physical demand during ITC is no different than similar Special Operations Forces and infantry training exercises, and while Marines are exposed to cyclic periods of severe negative energy balance and energy surplus, body mass is generally well maintained during the 9-month course.

Project 10: Supplementation Trial on Arginine with Metabolic Profiling (STAMP).

Specific Aim: To conduct a study to determine the effects of an acute dose of arginine on metabolism specifically related to the pituitary axis including prolactin and growth hormone.

Dietary supplements with arginine are popular among service members. Previously, the effects of tyrosine were investigated using metabolomics, and multiple metabolic changes were identified that could explain tyrosine's neuroendocrine effects. To determine if there is a common central mechanism explaining the effects of tyrosine and arginine (two dissimilar amino acids found in food), we conducted a study of the acute effects of arginine on multiple aspects of neuroendocrine function using classic laboratory assays and state-of-the-art metabolomic technologies. The study was designed to determine if a single dose of arginine could beneficially affect hormonal profiles of warfighters, thereby providing them with key physiological advantages. The double-blind, randomized, crossover design trial examining arginine versus placebo beverage treatments was conducted at PBRC in 2018, led by John Apolzan and Jennifer Rood in collaboration with Dr. Harris Lieberman at USARIEM **(Major Task 1)**. The primary study endpoints were growth hormone, prolactin and metabolomics. A list of the procedures and participant visits are detailed below.

Table 1. Procedures	SV 1	Meal Provision	Meal Provision	CV 1	CV 2	Meal Provision	Meal Provision	CV 3	CV 4
Informed Consent	X								
Brief Lifestyle Interview	X								
Medical History Form, demography, caffeine use form, & contact info	X								
CMEDS	X								
Metabolic Weight, Height, BMI, Waist/Hip Circumference, Bioelectrical Impedance	X								
Vitals (BP, pulse)	X								
Blood Draw (s)†	X*			X	X‡			X	X‡
Food Provision§ (Weekday)		X	X	X		X	X	X	
Metabolic Weight				X				X	
Treatment Beverage (provided by DOD)				X				X	
Peripheral Arterial Tonometry (PAT)				X				X	
Psychological Assessments including Fatigue+				X	X			X	X

* CBC and Chem 26 (Fasting)
§ Food provision is for the 2 days immediately prior to the clinic visit.
† Time points are baseline (fasting), 1.5 hr, 3.0 hr, and 6.0 hr at CV 1 and CV3. Study archives includes serum, plasma, and metabolomics at all time points. Further, at the 6.0 hour time point, please archive enough blood to potentially rerun all parameters.
+Questionnaires include: Visual Analogue Scales (VAS) for subjective ratings of appetite and Profile of Mood States (POMS),. See individual questionnaires for time points of assessment.
Modified Acute Sleep Questionnaire will be performed at home on at least 3 mornings between SV1 and initial provision and on each clinic visit morning.
‡ Please take sample 24 hr after beginning of treatment beverage consumption (i.e. time 0). Study archives includes serum, plasma, and metabolomics at all time points.

Procedures Involved

Overview:
To accomplish the objective, standardized meals were provided for two days preceding treatment for dietary consistency. Participants arrived at the center following a 10- to 12-hour fast. Blood samples were collected at baseline (fasting) and 1.5, 3, 6 (archived), and 24 hours post-treatment. Plasma prolactin, growth hormone, amino acids, glucose, insulin, triacylglycerols, thyroid hormones (TSH, T3 and T4), sex hormone binding globulin (SHBG), testosterone, cortisol, DHEA and citrulline are being measured. Metabolomics will be measured at three timepoints for each treatment. Peripheral arterial tonometry (PAT) was performed about two hours following treatment to examine endothelial function in response to nitrous oxide release. In addition, psychological status including fatigue was assessed using a standardized mood questionnaire. Fasting body weight was obtained on days of treatment consumption.

Dose:
A formulated drink containing about 10 grams of arginine or a placebo was provided. The only difference in the formulated drink between treatments was arginine.

Diet:
Isocaloric diets were provided for three days for each condition (six days total). The first two days were pretreatment and Day 3 was post-treatment. Day 3 could be slightly more energy dense (still isocaloric) so it was easier to consume in an abbreviated time span. Isocaloric was determined by estimated resting metabolic rate (RMR) * 1.7 physical activity level (PAL). The menu provided 12.5% protein, 30% fat and 57.5% carbohydrate. Arginine was maintained at 3-5 grams/day during the feeding period(s), and no high-arginine foods were provided.

Participants picked up food daily. Breakfast was consumed onsite (on days without a blood draw), and other meals could be packed out or consumed onsite. Meals were provided only on weekdays, with the first food provision day starting on a Monday or Tuesday.

Metabolomics and other blood parameters:
Blood parameter analysis will be performed only on samples collected at the baseline (fasting), 1.5-hour, 3-hour and 24-hour timepoints. A 6-hour timepoint was collected to be archived.

Blood parameters are being measured on samples collected at baseline, 1.5-hour, 3-hour and 24-hour timepoints:

- Growth hormone
- Prolactin
- Amino acids including citrulline
- Glucose
- Triglyceride
- Insulin
- Thyroid panel (TSH, T4, T3)
- Sex hormone binding globulin (SHBG)
- Testosterone
- Estradiol
- Stress hormones:
    - Cortisol
    - Dehydroepiandrosterone sulfate (DHEA-S)

All timepoints had additional blood archived (serum and plasma).

Samples collected at three timepoints for each treatment will be sent to Metabolon, Inc. for comprehensive metabolomic analysis.

For all analysis, when possible, high-sensitivity assays are being performed.

Peripheral Arterial Tonometry (PAT):
PAT occurred following the 1.5-hour blood draw.

Endothelial function was assessed using an Endo-PAT 2000 device (Itamar Medical Ltd., Caesarea, Israel). During the measurement, the subject lay supine with the hands at the level of the heart and fingers hanging freely. Fingertip probes were placed on both index fingers, and pulse wave amplitudes were detected and recorded during the study. After a roughly 10-minute baseline measurement, arterial flow was occluded using a cuff on the nondominant arm. The cuff was inflated to 60 mmHg above systolic pressure (but not less than 200mmHg) and then increased as needed to occlude vessel. After five minutes of occlusion, the cuff was rapidly deflated to allow reactive hyperemia. Pulse wave amplitudes were recorded again for at least five minutes.

The Endo-PAT software will be used to compare the arterial pressure ratio in the two fingers before and after occlusion. The reactive hyperemia index (RHI), a ratio of the average pulse wave amplitude measured over 60 seconds starting one minute after cuff deflation to the average pulse wave amplitude measured at the baseline, also will be calculated. The arm that is not occluded will serve as a control, and the ratio will be corrected for changes in the systemic vascular tone.

All acquisitions and analysis are being completed by a trained technologist.

Psychological Assessments:
Assessments occurred at baseline (fasting), 1.5 hours, 3 hours, 6 hours and 24 hours for Profile of Mood States and subjective ratings of appetite following the blood draw. Also, the psychological assessments were performed before the PAT at the 1.5-hour timepoint.

Profile of Mood States (POMS): The POMS has six subscales and 65 questions: tension (score range 0-36), depression (score range 0-60), anger (score range 0-48), confusion (score range 0-28), vigor (score range 0-32), fatigue (score range 0-28) and a total mood disturbance score (score range -32 to 200). Participants rated each of the 65 mood-related adjectives on a five-point scale in response to the question “How are you feeling right now?”

Visual Analog Scales (VAS) for subjective ratings of appetite: VAS were used to measure average ratings of satiety that participants experienced.

Assessments for the modified sleep acute sleep index were conducted the morning after every treatment day at participants’ homes and also the following day. Baseline sleep also was assessed on several occasions (minimum three mornings) after the screening visit but prior to initial meal provision. All questionnaires were completed at home for consistency.

Modified Pittsburgh Sleep Quality Index: The Pittsburgh Sleep Quality Index [Buysee, Reynolds, Monk, Berman, & Kupfer (1989)] is used to assess sleep quality and disturbances over a one-month time interval. This is a 19-item measure that consists of multiple component scores: subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medication and daytime dysfunction. The total score is derived by summing scores for component items. This measure has acceptable test-retest reliability, internal homogeneity and validity. We modified this questionnaire to examine sleep during the previous night.

Schedule of screening and clinic visits

Screening Visit 1: About 2.5 hours – Fasting (nothing to eat or drink other than water for at least 10 hours prior to visit)

This visit took place in the Outpatient Clinic of Pennington Biomedical Research Center and included the following procedures:

- Medical history questionnaire
- Review of current medication and supplement use
- Demographic and Lifestyle Questionnaire
- Lifestyle Interview to discuss subject's understanding of the study and for the study staff to know what kinds of barriers might have made it difficult for subject to participate in the study. Such lifestyle barriers include work or school schedule, outside activities and other responsibilities.
- Caffeine Use Form
- Height
- Weight
- Body Fat Percentage (Body Composition) by bioelectrical impedance (BIA)
- Circumferences: measurement of waist and hip
- Vital signs (blood pressure and heart rate)
- Sleep questionnaire that was taken home to obtain several baseline measurements
- Fasting blood draw (about one tablespoon) to determine health of kidneys, liver and electrolytes

Clinic Visit 1: About 7 hours – Fasting (nothing to eat or drink other than water for at least 10 hours prior to visit) after at least seven hours of rest the previous evening

This visit took place in the Inpatient Clinic of Pennington Biomedical and included the following procedures:

- Body weight
- Blood draws (~8 tablespoons): metabolomics, amino acids (protein), glucose and hormone levels. The baseline blood draw occurred between 7:30 a.m. and 8:30 a.m.
- Questionnaires: appetite and mood
- Treatment beverage consumption
- Peripheral Arterial Tonometry (PAT): Measurement of blood vessel function
- Lunch meal was provided after 6-hour blood draw and psychological assessments
- Dinner was provided via pack out

Clinic Visit 2: About 0.75 hours – Fasting (nothing to eat or drink other than water for at least 10 hours prior to visit) after at least seven hours of rest the previous evening

This visit took place in the Outpatient Clinic of Pennington Biomedical and included the following procedures:

- Blood Draw (about 2.5 tablespoons): metabolomics, amino acids (protein), glucose and hormone levels about 24 hours after initiating consumption of treatment beverage
- Questionnaires: appetite and mood

Clinic Visit 3: About 7 hours – Fasting (nothing to eat or drink other than water for at least 10 hours prior to visit) after at least seven hours of rest the previous evening

This visit took place in the Inpatient Clinic of Pennington Biomedical and included the following procedures:

- Body weight
- Blood draws (about 8 tablespoons): metabolomics, amino acids (protein), glucose and hormone levels. The baseline blood draw occurred between 7:30 a.m. and 8:30 a.m.
- Questionnaires: appetite and mood
- Treatment beverage consumption
- Peripheral Arterial Tonometry (PAT): measurement of blood vessel function
- Lunch meal was provided after 6-hour blood draw and psychological assessments
- Dinner was provided via pack out

Clinic Visit 4: About 0.75 hours – Fasting (nothing to eat or drink other than water for at least 10 hours prior to visit) after at least seven hours of rest the previous evening

This visit took place in the Outpatient Clinic of Pennington Biomedical and included the following procedures:

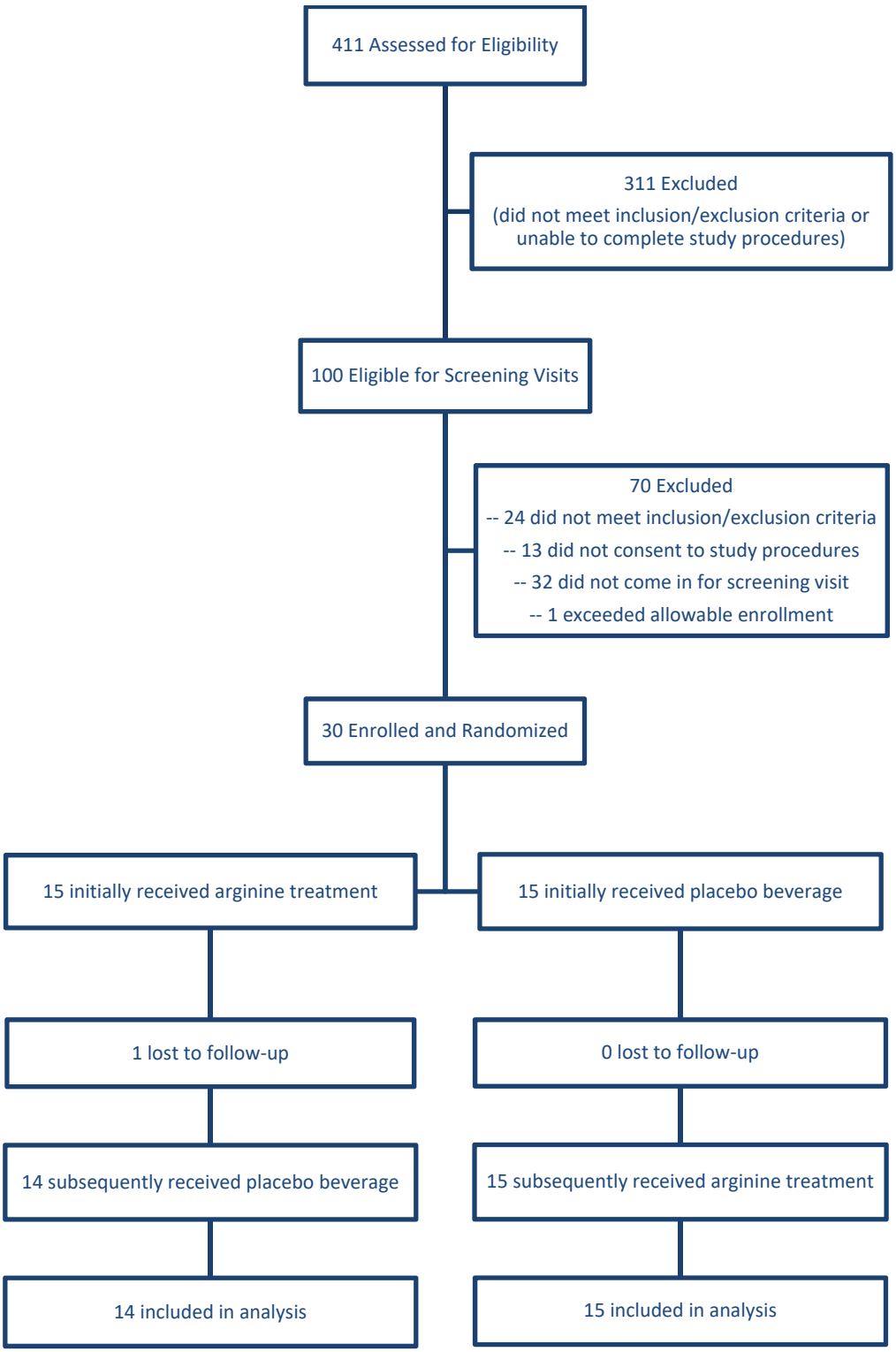
- Blood draw (about 2.5 tablespoons): metabolomics, amino acids (protein), glucose and hormone levels about 24 hours after initiating consumption of treatment beverage
- Questionnaires: appetite and mood

Data and Specimen Banking

The principal investigator and co-investigators have access to the specimens. The specimens are stored frozen (-80°C) indefinitely. Specimens will be appropriately transferred to Metabolon, Inc for analysis.

The study consort diagram is shown below.

Figure 2. STAMP Consort Diagram



The study was completed, and analysis of biospecimens, measures of peripheral arterial tonometry, questionnaires and psychological assessments **(Major Task 2)** is ongoing.

Thematic Area 4. Healthy Eating and Behavior

Project 11: H14-14. Nutritional Practices of Soldiers Consuming Meals in Military Dining Facilities (DFAC-Nutrition Study).

Specific Aim: To conduct a study to examine the efficacy of a dietary intervention to improve soldiers' omega-3 fatty acid status in a garrison feeding environment.

This project was led by LTC Asma Bukhari, Ph.D. (USARIEM) at the Natick Soldier Systems Center dining facility. The study was a prospective between-subjects repeated measures placebo-controlled study design with two groups (experimental and control). The control group consumed meals without change in any aspects of the dining facility (DFAC) operations. The experimental group was provided similar menu options but certain foods were swapped with like items with high n-3 and low n-6 polyunsaturated fatty acid (PUFA) ratios. PBRC prepared and shipped barcoded specimen collection tubes and specimen aliquot cryovials to the field site. USARIEM collected blood samples at two timepoints (week 0 and week 9) and shipped them to PBRC for the measurement of fatty acids, lipids and markers of inflammation **(Major Task 1)**. Dr. Catherine Champagne (PBRC) was an associate investigator on this project serving in an advisory role and assisting with data analysis.

PBRC analyzed specimens for markers of lipid function, fatty acids and measures of metabolism **(Major Task 2)**:

a. Blood assays were completed, including the following: cholesterol, homocysteine, HDL cholesterol, hsCRP, LDL cholesterol and triglycerides. (222 assays)
b. Red blood cell fatty acid assays were completed, including C14:0, C14:1, C16:0, C16:1, C18:0, C18:1c, C18:2c, C20:0, C18:3 n-6, C18:3 n-3, C20:2, C22:0, C20:3 n-6, C22:1, C20:3 n-3, C20:4, C22:2, C24:0, C20:5, C24:1 and C22:6. (819 assays)
c. Results were sent to the principal investigator at USARIEM electronically via the secure website https://safe.amrdec.army.mil/safe/.

An abstract on findings from the study was presented at a scientific meeting in 2017 **(Milestone #7)**:

> Bukhari AS, Lutz LJ, Smith TJ, Hatch AM, Hawes MR, O'Conner KL, Carrigan CT, McGraw SM, Champagne CM, Montain SJ. A food-based intervention in a military dining facility results in improvements in blood fatty acid profile. Food and Nutrition Conference and Exposition of the Academy of Nutrition and Dietetics, October 2017

Initial results from this study show that the intervention in the dining facility improved the blood fatty acids profiles. Additional analysis is underway, and data is being readied for publication.

Project 12: H15-04. Dining satisfaction and diet quality of soldiers eating at two Fort Bragg dining facilities (DFACS).

Specific Aim: To conduct a study to assess the quality of dietary intakes of Soldiers using food photography.

The purpose of this study led by LTC Renee Cole, Ph.D. (USARIEM) was to test the efficacy of strategic food placement, performance-based menu enhancement and point-of-service labeling on Special Operations Forces (SOF) Soldiers' nutritional intake and dining satisfaction. SOF Soldiers and students at the U.S. Army John F. Kennedy Special Warfare Center and School (SWCS) who consumed meals at the Tactical Human Optimization Rapid Rehabilitation and Reconditioning (THOR3) modified SWCS dining facility (DFAC), Fort Bragg, NC, were compared to Soldiers consuming meals at a standard Fort Bragg DFAC without extensive THOR3 intervention. The digital photography method was used to quantify food selections and intake of enrolled study participants at four timepoints. At each meal, foods and beverages selected by the Soldiers prior to and after eating were photographed using digital video cameras. As part of this project, PBRC piloted the use of a second camera at baseline in order to evaluate whether this simplified and enhanced accuracy of estimations **(Major Task 1)**.

Procedures and findings are detailed in the Technical Report included in the appendix of this report:

> Cole RE, Bukhari AS, Champagne CM, Hatch AM, McGraw SM, Allen R, Montain SJ. Technical Report T17-03. Evaluation of a Dining Facility Intervention on U.S. Army Special Operations Soldiers' Meal Quality, Dining Satisfaction, and Cost Effectiveness. Military Nutrition Division, U.S. Army Research Institute of Environmental Medicine; Nov 2016:1-85 (DTIC accession # AD1039585).

Daily nutrient content and Healthy Eating Index (HEI) scores were computed **(Major Task 2)**. Descriptive, pre to post t-test, and ANOVA statistical analysis from baseline to 4, 8 and 12-month post-intervention were performed (α=0.05; 80% power). A total of 688 (98% male; mean age of 25.6±2.9 yrs) Soldiers participated. At 12 months, individuals in the intervention group exhibited a significantly higher sodium-adjusted HEI score (70.3±8.7 points; +4.6 pts; p=0.005) compared to the control DFAC (average of 56.3±10.8 points over 12 months). The human performance program nutrition intervention exceeded the national HEI score (ranging 48-57 points). The improved HEI scores for participants were attributed to significant increases of 0.4 cups/day fruit, 0.4 cups/day red/orange vegetables, 0.8 cups/day whole grains, 0.9 cups/day protein-based legumes and 0.6 oz/day protein (p<0.05). USASOC patrons also exhibited significant reductions of 0.5 cups/day refined grains, 0.6 cups/day starchy vegetables, 0.9 cups/day milk (although 0.2 cups/day increase in yogurt), 11 g/day of oil and 6 g/day of solid fat (p<0.05). These data illustrate that education, introduction of healthy food options and revised cooking practices are effective interventions for improving Warfighter meal quality.

Eight abstracts on findings from this study were presented at scientific meetings **(Milestone #8)**:

1. Bukhari AS, Crombie AP, McGraw SM, Champagne CM, Allen R, Montain SJ, Young AJ. Impact of military dining facility serving modifications on the nutritional intake of special operations personnel. FASEB J 30:895.12, April 2016
2. Bukhari AS, Champagne C, Logan CM, Montain SJ, Cole RE. Key Insights from Foodservice Staff Regarding Operations at Military Dining Facilities. Military Health System Research Symposium, August 2016
3. Cole RE, Bukhari AS, Champagne CM, McGraw SM, Hatch AM, Logan CM, Spanbauer SM, Montain SJ. Healthy Eating Index Increased after Tactical Human Optimization, Rapid Rehabilitation and Reconditioning (THOR3) Dining Facility Menu Enhancement in Special Forces Operators. Military Health System Research Symposium, August 2016
4. Cole RE, Bukhari AS, Champagne CM, McGraw SM, Hatch AM, Logan CM, Spanbauer SM, Montain SJ. Adequate sleep is associated with healthy eating, physical activity and time spent inactive. Military Health System Research Symposium, August 2016
5. McGraw SM, Bukhari AS, Champagne CM, Hatch AM, Logan CM, Spanbauer SM, Montain SJ, Cole RE. Physically fit Soldiers eat healthier and feel nutrition impacts physical performance. Military Health System Research Symposium, August 2016
6. Cole RE, Bukhari AS, Champagne CM, McGraw S, Hatch AM, Logan CM, Spanbauer SM, Montain SJ. Healthy Eating Index Increased after Tactical Human Optimization, Rapid Rehabilitation and Reconditioning (THOR3) Dining Facility Menu Enhancement in Military Operators. Food and Nutrition Conference and Expo, October 2016
7. McGraw S, Bukhari AS, Champagne CM, Hatch AM, Logan CM, Spanbauer SM, Montain SJ, and Cole RE. Physically fit Soldiers eat healthier and feel nutrition impacts physical performance. Food and Nutrition Conference and Exposition of the Academy of Nutrition and Dietetics, October 2016
8. Cole RE, Bukhari AS, McGraw SM, Champagne CM, Hatch AM, Karl JP. Predictors of diet quality and appetite in soldiers eating at Military Dining Facilities. Military Health System Research Symposium, August 2017

An abstract on improved dietary assessment techniques using digital photography also was presented at a scientific meeting in 2016 **(Milestone #9)**:

> Champagne CM, Allen HR, Johnson CM, and Cole RE. Improved Techniques for Dietary Assessment Using the Food Photography Method. The Obesity Society Annual Meeting, November 2016

A manuscript on initial findings from the study was published in 2018:

> Cole RE, Bukhari AS, Champagne CM, McGraw SM, Hatch AM, Montain SJ. Performance nutrition dining facility intervention improves special operations soldiers' diet quality and meal satisfaction. J Nutr Educ Behav. 2018 Nov - Dec;50(10):993-1004. doi: 10.1016/j.jneb.2018.06.011. Epub 2018 Aug 29. PMID: 30172700

This study showed that implementing a performance-based program was feasible, effective and sustainable in a high-paced dining facility. Nutrition education in the classroom combined with the intervention promoted more appropriate food choices and supported optimal Soldier readiness. This study provided novel information on evidence-based performance nutrition and its role in warfighter fueling.

Project 13: H.E.A.L.T.H. II. Healthy Eating, Activity, & Lifestyle Training Headquarters.

Specific Aim: To conduct a study to test the efficacy of an intensive intervention to assist Soldiers in meeting standard for body fat/fitness. The intervention will use an internet/smartphone program, integrated remote monitoring devices and individualized weight management and exercise recommendations.

Eligible Soldiers were those who were >5% above the screening table weight (N=100, n=50 active, n=50 controls). Soldiers' adult family members were also eligible if their BMI was > 25. This project was led by Tiffany Stewart, Ph.D. (PBRC). It utilized a prospective design and tracked participants for a period of 6 months. The study was a randomized trial in which Soldiers were randomly assigned to one of two treatment arms: 1) immediate access to the *H.E.A.L.T.H.-II* intervention or 2) six-month delayed access to the *H.E.A.L.T.H.-II* intervention (wait-list control). Change in the outcome variables is being compared between the two groups at months 3 and 6. During month 6, the wait-list control group was given access to the *H.E.A.L.T.H.-II* intervention for a six-month weight-loss phase followed by a three-month weight maintenance phase (this treatment regimen is identical to the treatment delivered to the group that received the intervention during Year 1). Change in the wait-list control group is being evaluated with within group comparisons. Data was collected from three sources: 1) individual's height, weight and body fat, as collected by PBRC field managers, 2) APFT test data (if available), and 3) the *H.E.A.L.T.H. –II* intervention, which includes body weight, energy intake and activity data collected with remote devices. **(Major Task 1)**

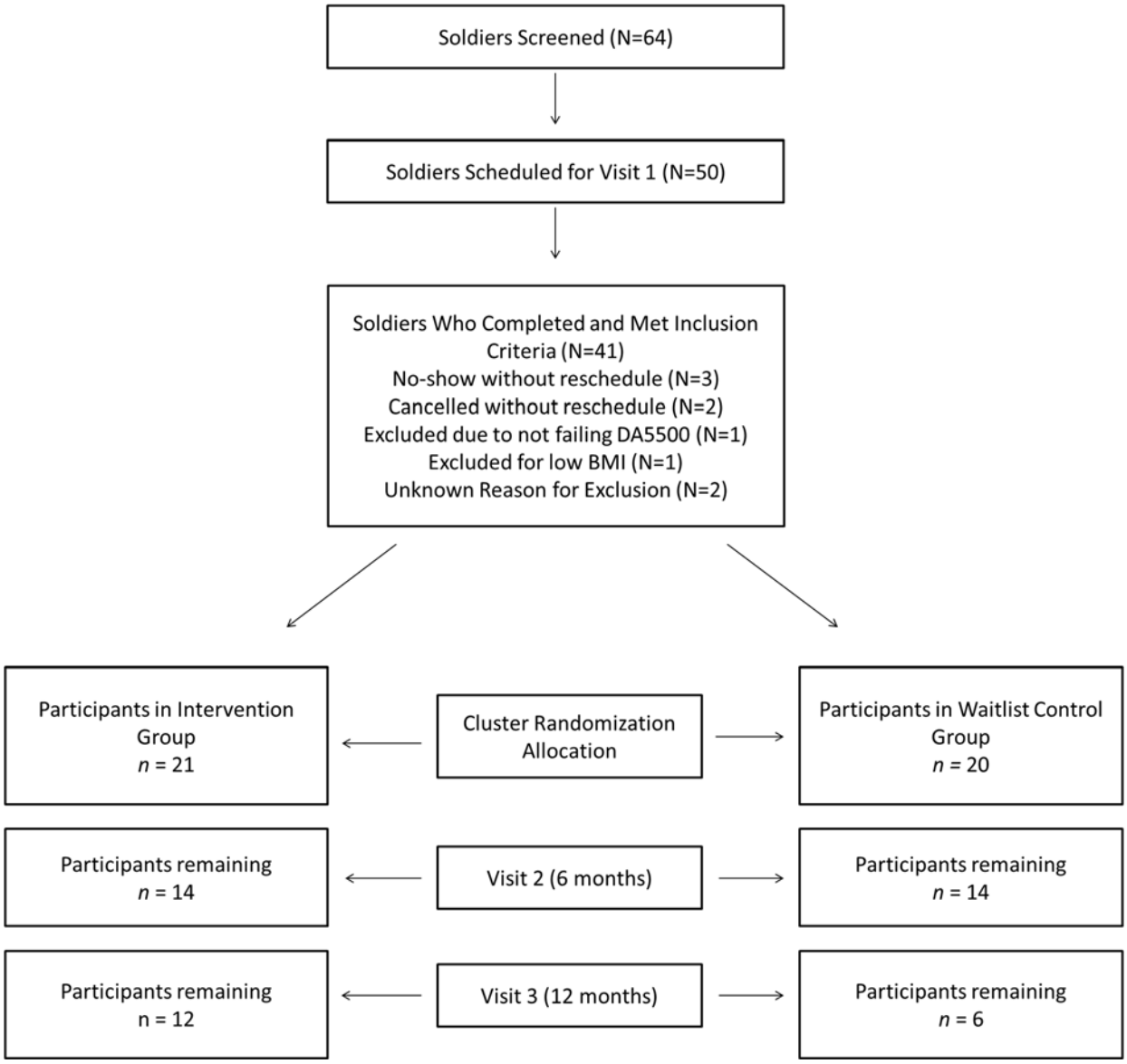
During Year 1, the objectives were: 1) establish initial study design; 2) obtain approval from the Institutional Review Board (IRB), 3) finalize intervention content, 4) promote the *Army H.E.A.L.T.H. Intensive* study, 5) randomize study participants, and 6) launch the *Army H.E.A.L.T.H. Intensive* intervention. These objectives were successfully completed in Year 1.

During Year 2, the objectives were: 1) continue promotion of the *Army H.E.A.L.T.H. Intensive* study, and 2) continue to randomize study participants. These objectives were completed during Year 2; however, due to a low budget for recruitment and mass deployments for natural disasters in Louisiana, recruitment and retention fell behind schedule.

During Year 3, the objectives were: 1) continue promotion of the *Army H.E.A.L.T.H. Intensive* study, 2) continue to randomize study participants, and 3) analyze preliminary data. In Year 3, many eligible Soldiers also were activated for duty and could not enroll or attend follow-up study visits, which led to the study not being fully recruited.

Recruitment resulted in 64 possible participants. After screening, 50 participants were scheduled for Visit 1. Of those, 41 completed and met inclusion criteria, three no-showed and rescheduling was unsuccessful, two cancelled Visit 1 and rescheduling was unsuccessful, one was excluded at

Visit 1 due to not failing DA5500, one was excluded at Visit 1 for low BMI and two were excluded for unknown reasons. At Visit 2 (Month 6), 13 participants did not complete the follow-up visit (7 active, 6 control). At Visit 3 (Month 12), 23 participants did not complete the follow-up visit (9 active, 14 control). Of those participants missing follow-up visits, 13 missed both (7 active, 6 control).



Army H.E.A.L.T.H. Intensive Intervention

Active intervention group participants had immediate access to the *Army H.E.A.L.T.H. Intensive* intervention online/Smartphone, counselor-assisted program from week 0 to month 6. This program included a variety of helpful tools to assist Soldiers in weight loss through an interactive lifestyle intervention. Users had access to the website/mobile app (including meal plan and nutrition tracking, fitness plan and fitness tracking, comprehensive data statistics and behavioral prompts to engage with the online program); two weekly emails from an interventionist, which included discussing

individual progress and barriers; two lessons per week (related to nutrition, physical activity, sleep, mind/body) delivered via text and email; a fitness tracker (which monitors physical activity and sleep); and a smart scale for daily weight tracking.

Website Main Features

Feature	Description
Body Mass Index (weight in kg/height in m^2)	Accurately (100%) calculated for users through a coded algorithm based on user-entered data for height and weight during registration.
Screening Table Weight	Accurately (100%) calculated for Soldiers through a coded algorithm based on user-entered data for height and weight during registration per AR 600-9 standards.
Body Fat Calculator	Application accurately (100%) calculates Soldier's body fat percentage based on self-reported circumference measurements for abdomen, neck and hips (women only) using a coded algorithm from the equations obtained from AR600-9. Users are then shown a graph that accurately (100%) compares their calculated percent body fat compared to their maximum allowed percent body fat according to the AR 600-9.
APFT Calculator	Accurately (100%) computes APFT scores based on user-entered number of reps for push-ups and sit-ups as well as time in minutes for the 2-mile run using a coded algorithm from the equations/scoring chart obtained from FM-21-20. Calculation is also available for modified APFT exercises such as swimming, biking and walking.
Meal Plan	A prescribed meal plan is provided to the user based on starting weight and activity and weight-loss goals. An algorithm accurately (100%) calculates estimated energy intake and energy expenditure to prescribe appropriate daily calorie consumption (based on body weight goals) and provides premade meals and substitution options that fit within those calorie goals.
Exercise Plan	A prescribed exercise plan is provided to the user based on starting activity level. Current exercise level is assessed through current frequency and duration of both strength and cardio exercise. An algorithm accurately (100%) pulls recommended exercises from a database appropriately categorized by difficulty and frequency.
APFT Training Plan	An APFT training plan that includes APFT specific exercises (based on amount of training time) is provided for Soldiers who input the date of an upcoming APFT test. An algorithm accurately (100%) pulls recommended exercises from a database of appropriately categorized exercises to meet the APFT goal.
Workout Generator	In addition to pre-programmed workouts, the application can provide users with a custom workout appropriate to their fitness level based on user-selected exercise equipment and muscle groups that they wish to target.
Exercise Intensity	User input of age and resting heart rate are used to accurately (100%) calculate user-specific training zones based on heart rate percentages.
Progress Charts	Application provides progress charts that accurately (100%) display changes over time of metrics such as calorie intake (from user-entered food

	logs), user-entered body weight, calories burned (from user-entered exercise logs), and user-entered APFT history. Application accurately (100%) aggregates data into daily, biweekly or monthly summaries.
Customized Interventionist Emails	Bi-weekly emails discussing individual progress and barriers with suggestions on how to change behaviors to improve health outcomes. These emails also introduce lesson information for the week and accompanying expectations.
Educational Lessons	Lessons are delivered bi-weekly to user accounts as they are "unlocked." Each lesson covers one of four topics: nutrition, physical activity, sleep or mind/body.
Behavioral Prompting	Users receive texts and emails to remind them to log their weight, food, activity and sleep dependent on their established habits in interacting with the program.

Promotion/Recruitment

The promotion strategy for *Army H.E.A.L.T.H. Intensive* program/website consisted of a 2-step program:

- Step 1: An awareness campaign that was designed to recruit eligible volunteers in the Louisiana Army National Guard (LANG) who met all inclusion criteria of the study. Volunteers were recruited by briefings to leadership and Soldiers/family members at LANG family days, state family workshops, drill weekends, annual training and Annual Enlisted and Officer Conferences.
- Step 2: A referral program designed to encourage active participants to refer a friend to sign up for the study in order to be eligible to win select promotional items.

The following table illustrates the website promotion schedule for distribution of promotion materials to full-time members and Traditional Guardsmen of the LANG. A typical day of promotion (during the week) required that Dr. Stewart and the Behavior Technology Laboratory (BTL) staff members make calls, have in-person meetings with the Louisiana National Guard's full-time Unit Readiness Non-Commissioned Officers, give presentations to all LANG full-time personnel, interact with Unit Family Readiness Groups (FRG) and attend monthly LANG Command update briefings. At each opportunity, the BTL staff members communicated the key components of the program and provided an overview of how the website could be used to help individuals achieve their diet/nutrition and exercise/fitness goals.

The majority of the interactions with the population occurred over drill weekends. This is the time period when the Project Manager had the greatest opportunity to communicate to a large portion of a unit's Soldiers. During drill, the Project Manager was responsible for educating Traditional Guardsmen on the *Army H.E.A.L.T.H. Intensive* program and eligibility requirements. Promotional items, fliers and posters were handed out to provide a phone number/email to follow up on interest in participation in the study, as well as other relevant recruitment information. Similarly, the *Army H.E.A.L.T.H. Intensive* program was able to develop a working relationship with the LANG Public Affairs Office (PAO), which allowed for the mass distribution of electronic promotional materials during the recruitment process.

Promotion Schedule

	Daily	Weekly	Monthly	Quarterly
Military Email			X	
Word of Mouth	X			
Newsletters			X	
Liaisons		X	X	X
Fliers		X		
Brochures		X		
Social Media Posts			X	X
Promotional Contests				X
Promo Material			X	

Key Research Accomplishments and Impact

- The Army H.E.A.L.T.H. *Intensive* program was promoted to Soldiers at Family Days, State Family Workshops, Drill, Annual Training, a promotional contest within the 256th and 225th Brigades, and Officer and Enlisted Annual Conferences.
- To date, 41 Soldiers have enrolled in the program.
- Preliminary Data analysis is underway.
- Valuable feedback from Soldiers using the website and mobile app has been incorporated into *Army H.E.A.L.T.H.* technology development of an iOS application for the advanced development process (collaboration with USARIEM). This program will be completed in 2019 and will be disseminated Army-wide.
- The results of this study have highlighted the importance of producing a scalable product with minimal burden and high efficacy.
- Building from the work on this project and other similar previous projects, Dr. Stewart submitted a recent grant application (Peer Reviewed Medical Research Program) to translate Army Resilience programming into a scalable iOS application similar to what is currently being done with the *Army H.E.A.L.T.H*. product.

Preliminary data is currently being analyzed **(Major Task 2**). Data will be disseminated through the following ways:

- Presentations on lessons learned at various Army meetings/conferences
- Outcomes manuscript

What opportunities for training and professional development has the project provided?

New methods were developed and implemented with the laboratory to assay endpoint metabolites. Laboratory support personnel received training and new skills for clinical chemistry assays.

How were the results disseminated to communities of interest?
Nothing to report

What do you plan to do during the next reporting period to accomplish the goals?
Nothing to Report

IMPACT

The goal of this series of projects was to assess and evaluate novel ways to sustain warfighter performance during high-intensity missions at home and abroad. A total of 13 projects were initiated in four focus areas: Metabolism and Performance, Stress and Inflammation, Nutrients and Resilience, and Healthy Eating and Behavior. This work was designed to address the scientific gaps in knowledge that need to be addressed in order to find solutions for issues facing our servicemen and women. Data derived from this program will be used to develop better models for predicting military dietary requirements, assess resilience and/or forecast performance capacity. I have highlighted some of the outcomes/conclusions from the work. All publications are listed further in the report and provide additional detail.

What was the impact on the development of the principal discipline(s) of the project?
Major findings from this project include the following:

1. Many times, warfighters are faced with intermittent periods of severe energy deficit that result in negative energy balance. Well-trained individuals are capable of adequately compensating to restore body mass during periods of refeeding.
2. High altitude can lead to acute hypoxia and can impair carbohydrate oxidation. However, if individuals are allowed to acclimate to altitude, this can alleviate the impairment.
3. There was no evidence that supplementing dietary intake with carbohydrates benefits aerobic performance in individuals who were at altitude (acclimated or non-acclimated).
4. Adequate calcium intake should be emphasized prior to initial military training. Calcium intake below the recommended levels leads to diminished bone health, potentially leading to greater susceptibility to injury.
5. Consuming sufficient energy (if possible) during periods of high-intensity training/missions will mitigate the negative effects of energy deficit.
6. Loss of lean mass is associated with decreased testosterone levels in individuals going through intensive military training exercises.
7. Recovery from periods of energy deficit is not enhanced by supplementing the diet with higher levels of protein intake.
8. The human performance program nutritional intervention illustrated that education, introduction of healthy food options and revised cooking practices are effective interventions for improving Warfighter meal quality.
9. Valuable feedback from Soldiers using the website and mobile app has been incorporated into *Army H.E.A.L.T.H.* technology development of an iOS application for the advanced development process (collaboration with USARIEM). This program will be completed in 2019 and will be disseminated Army-wide.

What was the impact on other disciplines?
Nothing to report

What was the impact on technology transfer?
Nothing to report

What was the impact on society beyond science and technology?
Nothing to report

CHANGES/PROBLEMS

Changes in approach and reasons for change

In the initial statement of work, the plan for the projects in the thematic area of Metabolism and Performance was to conduct a study to evaluate sex-specific risks and benefits of high-protein diets in physically active subjects. However, after completing two studies examining protein intake funded by a previous collaborative agreement (CROWN), we altered our hypothesis and focus to build upon the previous knowledge we had learned. Project 1 examined the physiological and psychological effects of testosterone during severe energy deficit and recovery. Project 2 examined substrate utilization, exercise performance and skeletal muscle response to energy deficit and altitude acclimatization.

To build upon the knowledge we obtained from Project 4 and to complement the work we had already concluded, we consulted with investigators at USARIEM and added six additional projects (Projects 5-10) to the Nutrients and Resilience thematic area.

Actual or anticipated problems or delays and actions or plans to resolve them
Nothing to report

Changes that had a significant impact on expenditures
Nothing to report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Nothing to report

Significant changes in use or care of human subjects
Nothing to report

Significant changes in use or care of vertebrate animals
Nothing to report

Significant changes in use of biohazards and/or select agents
Nothing to report

PRODUCTS

I. Publications, conference papers, and presentations

A. Journal publications

1. Physiological and psychological effects of testosterone during severe energy deficit and recovery: A study protocol for a randomized, placebo-controlled trial for Optimizing Performance for Soldiers (OPS). Pasiakos SM, Berryman CE, Karl JP, Lieberman HR, Orr JS, Margolis LM, Caldwell JA, Young AJ, Montano MA, Evans WJ, Vartanian O, Carmichael OT, Gadde KM, Harris M, Rood JC. Contemp Clin Trials. 2017 Jul;58:47-57. doi: 10.1016/j.cct.2017.05.001. Epub 2017 May 4. PMID: 28479217
2. Pasiakos Sm, Margolis LM, Murphy NE, McClung HL, Martini S, Gundersen Y, Castellani JW, Karl JP, Teien H, Madslien E, Stenberg PH, Young AJ, Montain SM, McClung JP. Effects of exercise mode, energy and macronutrient interventions on inflammation during military training. Physiol Rep. 2016 Jun;4(11). pii: e12820. doi: 10.14814/phy2.12820. PMID: 27273884 PMCID: PMC4908496
3. Margolis LM, Murphy NE, Martini S, Gundersen Y, Castellani HW, Karl JP, Carrigan CT, Teien H, Madslien E, Montain SJ, Pasiakos SM. Effects of supplemental energy on protein balance during 4-d arctic military training. Med Sci Sports Exerc. 2016 Aug;48(8):1604-12. doi: 10.1249/MSS.0000000000000944. PMID: 27054679
4. Karl JP, Margolis LM, Madslien EH, Murphy NE, Castellani JW, Gundersen Y, Hoke AV, Levangie MW, Kumar R, Chakraborty N, Gautam A, Hammamieh R, Martini S, Montain SJ, Pasiakos SM. Changes in intestinal microbiota composition and metabolism coincide with increased intestinal permeability in young adults under prolonged physiological stress. Am J Physiol Gastrointest Liver Physiol. 2017 Jun 1;312(6):G559-G571. doi: 10.1152/ajpgi.00066.2017. Epub 2017 Mar 23. PMID: 28336545
5. Sepowitz JJ, Armstrong NJ, Pasiakos SM. Energy balance and diet quality during the U.S. Marine Corps Forces Special Operations Command Individual Training Course. J Spec Oper Med. Winter 2017;17(4):109-113. PMID: 29256207
6. Cole RE, Bukhari AS, Champagne CM, McGraw SM, Hatch AM, Montain SJ. Performance nutrition dining facility intervention improves special operations soldiers' diet quality and meal satisfaction. J Nutr Educ Behav. 2018 Nov - Dec;50(10):993-1004. doi: 10.1016/j.jneb.2018.06.011. Epub 2018 Aug 29. PMID: 30172700

B. Books or other non-periodical, one-time publications

1. Cole RE, Bukhari AS, Champagne CM, Hatch AM, McGraw SM, Allen R, Montain SJ. Technical Report T17-03. Evaluation of a Dining Facility Intervention on U.S. Army Special Operations Soldiers' Meal Quality, Dining Satisfaction, and Cost Effectiveness. Military Nutrition Division, U.S. Army Research Institute of Environmental Medicine; Nov 2016:1-85 (DTIC accession # AD1039585).

C. Other publications, conference papers and presentations

1. Berryman CE, Karl JP, Cole RE, Kenefick RW, Margolis LM, Carbone JW, Ferrando AA, Lieberman HR, Young AJ, Pasiakos SM. Prolonged high altitude exposure exacerbates fat-free mass and fat mass loss during negative energy balance regardless of dietary protein intake. Experimental Biology, April 2017.
2. Derosier AN, Berryman CE, Karl JP, Wilson MA, Young AJ, Pasiakos SM. Higher-Protein Intake During Sustained Negative Energy Balance Attenuates Elevations in Resting Metabolic Rate at High Altitude (4300 m). Experimental Biology, April 2017.
3. Karl JP, Cole RE, Berryman CE, Kominsky MT, Radcliffe PN, Margolis LM, Young AJ, Pasiakos SM. Higher Protein Diet Suppresses Appetite at High Altitude. Experimental Biology, April 2017.
4. McClung JP, Hennigar SR, Berryman CE, Young AJ, Pasiakos SM. Prolonged High Altitude Exposure Results in Elevated Erythroferrone and Diminished Hepcidin Levels in Healthy Young Male Volunteers. Experimental Biology, April 2017.
5. Kenefick RW, Luippold AJ, Bradbury KE, Young AJ, Derosier AN, Wilson MA, Berryman CE, Pasiakos SM. No Impact of Carbohydrate Supplementation and Altitude Acclimatization on Aerobic Exercise Performance. American College of Sports Medicine, May 2017.
6. Young AJ, Berryman CE, Derosier AN, Kenefick RW, Wilson MA, Pasiakos SM. Effects of Acclimatization to High Altitude on Exogenous Carbohydrate Oxidation During Steady-State Exercise. American College of Sports Medicine, May 2017.
7. Karl JP, Margolis LM, Murphy NE, Martini S, Gundersen Y, Castellani JW, Carrigan CT, Teien HK, Madslien EH, Montain, SJ, Pasiakos SM. Increased Gastrointestinal Permeability During Prolonged Physical Stress is Associated with Lower Energy Intakes but not Dietary Macronutrient Composition. Experimental Biology, April 2016
8. Margolis LM, Murphy NE, Martini S, Gundersen Y, Castellani JW, Karl JP, Carrigan CT, Teien HK, Madslien EH, Montain SJ, Pasiakos SM. Energy not protein or carbohydrate intake attenuates whole-body protein loss during 4-d arctic military training. Medicine & Science in Sports & Exercise, June 2016
9. Karl JP, Margolis LM, Murphy NE, Martini S, Gundersen Y, Castellani JW, Carrigan CT, Teien HK, Madslien EH, Montain SJ, Pasiakos SM. High Energy Expenditure and Negative Energy Balance Modulate Composition and Metabolism of the Gut Microbiota. Experimental Biology, April 2017
10. Pasiakos SM, Berryman CE, Margolis LM, Sepowitz JJ, McClung HL, Lieberman HR, McClung JP, Ferrando AA. Changes in protein turnover, hormonal status, and body composition during physiologically demanding military training. Experimental Biology, April 2016
11. Sepowitz JJ, McClung HL, Berryman CE, Armstrong NH, Ferrando AA, Lieberman HR, McClung JP, Pasiakos SM. Supplementing an energy adequate high protein diet with additional protein is not necessary for recovery of lean body

mass after short-term starvation. American College of Sports Medicine Annual Meeting, June 2016

12. Scott JM, Gaffney-Stomberg E, Palmer JP, Daigle R, Kazman JB, McClung JP, Gasier HG. Vitamin D supplementation augment SIgA secretion rates in Marine Corps basic trainees. American College of Sports Medicine Annual Meeting, June 2016
13. Nakayama AT, Lutz U, McClung JP, Gaffney-Stomberg E. Calcium intake below the recommended dietary allowance is associated with lower tibia bone mineral content and strength in young adults entering initial military training. Experimental Biology, April 2017
14. Sepowitz HH, Armstrong NJ, Pasiakos SM. Effects of intermittent periods of severe negative energy balance on weight maintenance during US Special Operations Forces training. American College of Sports Medicine. June 2016
15. Bukhari AS, Lutz LJ, Smith TJ, Hatch AM, Hawes MR, O'Conner KL, Carrigan CT, McGraw SM, Champagne CM, Montain SJ. A food-based intervention in a military dining facility results in improvements in blood fatty acid profile. Food and Nutrition Conference and Exposition of the Academy of Nutrition and Dietetics, October 2017
16. Bukhari AS, Crombie AP, McGraw SM, Champagne CM, Allen R, Montain SJ, Young AJ. Impact of military dining facility serving modifications on the nutritional intake of special operations personnel. FASEB J 30:895.12, April 2016
17. Bukhari AS, Champagne C, Logan CM, Montain SJ, Cole RE. Key Insights from Foodservice Staff Regarding Operations at Military Dining Facilities. Military Health System Research Symposium, August 2016
18. Cole RE, Bukhari AS, Champagne CM, McGraw SM, Hatch AM, Logan CM, Spanbauer SM, Montain SJ. Healthy Eating Index Increased after Tactical Human Optimization, Rapid Rehabilitation and Reconditioning (THOR3) Dining Facility Menu Enhancement in Special Forces Operators. Military Health System Research Symposium, August 2016
19. Cole RE, Bukhari AS, Champagne CM, McGraw SM, Hatch AM, Logan CM, Spanbauer SM, Montain SJ. Adequate sleep is associated with healthy eating, physical activity and time spent inactive. Military Health System Research Symposium, August 2016
20. McGraw SM, Bukhari AS, Champagne CM, Hatch AM, Logan CM, Spanbauer SM, Montain SJ, Cole RE. Physically fit Soldiers eat healthier and feel nutrition impacts physical performance. Military Health System Research Symposium, August 2016
21. Cole RE, Bukhari AS, Champagne CM, McGraw S, Hatch AM, Logan CM, Spanbauer SM, Montain SJ. Healthy Eating Index Increased after Tactical Human Optimization, Rapid Rehabilitation and Reconditioning (THOR3) Dining Facility Menu Enhancement in Military Operators. Food and Nutrition Conference and Expo, October 2016
22. McGraw S, Bukhari AS, Champagne CM, Hatch AM, Logan CM, Spanbauer SM, Montain SJ, and Cole RE. Physically fit Soldiers eat healthier and feel nutrition impacts physical performance. Food and Nutrition Conference and Exposition of the Academy of Nutrition and Dietetics, October 2016

23. Cole RE, Bukhari AS, McGraw SM, Champagne CM, Hatch AM, Karl JP. Predictors of diet quality and appetite in soldiers eating at Military Dining Facilities. Military Health System Research Symposium, August 2017
24. Champagne CM, Allen HR, Johnson CM, and Cole RE. Improved Techniques for Dietary Assessment Using the Food Photography Method. The Obesity Society Annual Meeting, November 2016

II. Website(s) or other Internet site(s)
Nothing to report

III. Technologies or techniques
Nothing to report

IV. Inventions, patent applications, and/or licenses
Nothing to report

V. Other Products
Nothing to report

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

The projects in this award were carried out in collaboration with scientists from the U.S. Army Research Institute of Environmental Medicine; however, funds from this award covered personnel costs at Pennington Biomedical Research Center only. I have detailed the contributions of Pennington Biomedical Research Center employees below:

Name: Jennifer Rood
Project Role: Principal Investigator
ORCID ID: 0000-0001-5848-2987
Nearest Person Month Worked: 11.58
Contribution to Project: She oversaw/managed/coordinated the award and acted as the point of contact for interactions with USARIEM scientists and oversaw all regulatory processes. She also served as PI for project 1 and co-investigator for project 10.

Name: Ray Allen
Project Role: Computer support
Nearest Person Month Worked: 9.96
Contribution to Project: He provided computer support for project 13.

Name: John Apolzan
Project Role: Co-investigator
ORCID ID: 0000-0001-8492-7820
Nearest Person Month Worked: 1.39
Contribution to Project: He served as PI for project 10.

Name: Kate Blumberg
Project Role: research dietitian
Nearest Person Month Worked: 5.16
Contribution to Project: She provided visual estimation support for projects 11 and 12.

Name: Heather Brady
Project Role: behaviorist
Nearest Person Month Worked: 2.04
Contribution to Project: She helped develop the intervention and website for project 13.

Name: Catherine Carmichael
Project Role: research dietitian
Nearest Person Month Worked: 5.10
Contribution to Project: She served as project manager for projects 11 and 12.

Name: Owen Carmichael
Project Role: Co-investigator
ORCID ID: 0000-0002-0576-0047

Nearest Person Month Worked: 1.20
Contribution to Project: He served as co-investigator for project 1.

Name: Catherine Champagne
Project Role: Co-investigator
ORCID ID: 0000-0001-6127-1072
Nearest Person Month Worked: 4.98
Contribution to Project: She served as a co-investigator for projects 11 and 12.

Name: Bridget Conner
Project Role: Medical Technologist
Nearest Person Month Worked: 13.44
Contribution to Project: She provided analytical support in the clinical chemistry laboratory for projects 1-10. She prepared specimen collection containers, processed specimens, analyzed biological samples, and reported results.

Name: Allison Davis
Project Role: Psychology support
ORCID ID: 0000-0002-3124-7538
Nearest Person Month Worked: 1.52
Contribution to Project: She provided psychology support for project 1.

Name: Sarah Duet
Project Role: administrative assistant
Nearest Person Month Worked: 5.04
Contribution to Project: She provided administrative support for the entire award in year 1.

Name: Nathan Efferson
Project Role: computer support
Nearest Person Month Worked: 3.36
Contribution to Project: He provided computer support for project 1.

Name: Kishore Gadde
Project Role: Medical investigator
ORCID ID: 0000-0002-1856-5574
Nearest Person Month Worked: 0.77
Contribution to Project: He served as medical investigator for project 1.

Name: Melissa Harris
Project Role: project manager
ORCID ID: 0000-0001-9113-1695
Nearest Person Month Worked: 8.4
Contribution to Project: She served as the project manager for project 1.

Name: Valery Hymel
Project Role: research associate

Nearest Person Month Worked: 15.9
Contribution to Project: She provided analytical support in the Mass Spectrometry Laboratory. She prepared specimen collection containers, processed specimens, analyzed biological samples, and reported results.

Name: Steve Lee
Project Role: medical technologist
Nearest Person Month Worked: 14.94
Contribution to Project: He provided analytical support in the clinical chemistry laboratory for projects 1-10. He prepared specimen collection containers, processed specimens, analyzed biological samples, and reported results.

Name: Tarryn Pollard
Project Role: Psychology support
Nearest Person Month Worked: 2.04
Contribution to Project: She provided psychology support for project 13.

Name: Stacey Roussel
Project Role: medical technologist
Nearest Person Month Worked: 14.94
Contribution to Project: She provided analytical support in the clinical chemistry laboratory for projects 1-10. She prepared specimen collection containers, processed specimens, analyzed biological samples, and reported results.

Name: Jonathan Savoie
Project Role: research associate
Nearest Person Month Worked: 18.48
Contribution to Project: He provided analytical support in the Mass Spectrometry Laboratory. He prepared specimen collection containers, processed specimens, analyzed biological samples, and reported results.

Name: Tiffany Stewart
Project Role: Co-investigator
Nearest Person Month Worked: 4.44
Contribution to Project: She served as PI for project 13.

Name: Michael Switzer
Project Role: Research associate
Nearest Person Month Worked: 2.04
Contribution to Project: He served as project manager of project 13.

Name: Jamie Tuminello
Project Role: medical technologist
Nearest Person Month Worked: 21.12

Contribution to Project: She provided analytical support in the clinical chemistry laboratory for projects 1-10. She prepared specimen collection containers, processed specimens, analyzed biological samples, and reported results.

Name: Dawn Turner
Project Role: research dietitian
Nearest Person Month Worked: 4.22
Contribution to Project: She provided visual estimation and food photography support for projects 11 and 12.

Name: Nicole Wesley
Project Role: Research associate
Nearest Person Month Worked: 2.04
Contribution to Project: She assisted with implementation and administration of project 13.

Name: Edie White
Project Role: administrative assistant
Nearest Person Month Worked: 11.64
Contribution to Project: She provided administrative support for the entire award in years 2-4.

Total Expenditures:	$7,317,751.00
Personnel	$1,187,155.21
Students	$ 68,844.48
Fringe	$ 508,492.97
Travel	$ 63,244.36
Operating Services	$1,836,453.64
Other Charges	$ 675,094.42
Supplies	$ 611,392.22
Indirect Costs	$2,367,073.70

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to report

What other organizations were involved as partners?

Organization Name: United States Army Research Institute of Environmental Medicine, Military Nutrition Division
Location of Organization: Natick, MA
Partner's contribution to the project: Collaboration (as detailed in the Statement of Work)

SPECIAL REPORTING REQUIREMENTS

Collaborative Research to Optimize Warfighter Nutrition (CROWN) II

Proposal Log #: 1321003
W81XWH-14-1-0335

PI: Jennifer Rood **Org:** Pennington Biomedical Research Center **Award Amount:** $7,317,751.00

Study/Product Aim(s)

CROWN II proposes research in nutrition and metabolism to develop better models to assess resilience or performance capacity. The project enables USARIEM and PBRC scientists to work together on the design, execution, analysis and translation of research projects in four thematic areas:

- Metabolism and Performance
- Stress and Inflammation
- Nutritients and Resilience
- Healthy Eating and Behavior

Accomplishment: This project has provided the scientific basis to develop novel nutritional programs, products and strategies to promote Warfighter health, performance and resilience to operational stress.

Timeline and Cost

Activities FY	14	15	16	17
Initial Study Design and Execution for each Focus Area				
Follow Up Studies				
Biospecimen Analysis/Field Support				
Budget (M)	$2.377	$2.437	$2.492	$0.012

Updated: (11/30/2018)

Major Findings:

Many times warfighters are faced with intermittent periods of severe energy deficit that result in negative energy balance. Well-trained individuals are capable of adequately compensating to restore body mass during periods of refeeding.

High altitude can lead to acute hypoxia and can impair carbohydrate oxidation. However, if individuals are allowed to acclimate to altitude, this can alleviate the impairment. There was no evidence that supplementing dietary intake with carbohydrates benefits aerobic performance in individuals who were at altitude (acclimated or non-acclimated).

Adequate calcium intake should be emphasized prior to initial military training. Calcium intake below the recommended levels leads to diminished bone health, potentially leading to greater susceptibility to injury.

Loss of lean mass is associated with decreased testosterone levels in individuals going through intensive military training exercises

Recovery from periods of energy deficit is not enhanced by supplementing the diet with higher levels of protein intake.

Budget Expenditure to Date

Actual Expenditure: $7,317,751.00

APPENDIX

Contemporary Clinical Trials 58 (2017) 47–57

Contents lists available at ScienceDirect

Contemporary Clinical Trials

journal homepage: www.elsevier.com/locate/conclintrial

Physiological and psychological effects of testosterone during severe energy deficit and recovery: A study protocol for a randomized, placebo-controlled trial for Optimizing Performance for Soldiers (OPS)

Stefan M. Pasiakos[a,*], Claire E. Berryman[a,b], J. Philip Karl[a], Harris R. Lieberman[a], Jeb S. Orr[a], Lee M. Margolis[a], John A. Caldwell[b], Andrew J. Young[a,b], Monty A. Montano[c], William J. Evans[d,g], Oshin Vartanian[b,e], Owen T. Carmichael[f], Kishore M. Gadde[f], Melissa Harris[f], Jennifer C. Rood[f]

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[b] Oak Ridge Institute for Science and Education, Belcamp, MD, USA
[c] MyoSyntax Corporation, Worcester, MA, USA
[d] University of California at Berkeley, Berkeley, CA, USA
[e] Defence Research and Development Canada, University of Toronto, Canada
[f] Pennington Biomedical Research Center, Baton Rouge, LA, USA
[g] Duke University, Durham, NC, USA

ARTICLE INFO

Keywords:
Muscle mass
Hypogonadal
Eugonadal
Microbiome
Protein synthesis
Vigilance
Mood
Functional MRI
Negative energy balance
Calorie restriction

ABSTRACT

Background: The physiological consequences of severe energy deficit include hypogonadism and the loss of fat-free mass. Prolonged energy deficit also impacts physical performance, mood, attentiveness, and decision-making capabilities. This study will determine whether maintaining a eugonadal state during severe, sustained energy deficit attenuates physiological decrements and maintains mental performance. This study will also assess the effects of normalizing testosterone levels during severe energy deficit and recovery on gut health and appetite regulation.

Methods: Fifty physically active men will participate in a 3-phase, randomized, placebo-controlled study. After completing a 14-d, energy-adequate, diet acclimation phase (protein: 1.6 $g \cdot kg^{-1} \cdot d^{-1}$; fat: 30% total energy intake), participants will be randomized to undergo a 28-d, 55% energy deficit phase with (DEF + TEST: 200 mg testosterone enanthate per week) or without (DEF) exogenous testosterone. Diet and physical activity will be rigorously controlled. Recovery from the energy deficit (ad libitum diet, no testosterone) will be assessed until body mass has been recovered within ± 2.5% of initial body mass. Body composition, stable isotope methodologies, proteomics, muscle biopsies, whole-room calorimetry, molecular biology, activity/sleep monitoring, personality and cognitive function assessments, functional MRI, and comprehensive biochemistries will be used to assess physiological and psychological responses to energy restriction and recovery feeding while volunteers are in an expected hypogonadal versus eugonadal state.

Discussion: The Optimizing Performance for Soldiers (OPS) study aims to determine whether preventing hypogonadism will mitigate declines in physical and mental function that typically occur during prolonged energy deficit, and the efficacy of testosterone replacement on recovery from severe underfeeding.

Trial Registration: NCT02734238.

1. Introduction

Strenuous work and inadequate energy intake during military operations produce severe energy deficits, deplete body energy stores, result in fat-free mass (FFM) loss, degrade performance, and increase risk of injury [1–5]. The FFM loss induced by military operations is

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modifiable, as higher-protein diets [$> 0.8\ g \cdot kg^{-1} \cdot d^{-1}$ and within the Acceptable Macronutrient Distribution Range for protein (10–35% total energy intake)] have consistently been shown to spare FFM and preserve muscle anabolic sensitivity in situations where the energy deficit is ~40% of total daily energy expenditure (TDEE) [6–8]. However, under conditions of severe (~50–60% of TDEE) energy deficit, higher-protein diets fail to augment FFM retention [9,10]. Decrements in total body mass and FFM in men generally occur when circulating levels of testosterone are substantially reduced [11].

The reductions in testosterone that occur during severe energy deficit may diminish the efficacy of increasing protein intake to spare FFM. In healthy young males, the suppression of endogenous testosterone production has myriad adverse physiological consequences including reduced FFM, increased adiposity, and decreased muscle strength [12–15]. Finkelstein et al. [13] recently demonstrated that decreased testosterone levels (from 530 to 350 ng·dL-1), achieved by goserelin acetate administration to reduce endogenous testosterone and estradiol production, result in increased adiposity, and further reductions to ≤ 200 ng·dL-1 are accompanied by skeletal muscle atrophy and decreased muscle strength. Importantly, testosterone decreases of this magnitude occur during military training and sustained operations, and are associated with concomitant decreases in FFM [11,16–19]. Although dietary macronutrient manipulations have proven unsuccessful at mitigating the endocrine response to severe negative energy balance [20], pharmacologic interventions that restore anabolic hormone concentrations to normal levels have been shown to promote nitrogen retention despite energy deficit [21–23]. Whether preventing declines in testosterone during conditions simulating severe, sustained energy deficit enhances the FFM-sparing effects of a higher-protein diet has not been studied.

The potential influence of testosterone maintenance on physiological and body composition recovery from severe energy deficit remains unclear. In general, refeeding following energy deficit is marked by the preferential accumulation of adipose tissue and not FFM, a phenomenon known as “rebound fatness” [18]. It is possible that the loss of body fat during energy deficit elicits a persistent suppression of metabolic rate during recovery, whereas the reductions in FFM promote hyperphagia in recovery from energy deficit [24]. Thus, we suspect that if testosterone levels are maintained during severe energy deficit, FFM will be spared, reducing subsequent hyperphagia and relative fat mass gain during refeeding and thereby setting the conditions for a more favorable recovery.

1.1. Effects of testosterone on personality, mood, and cognition during energy deficit

Testosterone has the potential to augment cognitive performance [25–29], mood [30–34], assertiveness [35,36], and risk-taking [37–39]. A growing body of neuroimaging studies has suggested that testosterone may exert these effects by modifying the functioning of specific brain regions, including the amygdala and orbitofrontal cortex, that control emotion processing, self-restraint, and evaluation of threat [40–47]. Testosterone administration during a period of sustained energy deficit may reduce the adverse effects on mood associated with low testosterone levels. Furthermore, the effect of testosterone supplementation on sleep quantity and quality has not been examined, but there may be a relationship between endogenous testosterone levels and both sleep duration and length of wakefulness [48–50].

1.2. Effects of testosterone on appetite regulation during energy deficit

Appetite-mediating hormones, including peptide-tyrosine tyrosine (PYY), glucagon-like peptide-1 (GLP-1), and ghrelin contribute to the motivation to eat and hunger after weight loss and may underlie the common tendency for weight regain [51]. However, supporting evidence is predominantly derived from studies in obese populations. Given the high prevalence of dieting for weight loss and body weight cycling in non-obese populations (e.g., adolescents/adults with body image concerns, athletes of weight sensitive competitive sports, and military personnel required to meet body weight standards) and the increased risk of obesity that accompanies these behaviors [52], improving current understanding of adaptive responses of appetite-mediating hormones to weight management in non-obese populations is imperative.

1.3. Effects of testosterone on gut microbiome and intestinal permeability during energy deficit

Disruption or dysfunction of the gastrointestinal barrier can increase intestinal permeability, causing translocation of bacteria and their pro-inflammatory components [e.g., lipopolysaccharide] into systemic circulation [53]. The resulting low-grade systemic inflammation increases susceptibility to acute and chronic disease [54], increases risk for nutrient deficiency [55], adversely impacts cognitive and physical performance [53], and exacerbates gastrointestinal barrier dysfunction [56]. Evidence suggests testosterone may mediate intestinal permeability [57,58] and conversely, the gut microbiome may modulate testosterone levels [59].

1.4. Objectives, design and methods

1.4.1. Primary study objectives

I. Determine the extent to which maintenance of a eugonadal state by exogenous testosterone administration attenuates the effects of severe, sustained energy deficit on body composition (body mass, FFM and fat mass), skeletal muscle (mass, strength/power/endurance, proteomics, intramuscular regulators of metabolism, protein synthesis and proteolysis), metabolism (energy expenditure, substrate oxidation and nitrogen balance) and physiological status (androgens, stress and metabolic hormones, inflammation, biomarkers of nutritional status, circulating and intramuscular substrates and blood lipids).
II. Determine the effects of exogenous testosterone administration during severe, sustained energy deficit on subsequent recovery of body composition, skeletal muscle, and metabolic and physiological status.

1.4.2. Secondary study objective

III. Determine the effects of severe, sustained energy deficit and associated hypogonadism on mental fatigue and other aspects of mood, cognitive performance, brain function and sleep.
IV. Determine the extent to which the detrimental effects of sustained energy deficit on mood, cognitive performance, and sleep are attenuated by pharmacological testosterone treatment.

1.4.3. Tertiary study objectives

V. Determine the effect of testosterone maintenance on appetite and adaptive responses of appetite-mediating hormones during energy deficit and body mass recovery in non-obese adults.
VI. Determine the effects of energy deficit with and without testosterone treatment on gut microbiota composition, function, and activity.
VII. Identify associations between gut microbiota composition and function, host energy/substrate metabolism, body mass change, and the composition of body mass loss and regain.

1.5. Institutional review board approval and trial registration

The study protocol was approved by the Institutional Review Board

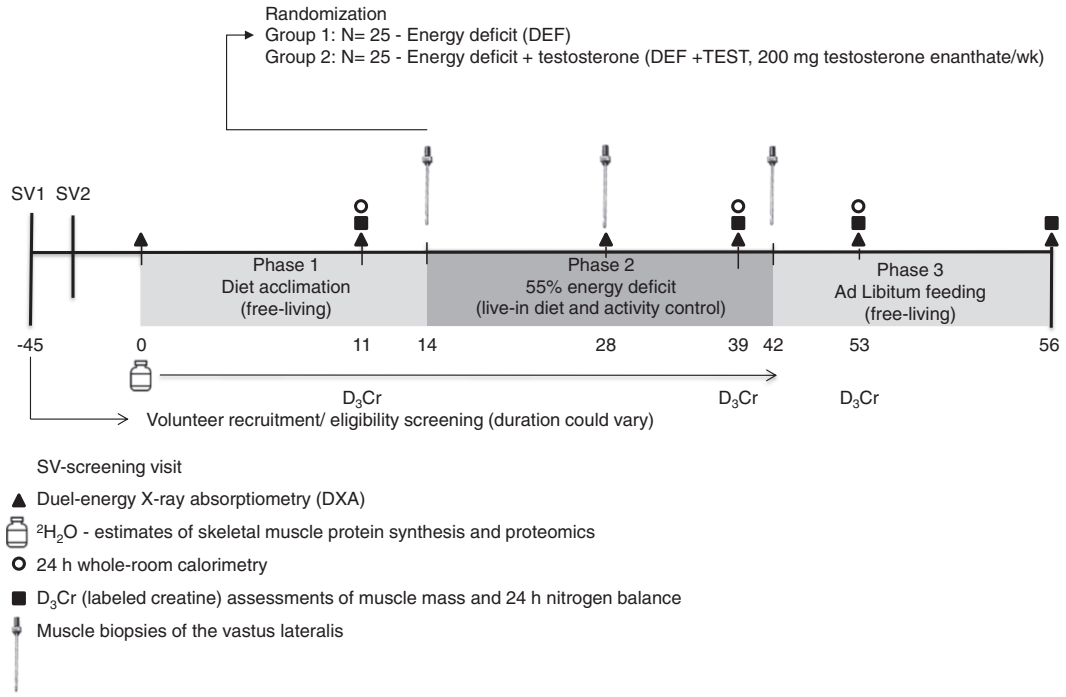

Fig. 1. Experimental design.

of the Pennington Biomedical Research Center (PBRC, Baton Rouge, LA, USA; protocol 2015-063-PBRC OPS) and the US Army Human Research Protections Office (Ft. Detrick, Fredericksburg, MD, USA). The ClinicalTrials.gov identifier is NCT02734238.

1.6. Study design

Physically active men will be recruited for a 3-phase, randomized, placebo-controlled study to assess physiological and psychological responses to testosterone administration, at a dosage designed to maintain eugonadal status (Fig. 1). Phase 1 will be free-living and begin immediately after baseline measures are complete. Participants will be prescribed and provided individualized, 14-d eucaloric lead-in diets with 1.6 g protein·kg^{-1}·d^{-1}, 30% of total daily energy requirements from fat, and the remaining energy from carbohydrate. The 14-d lead-in diet will ensure sufficient time to acclimate to the prescribed study diet. After completing phase 1, participants will be randomly assigned to one of two, highly controlled (live-in, phase 2), 28-d treatment groups: energy deficit (DEF, 55% of estimated TDEE) or energy deficit + testosterone maintenance (DEF + TEST). Protein intake (1.6 g protein·kg^{-1}·d^{-1}, based on day 14 total body mass) will remain constant throughout the 28-d intervention, fat will contribute 30% of total energy, and the remaining energy will be derived from carbohydrate. Exercise-induced energy expenditure (EIEE) will be increased 50% above TDEE established during phase 1 for all participants (Phase 1 EIEE will be added to the increased EIEE for phase 2). Energy intake will be 45% of the elevated TDEE.

After completing phase 2, participants will be allowed to return to their habitual diet and physical activity patterns, and total body mass and skeletal muscle mass recovery from the intervention (free-living, phase 3) will be assessed. Recovery will be assessed to determine when body mass has returned to within ± 2.5% of initial body mass (end of study, EOS). The duration of phase 3 will vary by participant, depending on each participant's rate of body mass regain (42-d maximum for phase 3).

1.7. Randomization

On day 14, immediately after phase 1 testing, participants will be randomized to one of two experimental groups (DEF or DEF + TEST). A randomized block design will be used and based on age stratification (< 29 or ≥ 29 y).

1.8. Participants

Fifty physically active men will complete the OPS study. Participants must be 18–39 y, physically active (at least 2-d/wk aerobic and/or resistance exercise) and meet the age-specific US Army body composition standards according to Army Regulation 600-9 [60]. All inclusion and exclusion criteria are listed in Table 1.

1.9. Eligibility determination

The OPS study will use a multi-stage screening process. Potential participants will undergo a web and/or telephone screen to determine initial eligibility based on inclusion and exclusion criteria (Table 1). They will be provided detailed information and, if still interested, undergo two screening visits. During the first visit, the study consent form will be reviewed and signed. Anthropometric data will be measured; study dietitians will meet with potential participants and review study diet requirements with them. Participants also will complete a Physical Activity Readiness Questionnaire and cardiovascular disease risk assessment during the first screening visit.

Participants who maintain eligibility will be scheduled for a second screening visit. At that time participants will undergo a complete physical exam, fasting blood draw, and urine collection. Participants who maintain eligibility will wear an accelerometer for 1 wk. prior to the start of phase 1. During this time, participants will complete a 3-d food record (2 weekdays and 1 weekend day) and 3-d activity log to assess habitual dietary and activity patterns.

1.10. Habitual physical activity levels

Habitual physical activity will be determined from 7-d acceler-

Table 1
Study inclusion and exclusion criteria.

Inclusion criteria	Exclusion criteria
1. Men aged 18–39 years	1. Musculoskeletal injuries that compromise exercise capability
2. Physically active (at least 2 d/wk aerobic and/or resistance exercise)	2. Diagnosed cardiometabolic disorders (i.e., hypertension, hyperlipidemia, kidney disease, diabetes)
3. Participants cannot be taking any prescription medications or must be willing to refrain from all medication use prior to and throughout the entire study period, unless provided/approved by the study physician	3. Systolic blood pressure > 150 or diastolic blood pressure > 95 mm Hg
4. Willing to refrain from alcohol, smoking, e-cigarettes and use of any nicotine product, caffeine, and dietary supplement use throughout the entire study period	4. History of chronic obstructive pulmonary disease, obstructive sleep apnea, gastrointestinal disease, kidney stones, or breast or prostate cancer
5. Willing to live on the PBRC inpatient unit for 28 d	5. Allergies or intolerance to study foods
6. Meets age-specific US Army body composition standards according to Army Regulation 600–9 [42], which includes estimates of percent body fat based on height, weight, and neck and waist circumference	6. Vegetarian practices
7. Total testosterone concentration is within the normal physiological range (300–1000 ng/dL)	7. History of complications with lidocaine
	8. Anabolic steroid, human growth hormone, or nutritional testosterone precursor like supplement use within the past 6 mo
	9. Use of antibiotics, except topical antibiotics, within 3 mo of study participation
	10. Colonoscopy within 3 mo of study participation
	11. Chronic use of laxatives, stool softeners, antacids, or anti-diarrheal medications (≥ once/wk)
	12. Restrained eater as measured by the Three-Factor Eating Questionnaire
	13. Adults unable to consent
	14. Women
	15. Prisoners
	16. Metal implants, claustrophobia, or head size incompatible with MRI equipment
	17. Findings of lab results: prostate-specific antigen > 3 ng/mL, Hematocrit > 50%, or positive urine drug screening
	18. Prospective participants can also be excluded based on the study team's clinical judgement

ometer and 3-d activity data obtained during the screening period. Physical activity patterns will be maintained at pre-study levels during phase 1. Physical activity will be verified using an accelerometer and physical activity logs during phases 1 and 3; physical activity will be highly controlled and monitored during phase 2.

1.11. Body composition

Height will be measured using a stadiometer (Harpenden Stadiometer, Holtain Company, UK). Body mass will be assessed after an overnight fast using a calibrated digital scale (GSE Inc. Model 450, GSE Scale Systems, Novi, MI) during each screening visit and daily during phase 1 and phase 2. After completing phase 2, participants will report to the PBRC approximately once/wk. for body mass measures; each participant also will be provided a scale to take home for daily body mass measurements during phase 3. Body composition will be determined using dual energy X-ray absorptiometry (DXA, Lunar iDXA, GE Healthcare, Madison, WI) on days 0, 11, 28, 39, 53 and at the end of the study (EOS). Estimates of skeletal muscle mass will be determined using the creatine (methyl-d_3) dilution method on days 11, 39, 53, and EOS [61]. Study procedures and corresponding measurement collection days are presented in Table 2.

1.12. Aerobic capacity

Aerobic capacity (i.e., peak oxygen uptake, VO_{2peak}) will be measured on day 0 of phase 1 using an indirect open circuit respiratory system (ParvoMedics TruOne 2400, East Sandy, UT) and a progressive intensity treadmill test (i.e., constant speed but increased grade until volitional exhaustion in 2 min stages; Trackmaster TMX425CP, Newton, KS). Aerobic capacity will be used as a reference point to determine the appropriate exercise workloads necessary to meet the energy requirements for phase 2. Participants will perform this assessment at standard ambient indoor temperature and humidity conditions.

1.13. Testosterone and placebo administration

Participants will receive either 200 mg testosterone enanthate or placebo (1 mL sesame oil) by intramuscular injection on days 15, 21, 28, and 35. Based on previous dose-response studies, 200 mg of testosterone enanthate was chosen as an effective dose to maintain testosterone within normal physiological ranges while minimizing risk of adverse secondary health effects [e.g., abnormal hemoglobin, hematocrit, and prostate-specific antigen (PSA) – all of which are monitored biweekly by a study-independent physician] [12,62,63].

1.14. Endocrine, hematological and physiological biomarkers

Blood samples will be collected after an overnight fast biweekly on days 0, 14, 28, 42, 56 and EOS. Blood samples will be analyzed for total testosterone, free testosterone, luteinizing hormone (LH), follicle-stimulating hormone (FSH), sex hormone binding globulin (SHBG), insulin-like growth factor-1 (IGF-1), estradiol, insulin, cortisol, and complete blood count with differential. In addition, blood samples collected on days 0, 14, 42, 56 and EOS will be analyzed for amino acids, lipopolysaccharide-binding protein (LBP), and a comprehensive metabolic panel will be conducted. PSA will be assessed on days 14 and 42. In addition, testosterone measurements will be taken every 90-d (starting from day 42) to assure full recovery of endogenous testosterone production (testosterone concentration ≥ 300 ng/dL) once the study has been completed.

1.15. Experimental physical activity

Physical activity will be highly-controlled and supervised starting on day 15 when phase 2 begins. Varied low-, moderate-, and high-intensity (40–85% of predetermined VO_{2peak}) endurance-type exercise will be performed during the 28-d live-in phase to increase TDEE 50% above phase 1 (phase 1 EIEE + 50% of phase 1 TDEE = phase 2 EIEE). Intensity is defined as < 40% of heart rate reserve (HRR) for low intensity, 40–59% of HRR for moderate intensity, and 60–89% of HRR for high intensity [64]. Energy expenditure will be achieved by

Table 2
Schedule of study procedures.

Procedure	SV1	SV2	Phase 1	Phase 2	Phase 3	End of study
			0–14	15–42	43–84	EOS-EOS + 3
Informed consent	X					
Neck/waist circumference	X					
Height	X					
Weight	X	X	X (15)	X (28)	X (12)	X (4)
Head circumference		X				
Medication use	X	X				X (1)
Barriers interview		X				
Vitals	X	X		X (28)		
Medical history & physical exam	X					
ECG		X				
Blood draw with archives		X	X (3)	X (2)	X (2)	X (2)
Urine collection		X	X (3)	X (3)	X (3)	X (3)
Food diary		X (3)			X (3)	
Activity log		X (3)	X (15)	X		
Questionnaires	X	X	X	X	X	X
Heavy water			X (15)	X (28)		
Food intake test with blood draws			X (1)		X (1)	X (1)
Accelerometer		X	X (15)	X (28)	X (42)	X (4)
DXA scan			X (2)	X (2)	X (1)	X (1)
fMRI			X (3)	X (3)	X (3)	
Cognitive testing			X (8)	X (9)	X (1)	X (1)
Exercise VO2 max test			X (1)			
Muscular strength test			X (2)	X (1)	X (1)	X (1)
Adverse event assessment		X	X (3)	X (4)		X (1)
In-house meals			X (15)	X (28)		
24 Hour stay			X (1)	X (28)	X (1)	
Stool collection			X (1)	X (2)		X (1)
D3-Creatine (D3Cr) consumed			X (1)	X (1)		X (1)
Muscle biopsy			X (3)	X (4)		
24 hour metabolic chamber			X (1)	X (1)	X (1)	
Randomization			X			
Cycle session			X (2)	X (1)		
Sugar substitute test			X (1)	X (1)		X (1)
Saliva collection			X (3)	X (7)		
Injection				X (4)		
Exercise sessions				X		

Note: The number in parentheses denotes the quantity of times each procedure is completed during the designated phase.

performing at least one, but no more than four, exercise sessions/d, using a variety of endurance-type modalities [outdoor walking, treadmill (walk, run, and load carriage w/weighted vest equal to 20–35% body mass), cycle ergometer, and elliptical]. Exercise intensity will be verified biweekly and adjusted accordingly using an open circuit indirect calorimeter.

Light calisthenics will be incorporated into the exercise regimen approximately every 3–4 d. Standardized energy expenditures will be determined for calisthenic exercises and integrated into individual exercise prescriptions to meet target expenditure using the Compendium of Physical Activities [65]. Calisthenics will not be done within 48 h of conducting muscle biopsies, muscle strength or endurance testing. On designated light exercise (days 21, 28, 35) or testing (days 14 and 42) days, participants will only perform half the exercise they perform on all other days (i.e., exercise will be walking at a low intensity). Dietary energy intake will be adjusted to account for the reduced level of exercise to maintain the 55% energy deficit.

1.16. Experimental diet

Dietary energy intake will be initially determined for study participants using the Mifflin St. Jeor Equation with an activity factor of 1.3 to account for activities of daily living, and in combination with results from the 7-d accelerometer data and 3-d activity logs collected during screening visits. Diets will provide 1.6 ± 0.2 g protein·kg^{-1}·d^{-1} distributed equally across meals, 30% of total daily energy requirements from fat, with the remaining energy derived from carbohydrate. Registered dietitians will develop individualized 6-d menus (consisting of breakfast, lunch, dinner, snacks, and beverages). The energy content of the phase 1 diet will be sufficient to maintain body mass within ± 2%. Intake will be adjusted incrementally (± 200 kcal every 3 d) to achieve energy balance. Energy expenditure data from the whole-room calorimetry assessment on day 11 (phase 1) also will be used to more accurately prescribe phase 2 TDEE.

Participants will consume breakfast daily at the PBRC metabolic kitchen during phase 1. Participants will be weighed by research staff before breakfast is provided. Lunch, dinner, snacks, and remaining beverages (water ad libitum) will be packed and consumed offsite. Dietary compliance will be verified daily by assessing foods/beverages remaining in returned coolers and using questionnaires that allow participants to list any deviations from the provided diet. Participants will remain onsite on day 14 after testing has been completed, undergo randomization, and begin the 28-d experimental phase (phase 2) the next morning. Dietary energy intake will be 45% of total daily energy expenditure after accounting for the 50% increase in TDEE (i.e., additional EIEE), resulting in a 55% total daily energy deficit.

The micronutrient content of the phase 1 diet will be consistent with current national recommendations. Micronutrient intake during the 28-d, 55% energy deficit will likely be diminished. However, to maintain operational relevance and to explore the impact of sustained, severe energy deficit on micronutrient-related markers of nutritional status, intake will not be augmented with supplementation. Sample menus for phase 1 and 2 are provided in Table 3.

Participants will resume their self-selected, ad libitum diet during the recovery phase (phase 3). Participants will complete 3-d food

Table 3
Sample menus for phase 1 (2700 kcal/d, weight maintenance) and phase 2 (1800 kcal/d, energy deficit) diets.

Food item	Phase 1 food item weights (g)	Phase 2 food item weights (g)
Breakfast		
Grits, Quaker, instant	50.0	29.7
Butter, salted	18.0	8.5
Canadian bacon	132.0	179.0
Egg white, raw weight	125.0	106.0
Orange juice	248.8	0
Milk, skim	236.0	250.2
Lunch		
Pita bread, white	55.0	0
Turkey breast, oven roasted	130.0	130.0
Mayonnaise, regular	24.0	0
Cheese, Swiss, sliced	40.0	45.0
Pretzels, Rold Gold, tiny twists	20.0	12.7
Lettuce, romaine, raw	23.5	90.0
Tomato, raw, sliced	20.0	40.0
Ranch dressing, regular	0	12.0
Dinner		
Lemon sage chicken breast	145.0	145.0
Long grain & wild rice blend, Uncle Ben's	200.0	110.0
Capri vegetable mix	130.0	80.0
Dinner roll, whole wheat, Rich's	60.0	0
Butter, salted	20.0	14.0
Pears, canned in juice	190.0	0
Greek yogurt, Chobani fat-free vanilla	0	116.6
Snack		
Pretzels, Rold Gold, tiny twists	57.0	40.3
Raisins, seedless	52.6	37.1
Crackers, Cheeze It's	30.0	21.2
M & M's, regular candies	15.0	10.6

records (1 weekend day, 2 weekdays) each week prior to day 56 and prior to EOS, which will be reviewed with registered dietitians at PBRC for continued monitoring and assessment.

1.17. Deuterium labeling, muscle biopsies and determination of muscle protein synthesis of individual proteins throughout the proteome

Deuterium (^2H_2O) labeling, muscle biopsies, and proteomic analyses will be used to determine protein synthesis rates in response to the intervention, and muscle composition based on histologic characterization in response to the intervention. Participants will consume ^2H_2O (70%; Cambridge Isotope Laboratories, Andover MA, USA) beginning on day 0 and ending on day 42. Days 0–7 will serve as an isotopic priming phase, where participants will consume 3, 50 mL doses (150 mL total) each day to achieve a target enrichment of 1–2%. Participants will consume 2, 50 mL doses (100 mL total) each day for the remainder of the study (days 8–42) to maintain isotopic enrichment of body water, which will be determined from blood collected during the muscle biopsy days and periodic saliva samples.

While participants are under local anesthesia (1% lidocaine), muscle biopsy samples from the vastus lateralis will be collected using a 5-mm Bergstrom needle with manual suction on days 14, 28, and 42. Participants will undergo three muscle biopsy procedures (pre-exercise, 60 min and 360 min post-exercise) on days 14 and 42 (contralateral leg from day 14). One muscle biopsy will be performed on day 28 (mid-point of the intervention). Participants will be fasted overnight for the biopsy on day 28 and both the pre-exercise and 60 min post-exercise biopsies on day 14 and 42. Participants will be provided a standardized meal (25% of energy requirements) after the 60-min post-exercise biopsy. The exercise bout will include 60 min cycle ergometry at 65–75% $VO2_{peak}$ (Lode Excalibur Cycle Ergometer, Netherlands).

1.18. Muscular strength and power

Muscular strength/power will be measured with isometric and isokinetic knee extension tests on days 13, 41, 55, and at EOS. Testing will take place prior to all daily exercise bouts. Isometric quadriceps strength, maximal power and muscular endurance will be quantified using an isokinetic dynamometer (Biodex Medical Systems, Shirley, NY).

1.19. Energy expenditure, substrate oxidation and nitrogen balance

Energy expenditure, substrate oxidation, and nitrogen balance will be measured for a 24-h period in a metabolic chamber on days 11, 39, and 53. The metabolic chamber stays will begin before breakfast but after completing the DXA scan (~0800), and end before breakfast the following morning (~0800). Participants will receive the same meals, snacks, and beverages during each chamber stay. While in the metabolic chamber, participants will perform the same amount of daily exercise typically performed during that respective phase (i.e., phase 1, 2, or 3). For uniformity, all exercise will be performed on a cycle ergometer. Lights will be turned off at 2230 and participants will be awakened at 0630. To determine nitrogen balance, all urine will be collected for measurement of urinary nitrogen and creatinine excretion rates. Energy expenditure will be calculated by indirect calorimetry corrected for urinary nitrogen excretion and respiratory quotient. Energy expenditure and substrate oxidation will be calculated for the 24-h period and also partitioned between rest, exercise and sleep.

1.20. Cognitive and behavioral assessments

A comprehensive battery of questionnaires, evaluations, cognitive performance tests, and personality assessment will be administered periodically throughout each phase after undergoing appropriate training and familiarization (Table 4).

1.21. Functional MRI assessments

Participants will perform a total of six behavioral tasks (~1 h/task) within a 3 T MRI machine (Discovery 750w, GE Healthcare, Waukesha, WI) during each study phase while fMRI data is collected (Table 4). The tasks probe executive functioning, emotion processing, risk taking, and aggression. Participants wear a 32-channel phased-array head coil and view visual stimuli through a rear-mounted mirror-projector system. A respiratory monitoring belt and pulse oxygenation sensor allow post-hoc correction of physiological influences on fMRI.

1.22. Sleep assessments

Actigraphic assessment of daily spontaneous motor activity, sleep and circadian rhythms will be conducted throughout the study using the Fatigue Science ReadiBand™ actigraph. The wrist-worn ReadiBand™ has been validated in comparison to polysomnography, and shows concordance of 90% or greater in terms of sleep-scoring accuracy. The Standards of Practice Committee of the American Academy of Sleep Medicine (2007) has concluded that actigraphy provides an accurate estimate of sleep patterns in healthy people [66]. The ReadiBand™ actigraph will be worn continuously (24 h/d) on the dominant wrist during each phase. Sleep quantity, quality, and sleep/wake timing scores will be calculated.

1.23. Appetite and endocrine mediators of appetite

Assessment of appetite and endocrine mediators of appetite will be completed after 1 wk. of diet acclimation (day 7, phase 1), on day 43 (start of phase 3), and at EOS. Participants will receive a fixed-portion breakfast meal (0 min) and an ad libitum lunch meal (180 min). Subjective appetite ratings and blood samples will be collected 15 min before starting the breakfast meal, at the start of the meal, and then 30, 60, 120, and 180 min after meal start. Appetite also will be rated after consuming the lunch test meal. On day 43, additional blood samples and subjective appetite ratings will be collected after the lunch test meal. Blood will be analyzed for serum leptin, insulin, and glucose, and plasma for acyl ghrelin and des-acyl ghrelin.

The fixed-portion test meal will be served as a breakfast meal, have mixed macronutrient content, and be prepared according to a standard procedure. For each participant the energy content of the meal will be equivalent to 20% of the TDEE on study days 0–14. Water in the amount of 240 g will be provided during the meal. Participants will be instructed to consume all of the water before completing the meal and will not be permitted additional water during the meal.

A lunch meal will be consumed ad libitum 180 min following provision of the breakfast meal. The lunch meal will be of mixed macronutrient content, consist of a single item, and be prepared according to a standard procedure by research staff. A portion calorically equivalent to at least 75% of the TDEE for that individual on study days 0–14 will be served to ensure that food intake is not limited by the amount served. Participants will be instructed to eat until comfortably full, and be permitted to eat as much or as little as desired. There will be no restrictions on meal duration. The amount of uneaten food will be weighed and the energy content of the portion consumed calculated. Water in the amount of 240 g will be provided. Participants will be instructed to consume all of the water before completing the meal and will not be permitted additional water during the meal.

Diets will be adjusted on day 7 to account for the appetite assessment. In the intervals between meals, participants will be required to drink 360 mL water, and will not be permitted access to any additional food or beverage.

1.24. Gut microbiome composition and activity, and intestinal permeability

Fecal samples will be collected at 4 time points (end of phase 1, mid

Table 4
Cognitive and behavioral test battery and fMRI tasks.

Test	Description	Familiarization Days	Criterion test
The Minnesota Multiphasic Personality Inventory [71]	Provides a broad understanding of a person's basic personality characteristics. Results are provided in t-scores on several traditional clinical scales as well as on clinical sub-scales and three validity scales.	None	5, 40
The Buss–Perry Aggression Questionnaire [72]	Statements (29-item) are ranked along a 5 pt. continuum from "extremely uncharacteristic of me" to "extremely characteristic of me." Results are shown in terms of scores on 4 scales: physical aggression, verbal aggression, anger, and hostility.	0, 2, 4, 6, 8, 10	13, 15, 20, 22, 27, 29, 34, 36, 41, 54, EOS
Profile of Mood States (POMS) Questionnaire [73]	An inventory of subjective mood states (65-item). Results consist of six mood sub-scale scores (tension, depression, anger, vigor, fatigue, and confusion).	0, 2, 4, 6, 8, 10	13, 15, 20, 22, 27, 29, 34, 36, 41, 54, EOS
Satiety Labeled Intensity Magnitude (SLIM) Questionnaire [74]	A vertical, 100 mm, bidirectional scale anchored by the terms "greatest imaginable fullness" and "greatest imaginable hunger." Volunteer marks the scale anywhere along the axis corresponding to their level of hunger or fullness "right now" to assess subjective feelings of hunger and fullness.	0, 2, 4, 6, 8, 10	13, 15, 20, 22, 27, 29, 34, 36, 41, 54, EOS
Objective Risk Assessment			
Balloon Analogue Risk Task (BART) [75]	Objective is to keep a simulated balloon inflated without popping (30 trials). There is a risk-learning component as some balloon colors pop with less inflation and others with more, while a third category is unpredictable. Designed to measure willingness to take risks versus "play it safe."	0, 2, 4, 6, 8, 10	13, 15, 20, 22, 27, 29, 34, 36, 41, 54, EOS
Objective Cooperation/Dominance			
The Ultimatum Game	This is a 2-player game in which one player is given a specific amount of money which he then decides to split with a second player. In the version used here, the player deciding upon the split is being simulated by computer and the other player is the research participant. Once the split is proposed, the research participant either accepts or rejects the deal. If the deal is rejected, neither player gets anything. This is a test of negotiation and cooperation.	0, 2, 4, 6, 8, 10	13, 15, 20, 22, 27, 29, 34, 36, 41, 54, EOS
Beliefs, Intentions, and Desires-of-Others			
Reading the Mind in the Eyes Test [76]	Volunteers are presented a series of single images of a person's eyes (and immediate eye region) along with 4 emotion-descriptive words, and asked to choose the word that best describes the emotional or mental state of the person in the image (36 images total). Tests ability to attribute or infer beliefs, intentions and desires of others.	0, 2, 4, 6, 8, 10	13, 15, 20, 22, 27, 29, 34, 36, 41, 54, EOS
Cognitive/Vigilance			
Scanning Visual Vigilance Task [77]	Volunteers scan a computer screen to detect the occurrence of infrequent, difficult to detect stimuli that appear randomly on a computer screen for 2 s. Upon detection, the participant will press a button as rapidly as possible. Detection accuracy and response time are recorded, as are false alarms. Assesses visual vigilance.	0, 2, 4, 6, 8, 10	13, 15, 20, 22, 27, 29, 34, 36, 41, 54, EOS
Psychomotor Vigilance Test (PVT) [78]	Test requires subjects to sustain attention and respond rapidly and accurately to a series of numerical time-count stimuli that appear on a computer screen by pressing a button. Reaction time and response accuracy as well as response lapses are scored. Test of vigilance and visual reaction time.	0, 2, 4, 6, 8, 10	13, 15, 20, 22, 27, 29, 34, 36, 41, 54, EOS
Matching to Sample Test [79,80]	Volunteer views an 8 × 8 matrix of a red and green checkerboard for 4 s, followed by a variable delay. After the delay, the original sample matrix and a second matrix that differs slightly (in that the color sequence two squares are reversed) are presented. The participant has 15 s to select the matching matrix. Assesses short-term spatial memory and pattern recognition skills.	0, 2, 4, 6, 8, 10	13, 15, 20, 22, 27, 29, 34, 36, 41, 54, EOS
Grammatical Reasoning Test (adapted from the Baddeley Grammatical Reasoning Test) [81]	The letters AB or BA follow a statement. Participant decides whether each statement correctly describes the order of the two letters by pressing a button. Statements can be active/passive or positive/negative, and a given letter may precede/follow the other letter. Assesses language-based logical reasoning.	0, 2, 4, 6, 8, 10	13, 15, 20, 22, 27, 29, 34, 36, 41, 54, EOS
Borg Rating of Perceived Exertion Scale (RPE)	Administered during exercise sessions. The RPE scale assesses self-reported perceived physical exertion.	0, 2, 4, 6, 8, 10	13, 15, 20, 22, 27, 29, 34, 36, 41, 54, EOS
N-back Task	Participants monitor the identity or location of a series of verbal (letters) stimuli and indicate when the presented stimulus is the same as the one presented "n" trials back (e.g., 0, 1, 2, or 3). Measures response time and accuracy to test working memory.	0, 2, 4, 6, 8, 10	13, 15, 20, 22, 27, 29, 34, 36, 41, 54, EOS
fMRI Tasks			
The Gambling Task (life-cash version) [82]	Participants choose between a high-risk, high-reward outcome and a low-risk, low-reward outcome. The outcome could either be in the form of saving civilian lives, or earning cash. This task assesses the propensity for high-risk, high-reward decisions, across differing reward domains (lives and cash respectively)	5	5, 36, 55
Provoked Aggression Task [83]	Participant is fitted with an MR-safe device designed to apply an electrical stimulus to a site on the arm (STM100C, BioPac Systems, Inc). The participant engages in a simple game with a digital opponent; after each round of the game, the winner chooses the intensity of the applied stimulus (modulated by the number of electrical stimuli applied to the arm) to the opponent. The participant is not told that there is no real human opponent, and both the outcomes of the trials and the intensities of the electrical stimulus applied by the digital opponent are actually preordained. The digital opponent provokes the participant by increasing the intensity of stimulus across winning trials. This task assesses the propensity toward retaliatory aggression, i.e. escalating stimulus intensity applied by the participant in response to such provocation.	5	5, 36, 55
Multi-Source Interference Task [84]	Participant is shown a string of three numbers including digits 1 to 3, and	9	9, 37, 56

(continued on next page)

Table 4 *(continued)*

Test	Description	Familiarization Days	Criterion test
	requires the participant to use a 3-button response box to identify which of the three numbers appears only once in the string. There are two sources of interference: the two other distracting numbers in the string and positioning of the probe number that is discordant with the position of the corresponding button on the response box. The task assesses inhibitory control in the face of such interference.		
AX Continuous Performance Task [85]	Participants click a response button when they observe an X that has been followed by an A in a stream of characters displayed on a screen. This task assesses working memory.	9	9, 37, 56
Attention Network Task	Participants are presented with a line of arrows and must click a left-hand or right-hand button depending on whether the center arrow points to the left or right, respectively. Flanking arrows pointing in the opposite direction of the center arrow provide interference. A spatial cue indicates where on the screen the line of arrows are about to appear. This task assesses multiple aspects of attentional and inhibitory control, including spatial attention and how it is modified by the presence of interference.	12	12, 38, 57
Emotional Reactivity Task [41]	Participants are presented with a probe image of a human face shown in the center of the screen which must be matched to a face shown either on the left or right side of the screen. The facial expression shown in the probe image has differing emotional valence (i.e., angry or neutral). This task assesses brain reactivity to emotional stimuli even when the task does not require overt emotion processing.	12	12, 38, 57

and end of phase 2, and EOS) to assess gut microbiota composition and function. At each time point participants will be given a ≤ 72 h window to collect a usable sample. Samples will be processed as soon as possible and within 12 h of defecation. 16 s rRNA gene sequencing and short-chain fatty acids will be analyzed, and additional samples stored for metagenomics, transcriptomics, and metabolomics, analyses.

1.25. Urine collection and assessment of gastrointestinal permeability

Differential sugar absorption tests will be used to provide a functional assessment of gastrointestinal permeability [67]. For this test participants will consume 2 g sucralose dissolved in 180 mL water on the morning of study days 11, 39, and EOS. Prior to testing days, participants will undergo a 2-d washout period of sucralose-containing beverages and foods. Participants will collect all urine produced 24 h after sugar substitute ingestion. Sucralose excretion will provide a measure of whole gut permeability [67].

1.26. Determination of eating attitudes and behaviors

Several questionnaires addressing eating behaviors and food cravings will be administered with participants in a fasted state during screening visit 2 and on study days 14, 42, and EOS. The Three Factor Eating Questionnaire will be used to measure hunger, dietary restraint, and disinhibition [68]. Food cravings will be assessed using the Food Cravings Questionnaire-trait (FCQ-trait) and the Food Cravings Inventory 2 (FCI-2), which measures the frequency of cravings for specific types of foods.

1.27. Statistical power and sample size

Relevant data (means ± SD) demonstrating the effects of moderate-to-severe energy deficit on FFM were used to determine statistical power and sample size (Table 5). The percentage total body mass lost (2.7 kg) attributed to FFM during a 21 d, higher-protein (1.6 $g \cdot kg^{-1} \cdot d^{-1}$), moderate energy deficit was approximately 30% (0.8 kg) [8]. However, the proportion of total body mass loss (5.8 kg) attributed to FFM in response to a short-term (~7 days) military training-induced, severe energy deficit was approximately 55% (3.1 kg) [69]. Based on these results, and given that the proposed study will induce a 55% energy deficit for 28 d in men consuming a higher-protein diet (1.6 $g \cdot kg^{-1} \cdot d^{-1}$), FFM will likely account for 40% of the total body mass loss. Maintaining testosterone within normal physiological ranges is expected to attenuate the loss of FFM by 25%, such that those assigned to the testosterone group will lose proportionally 30%, the same percentage of FFM lost in our previous study when testosterone levels were, largely, unchanged in participants consuming a higher-protein diet (1.6 $g \cdot kg^{-1} \cdot d^{-1}$), energy deficient diet for 21 d [20]. Based on these estimates, the sample size necessary to determine the estimated differences between treatments is 22 per group with 90% power. However, based on previous variability in FFM loss in response to moderate-to-severe energy deficit, a more conservative estimate of 25 participants per group will be used. To account for possible attrition (20%), 30 participants will be assigned to each group (60 total participants). Enrollment will stop once 50 participants have completed the study.

Table 5
Statistical power and sample size justification based on change in lean body mass.

DEF	3.0 ± 0.75[a]
DEF + TEST	2.25 ± 0.75
Effect Size	1.0
Alpha	0.05
Power	0.90
Sample size	22 per group
Expected variability	25 per group
20% study attrition	60 participants total[2]
75% eligibility screen failure	240 total consented individuals

[a] Hypothesized effects (mean ± SD); DEF, 55% energy deficit; DEF + TEST, 55% energy deficit + testosterone. [2]Enrollment will stop once 50 participants (25 per group) complete the study.

1.28. Data management

Study participants will be assigned unique subject identification (ID) numbers. Study subject ID numbers will be used on all data collection instruments, to include questionnaires, data collection forms, biological specimen tubes, and computer records. All data are entered into a fully integrated and automated data management system that has been fully validated and undergoes continuous quality assurance by the PBRC Research Computing Core. All data are backed up daily, and the Research Computing Core at PBRC oversees all data management.

1.29. Statistical analysis

All data analyses will be based on the intention-to-treat principle using SPSS statistical software, unless otherwise noted. Data will be examined quantitatively and graphically for outliers and artifacts that might have an undue impact on the analyses. Logarithmic or similar transformations will be applied when necessary (i.e., when data are not normally distributed) to ensure the validity of statistical procedures (e.g., circulating and skeletal muscle markers of inflammation, intramuscular regulators of metabolism, etc.). The familywise error rate for related outcomes will be controlled using the Bonferroni correction. All tests will be two-sided, considered statistically significant at $P < 0.05$, and performed by a statistician.

1.30. Physiological and metabolic outcomes

Generalized linear mixed-effect models (GLMM) will be used to test the effects of testosterone on changes in body composition, skeletal muscle function and composition, protein dynamics, metabolism, and biomarkers of physiological status. Mixed-models will include subject as a random factor, study day and group (DEF vs. DEF + TEST) as fixed factors, and the day-by-group interaction. When a statistically significant day-by-group interaction is detected ($P < 0.05$), all possible within- and between-group comparisons will be completed, and the familywise error rate adjusted using the Bonferroni correction.

1.31. Cognitive and behavioral outcomes

GLMM also will be used to analyze ReadiBand activity/sleep-monitoring data (sleep duration, sleep latency, and sleep efficiency) and fMRI data. For each fMRI task, outcomes of interest from the fMRI data will be summary measures of fMRI signals within brain regions of interest previously implicated as critical to the task. Individualized scale scores from each of the questionnaires, the MMPI-2, and the Reading the Mind in the Eyes Test, the scores from the Ultimatum Game, the number of points earned on the Balloon Task, and dependent measures (such as accuracy scores and reaction times) on the vigilance, memory, and reasoning tasks will be analyzed with a series of GLMM. Mixed-models will include subject as a random factor, time-in-study and group as fixed factors, and the time-in-study-by-group interactions. Significant main effects and interactions will be pursued with Analysis of Simple Effects followed by appropriate linear contrasts and pairwise comparisons. The familywise error rate will be adjusted using the Bonferroni correction. Questionnaire outcomes will be analyzed at 7 time points. The specific sessions chosen for this analysis will depend on whether or not acute testosterone dose effects are found in a separate examination of immediate pre/post-dose effects.

1.32. Appetite and gut health outcomes

GLMM will be used to test the effects of testosterone maintenance on changes in appetite, postprandial endocrine responses, eating behaviors, intestinal permeability, and short-chain fatty acid concentrations over time. Postprandial appetite ratings and responses of endocrine mediators of appetite will be summarized using area under the curve and peak/nadir concentration prior to analysis. Mixed-models will include subject as a random factor, study day and group as fixed factors, and the day-by-group interaction. When a statistically significant time-by-group interaction is detected ($P < 0.05$), all possible within- and between-group comparisons will be completed, and the familywise error rate adjusted using the Bonferroni correction.

Metabolomics data will be visualized using hierarchical clustering and principal components analysis. Taxonomic data will be visualized using hierarchical clustering and principal coordinates analysis of beta (i.e., between samples) diversity scores (e.g., Bray-Curtis, and weighted and unweighted UniFrac). Alpha (i.e., within-sample) diversity will be calculated for taxonomic data using Shannon, Simpson and Chao1 indices [70]. GLMM will be used to test the effects of testosterone maintenance on changes in metabolite concentrations, alpha diversity, and the relative abundance of individual taxa over time. Mixed-models will include subject as a random factor, study day and group as fixed factors, and the day-by-group interaction. The Benjamini-Hochberg correction will be used to control the false discovery rate for main effects of time, group, and the time-by-group interactions resulting from models that include "– omic" data. When a statistically significant time-by-group interaction is detected (FDR-adjusted $P < 0.05$), post hoc within- and between-group comparisons will be completed and the familywise error rate adjusted using the Bonferroni correction.

2. Discussion

There is a critical need for effective and feasible interventions that sustain and optimize the health and performance of service members during real-world training and combat operations. This interventional trial will delineate the contribution of testosterone declines, and benefits of testosterone replacement, on complex markers of physiological and psychological status during prolonged, severe energy deficit, addressing a direct and consistently observed gap in knowledge.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.cct.2017.05.001.

Abbreviations

DEF 55% energy deficit diet group with placebo administration-DEF + TEST55% energy deficit diet group with testosterone administrationDXAdual-energy X-ray absorptiometryEIEEexercise-induced energy expenditureEOSend of studyFCI-2Food Cravings Inventory 2FCQ-traitFood Cravings Questionnaire-traitfMRIfunctional magnetic resonance imagingHRRheart rate reserveFFMlean body massMMPI-2Minnesota Multiphasic Personality Inventory–2OPSOptimizing Performance for SoldiersPBRCPennington Biomedical Research CenterTDEEtotal daily energy expenditure

Trial status

This trial is currently recruiting participants and was approximately 50% complete as of 1 January 2017.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

SMP, CEB, JPK, and HRL were responsible for the conception and design of the study, and manuscript writing. JSO, LMM, JAC, AJY, WJE, and OV were responsible for the conception and design of the study and critical revision of the manuscript. KMG was responsible for medical oversight and critical revision of the manuscript. OTC, MH, and JCR were responsible for conception, design, and conduct of the study, and critical revision of the manuscript. All authors read and approved the final version of the manuscript.

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ORIGINAL RESEARCH

Effects of exercise mode, energy, and macronutrient interventions on inflammation during military training

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Abstract

Load carriage (LC) exercise may exacerbate inflammation during training. Nutritional supplementation may mitigate this response by sparing endogenous carbohydrate stores, enhancing glycogen repletion, and attenuating negative energy balance. Two studies were conducted to assess inflammatory responses to acute LC and training, with or without nutritional supplementation. Study 1: 40 adults fed eucaloric diets performed 90-min of either LC (treadmill, mean ± SD 24 ± 3 kg LC) or cycle ergometry (CE) matched for intensity (2.2 ± 0.1 VO_{2peak} L min^{-1}) during which combined 10 g protein/46 g carbohydrate (223 kcal) or non-nutritive (22 kcal) control drinks were consumed. Study 2: 73 Soldiers received either combat rations alone or supplemented with 1000 kcal day^{-1} from 20 g protein- or 48 g carbohydrate-based bars during a 4-day, 51 km ski march (~45 kg LC, energy expenditure 6155 ± 515 kcal day^{-1} and intake 2866 ± 616 kcal day^{-1}). IL-6, hepcidin, and ferritin were measured at baseline, 3-h post exercise (PE), 24-h PE, 48-h PE, and 72-h PE in study 1, and before (PRE) and after (POST) the 4-d ski march in study 2. Study 1: IL-6 was higher 3-h and 24-h post exercise (PE) for CE only (mode × time, $P < 0.05$), hepcidin increased 3-h PE and recovered by 48-h, and ferritin peaked 24-h and remained elevated 72-h PE ($P < 0.05$), regardless of mode and diet. Study 2: IL-6, hepcidin and ferritin were higher ($P < 0.05$) after training, regardless of group assignment. Energy expenditure ($r = 0.40$), intake ($r = -0.26$), and balance ($r = -0.43$) were associated ($P < 0.05$) with hepcidin after training. Inflammation after acute LC and CE was similar and not affected by supplemental nutrition during energy balance. The magnitude of hepcidin response was inversely related to energy balance suggesting that eating enough to balance energy expenditure might attenuate the inflammatory response to military training.

Introduction

Endurance exercise elicits an increase in IL-6, a proinflammatory cytokine that, in turn, triggers hepatic release of hepcidin, a regulator of iron status (Peeling 2010; Peeling et al. 2014). A single bout of traditional weight-bearing and nonweight-bearing endurance exercise acutely increases circulating IL-6 and hepcidin concentrations to a similar degree (Sim et al. 2013, 2014a). However, repeated bouts of weight-bearing endurance exercise (i.e., training) produce a chronic inflammatory response that elevates basal hepcidin levels to a greater extent than repeated bouts of nonweight-bearing endurance exercise (Sim et al. 2014b). The authors of that study suggested that the elevated levels of hepcidin may alter iron status in athletes participating in weight-bearing exercise

training programs (Sim et al. 2014b). The inflammatory responses to military training may exceed those observed after traditional endurance exercise training, as the unaccustomed activities encountered during military training differ greatly from traditional exercise. For example, load carriage (LC) is not typically performed during traditional exercise training, but is commonly performed for prolonged periods at low to moderate intensities with loads ranging from 20 to 60 kg during military training (Nindl et al. 2013). Whether the physiological strain of LC elicits an inflammatory and hepcidin response that exceeds responses produced during training employing traditional endurance exercise has not been determined.

Military training can produce severe energy deficits that deplete endogenous energy stores (Margolis et al. 2014). Depleted endogenous energy stores may exacerbate inflammation and the hepcidin response to exercise, as low glycogen status upregulates postexercise skeletal muscle IL-6 gene expression (Keller et al. 2001; Steensberg et al. 2001) and increases plasma levels of IL-6 (Steensberg et al. 2000). It has been hypothesized that carbohydrate supplementation might attenuate postexercise inflammation by limiting glycogen depletion during sustained endurance exercise and enhancing glycogen repletion during recovery, although the data supporting this hypothesis are largely equivocal (Cox et al. 2008, 2010; Sim et al. 2012; Badenhorst et al. 2015). Some studies suggest that combining whey protein with carbohydrate attenuates the inflammatory response to exercise (Kerasioti et al. 2013), although other studies fail to substantiate such an effect (Nelson et al. 2013; Rowlands et al. 2008). Of note, each of these studies was performed in laboratory settings under controlled periods of energy balance. To what extent carbohydrate, protein, or combined supplementation attenuates the inflammatory responses to arduous military training (McClung et al. 2013; Margolis et al. 2014) has not been tested.

In this study, two experiments were conducted to assess inflammation and hepcidin responses to military unique endurance exercise tasks, with or without supplemental nutrition. Study 1 compared inflammatory and hepcidin responses to LC and intensity-matched, nonweight-bearing exercise, cycle ergometry (CE), and evaluated whether consuming a combined protein and carbohydrate supplement mitigated the inflammatory response. We hypothesized that the postexercise inflammatory and hepcidin responses to acute LC would be more pronounced than traditional CE, and that combined carbohydrate and protein supplementation would attenuate the acute response. Study 2 assessed the efficacy of a carbohydrate- or protein-based nutrition intervention on inflammation and hepcidin responses during a multi-day military training operation in which participants performed repeated days of LC and performed activities that produced severe energy deficits. We expected carbohydrate-based supplemental nutrition to mitigate inflammatory and hepcidin responses during multiday military training to a greater extent than protein-based supplementation.

Methods

Study 1: Volunteers and experimental design

Forty adults (37 males and 3 females) participated in this randomized, double-blind, placebo-controlled study after providing informed, written consent from October 2012 to November 2013 (Pasiakos et al. 2015). All study procedures were conducted at the US Army Research Institute of Environmental Medicine (USARIEM, Natick, MA). Volunteers were military personnel from the US Army Natick Research, Development and Engineering Center, Human Research Volunteer recruit platoon, and civilians from the local area. Volunteers were required to be between the ages of 18–39 years, weight stable (±2 kg for a period of 2 months), physically fit (peak oxygen uptake, VO_{2peak} 40–60 mL kg^{-1} min^{-1}), and have a body mass index (BMI) between 22 and 29 kg m^{-2}. A medical screening was also conducted to ensure that potential volunteers could safely participate in the study. This study was approved by the Institutional Review Board at USARIEM and registered at www.clinicaltrials.gov as NCT01714479.

Volunteers were randomly assigned to one of four experimental groups (Fig. 1A). All four groups performed a single 90-min exercise bout. Two groups performed nonweight-bearing, traditional endurance exercise (CE) and the other two performed LC (i.e., a military-like, occupational exercise) exercise. One of each of the exercise groups received combined essential amino acid and carbohydrate supplement (SUPP, 223 kcal) drinks to consume during exercise, and the other groups received flavor-matched, nonnutritive control (CON, 22 kcal, 5 g carbohydrate) drinks. The essential amino acid/carbohydrate treatment provided 10 g of essential amino acids (0.7 g histidine, 0.7 g isoleucine, 3.6 g leucine, 1.2 g lysine, 0.3 g methionine, 1.4 g phenylalanine, 1.0 g threonine, and 1 g valine) and 46 g of carbohydrate (maltodextrin and fructose at a 2:1 ratio). The composition of the SUPP treatment was based on previous work demonstrating a muscle protein synthetic advantage of consuming small doses of leucine-enriched essential amino acids during steady-state exercise (Pasiakos et al. 2011). However, the treatment also included carbohydrate to test a palatable, eat-on-the move, combat ration recovery item that provides not only essential amino acids, but also

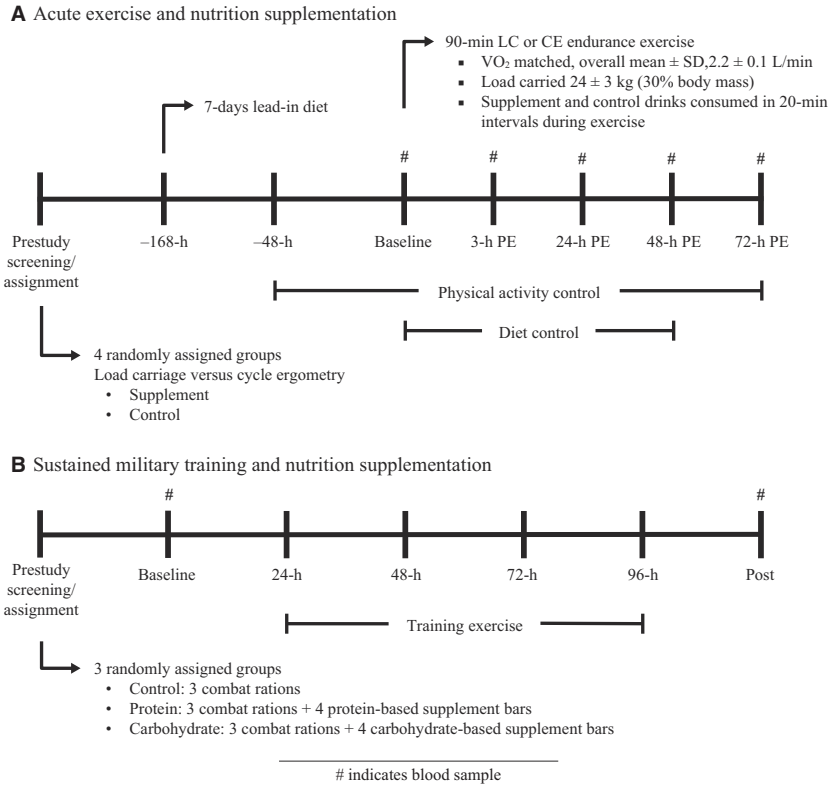

Figure 1. Study designs. (A) Experimental design for assessing the inflammatory responses to acute, 90-min load carriage (LC) or cycle ergometry (CE) intensity-matched exercise bouts, with or without combined essential amino acid and carbohydrate supplementation. Biochemical assays were performed using blood collected at baseline, 3-h postexercise (PE), 24-h PE, 48-h PE, and 72-h PE. Dietary intake was individually prescribed to maintain body mass and provided to research volunteers as US military combat rations. (B) Experimental design for assessing the effects of carbohydrate and protein supplementation on inflammation before (PRE) and immediately after (POST) completing a 4-day arctic military training operation.

energy in the form of carbohydrate to sustain activity during military operations (Jeukendrup 2014). Inflammation (IL-6), hepcidin, and ferritin, because ferritin is also induced by inflammation, were assessed at baseline (pre-exercise), postexercise, and once daily during a 72-h recovery period. Creatine kinase (CK) was measured as a surrogate marker of muscle damage to differentiate skeletal muscle strain between LC and CE.

Diet and physical activity

Dietary intake and physical activity were controlled to minimize any potential confounding effects on outcome variables. Dietary intake was individually prescribed based on 3-day diet and activity records at baseline and dietary compliance was confirmed by conducting 24-h dietary recalls every 2 days during a 7-day prestudy lead-in phase (Food Processor SQL®, version 10, ESHA Research, Salem, OR). Overall (mean ± SD) energy (2607 ± 468 kcal day^{-1}), carbohydrate (344 ± 78 g day^{-1}), protein (111 ± 23 g day^{-1}), and fat (92 ± 26 g day^{-1}) intakes were similar across groups, and body mass was stable during the lead-in phase (Pasiakos et al. 2015). Eucaloric diets were then standardized across groups beginning with dinner the evening before the experimental exercise session, and ending with dinner 48-h post exercise. Volunteers were instructed to maintain activity levels reported at baseline for the first 5 days of the lead-in phase. All resistive and endurance-type physical activity was then prohibited from 48-h before the experimental exercise session until after the 72-h data collection period was completed.

Experimental load carriage and cycle ergometry

Volunteers performed LC by walking on a treadmill while wearing a weighted vest equivalent to 30% of baseline body mass. A Lode (BV, Netherlands) ergometer was used for the CE exercise bouts. Baseline VO_{2peak} and associated heart rates at maximal and submaximal levels were used

to establish a target exercise intensity of 2.4 L min^{-1} for both the LC and CE trials. Absolute exercise intensity was matched between LC and CE by adjusting the speed and grade for LC and power for CE. By matching the intensity and, ultimately, energy cost, the effects of possible differences in mechanical force and contractile properties of LC and CE from the relative intensity and energy cost of the exercise bout were isolated. A 90-min familiarization trial was conducted at least 1 week before the experimental session to ensure the accuracy of the exercise prescription and the ability of the volunteer to complete the prescribed exercise bout. Heart rate was monitored continuously, and indirect calorimetry (ParvoMedics, Sandy, UT) was used to verify exercise intensity (15-min intervals). Workloads were adjusted to maintain the desired exercise intensity.

The experimental LC and CE sessions were conducted in the morning following a 12-h fast. Volunteers began the 90 min intensity-matched LC or CE exercise bout after baseline blood sampling. Exercise intensity was verified (and adjusted accordingly) every 30-min and adjustments were made based on indirect calorimetry. Overall intensity was not different between groups: oxygen uptake was 2.2 ± 0.1 L min^{-1}, energy expenditure was 1000 ± 60 kcal•90-min^{-1} and average load carried for LC groups was 24 ± 3 kg. Volunteers consumed equal volumes (500 mL total, 125 mL per serving) of either the SUPP or flavor-matched, nonnutritive CON drinks in 30-min intervals, beginning at the start of the exercise session and ending after completing the 90-min bout. Study staff and volunteers were blinded and supplements were prepared and coded by an independent third party (Combat Feeding Directorate, US Army Natick Soldier Systems Center, Natick, MA) to eliminate bias. Postexercise (PE) and recovery blood samples were obtained 3-h, 24-h, 48-h, and 72-h to assess temporal changes in study outcomes.

Study 2: volunteers and experimental design

Seventy-three Norwegian Soldiers (71 males and 2 females) stationed in Skjold, NO participated in this randomized controlled study after providing informed, written consent (Margolis et al. 2016). Volunteers were scheduled to participate in a 4-day winter military training program as part of their routine training in January of 2015. This study was approved by the Human Use Review Committee at the US Army Research Institute of Environmental Medicine (Natick, MA, USA) and the Regional Committees for Medical and Health Research Ethics (REK sør-øst, Oslo, NO). This trial was registered at www.clinicaltrials.gov as NCT02327208.

Volunteers were block randomized by body mass to one of three dietary treatment groups for the duration of the training program: three Norwegian arctic combat rations alone (control, CON), three rations plus four whey protein-based snack products (PRO), and three rations plus four carbohydrate-based snack products (CHO). The total number of rations provided was based on local Norwegian Army command policy for this specific training program. Volunteers began consuming the daily allotment of three Norwegian arctic combat rations (provided by study staff) 2 days before the start of the training program. Three combat rations provided 3487 kcal day^{-1}, 141 g day^{-1} protein, 435 g day^{-1} carbohydrate, and 126 g day^{-1} fat if consumed in their entirety. Volunteers assigned to PRO and CHO were provided four snack bars beginning on day one of the training program and were instructed to consume the bars either between meals, after a prolonged period of cross-country skiing, or before bed. Four PRO snack products provided approximately 1062 kcal day^{-1}, 85 g day^{-1} whey protein, 102 g day^{-1} carbohydrate, and 35 g day^{-1} fat, whereas four CHO products provided approximately 1058 kcal day^{-1}, 11 g day^{-1} whey protein, 189 g day^{-1} carbohydrate, and 29 g day^{-1} fat, if consumed in their entirety (Margolis et al. 2016). The snack bars were manufactured by the Combat Feeding Directorate (Natick, MA) and designed to be isocaloric and similar in serving size, taste, and textural qualities to blind volunteers and study staff (only on treatment groups) to eliminate bias. No additional food or dietary supplements were permitted for any group and volunteers were instructed to only eat the bars and rations they received and not trade or share the bars with other Soldiers. Fasted blood samples (0500–0700 h) obtained before (PRE) and within 8 h of completing the 4-day training program (POST) were used to assess CK, IL-6, hepcidin, and ferritin (Fig. 1B).

4-day winter training program and experimental dietary intake

The training program consisted of sustained periods of low-to-moderate intensity physical activity, primarily LC (51 km cross-country skiing), with intermittent periods of rest and more intense activity. Volunteers carried approximately 45 kg of gear in a weighted pack while skiing during the 4-day program and were encouraged to maintain hydration by drinking fluids ad libitum.

Energy and macronutrient intake were determined using ration-specific food logs. Volunteers were provided with cards containing a list of all the items in the provided rations, and were trained to record the percentage of each item and number of snack bars consumed. Food logs were collected daily, with trained Registered

Dietitians verifying items consumed with each participant. The amount of each ration item consumed was subtracted from the known initial amount to calculate energy, protein, carbohydrate, and fat intake. These data were used in combination with doubly labeled water (DLW) estimates of energy expenditure to calculate energy balance. In brief, energy expenditure was assessed in a subset of volunteers ($n = 14$ per group) using DLW (0.23 g of $H_2{}^{18}O$ per total body water (TBW) (kg) and 0.15 g of ^2H_2O per TBW (kg); Sigma-Aldrich, St. Louis, MO). Volunteers were consumed the DLW at baseline, daily urine samples (morning void) were collected to determine isotopic elimination rates during the training exercise, and used to calculate energy expenditure (Schoeller et al. 1986). To account for the influence of fat-free mass on energy expenditure, regression modeling, with fat-free mass (measured by skinfold) as a covariate, was conducted. The predictive equation generated [kcal day^{-1} = 1291 + (69.1 × fat-free mass)] was also used to estimate energy expenditure for the study volunteers not dosed with DLW (Redman et al. 2014; Margolis et al. 2016). Dietary intake, energy expenditure, and resulting energy balance have been reported previously (Margolis et al. 2016), but are included in this manuscript in order to explore associations between diet and markers of inflammation, muscle damage, and iron status.

Biological analyses

Blood samples, with the exception of 3-h post exercise (PE) for study 1, were collected after an overnight fast by antecubital venipuncture (Vacutainer; Becton Dickson, Franklin Lakes, NJ). Serum was isolated, frozen, and shipped on dry ice to the Pennington Biomedical Research Center (Baton Rouge, LA) for analysis of CK (Beckman Coulter DXC 600 Pro, Beckman Coulter, Brea CA), IL-6 (Milliplex MAP; Millipore, Billerica, MA), hepcidin (DRG International, Inc, Springfield, NJ) and ferritin (Siemens Medical Solutions USA Inc, Malvern, PA).

Statistical analyses

One-way ANOVA was used to confirm homogeneity of groups within each study. All variables were examined for normality. Variables exhibiting nonnormal distributions were transformed for analysis. Mixed model repeated measures ANOVA was used to determine main and interactive effects of exercise mode (LC vs. CE), drink (EAA vs. CON), and time (baseline, 3-h, 24-h, 48-h, and 72-h post exercise) for study 1. Akaike's information criterion was used to determine the appropriate covariance model (Burnham and Anderson 2002). Repeated measures ANOVA was performed to assess dietary treatment (CON, CHO, and PRO) and time (PRE and POST) for study 2. Bonferroni adjustments were used for post hoc comparisons if interactions were observed. Associations between IL-6, hepcidin, ferritin, and the change from PRE and POST were determined using Pearson correlation coefficients for the complete dataset. Pearson correlation coefficients were also determined to assess relationships between dietary intake (carbohydrate, protein, and fat), energy expenditure, and energy balance with IL-6, hepcidin, ferritin, and the change from PRE and POST. Significance was $P < 0.05$ and data were analyzed using SPSS (Version 21.0, 2010, SPSS Inc, Chicago, IL) and expressed as means ± SD.

Results

Study 1

Baseline volunteer characteristics were not different between dietary treatment groups (Table 1) (Pasiakos et al. 2015). Dietary intake during the recovery phase was similar between dietary treatment groups and averaged 2788 ± 239 kcal day^{-1}, 112 ± 14 g day^{-1} protein, 373 ± 28 g day^{-1} carbohydrate, and 97 ± 10 g day^{-1} fat (Table 2).

Table 1. Volunteer characteristics for study 1 and 2

	Age (years)	Height (cm)	Body mass (kg)	BMI (kg m^{-2})	Peak VO_2 mL kg^{-1}min^{-1}
Study 1					
LC-CON	24 ± 5	177 ± 8	77 ± 10	25 ± 3	51 ± 5
LC-SUPP	22 ± 3	178 ± 5	81 ± 10	25 ± 3	51 ± 4
CE-CON	22 ± 4	175 ± 8	78 ± 11	25 ± 2	50 ± 4
CE-SUPP	22 ± 2	177 ± 7	84 ± 10	26 ± 2	49 ± 4
Study 2					
CON	19 ± 2	182 ± 7	77 ± 6	23 ± 2	–
CHO	20 ± 1	180 ± 6	78 ± 9	24 ± 2	–
PRO	20 ± 1	184 ± 7	78 ± 9	23 ± 2	–

Data are means ± SD. $N = 10$ per group for LC-CON (load carriage + nonnutritive control), LC-SUPP (load carriage + essential amino acid and carbohydrate supplement), CE-CON (cycle ergometry + nonnutritive control), and CE-SUPP (cycle ergometry+ essential amino acid and carbohydrate supplement). $N = 18$ for CON (control, 3 rations only), 27 for CHO (3 rations + 4 carbohydrate-based snacks), and 28 for PRO (3 rations + 4 protein-based snacks). Peak VO_2 (aerobic capacity assessed for experiment 1 only using indirect calorimetry, ParvoMedics, Sandy, UT). Data were analyzed for homogeneity using a one-way ANOVA. No differences were observed between groups.
BMI, body mass index; CE, cycle ergometry.

Table 2. Energy and macronutrient intake for the 72-h recovery period in study 1 and 4-d arctic military training operation in study 2

	Energy kcal day^{-1}	Carbohydrate g day^{-1}	Protein g day^{-1}	Fat g day^{-1}
Study 1				
LC-CON	2769 ± 298	373 ± 39	108 ± 15	97 ± 12
LC-SUPP	2804 ± 201	376 ± 20	114 ± 12	96 ± 10
CE-CON	2714 ± 230	361 ± 28	109 ± 16	97 ± 10
CE-SUPP	2858 ± 231	382 ± 26	118 ± 14	99 ± 10
Study 2				
CON	2506 ± 410^{ac}	312 ± 47^{a}	100 ± 15^{b}	91 ± 20^{a}
CHO	3131 ± 632^{b}	434 ± 86^{b}	98 ± 22^{b}	107 ± 24^{a}
PRO	2824 ± 599^{ab}	321 ± 77^{a}	148 ± 25^{a}	102 ± 23^{a}

[1]Data are means ± SD. $N = 10$ per group for LC-CON load carriage + nonnutritive control), LC-SUPP (load carriage + essential amino acid and carbohydrate supplement), CE-CON (cycle ergometry + nonnutritive control), and CE-SUPP (cycle ergometry+ essential amino acid and carbohydrate supplement). $N = 18$ for CON (control, 3 rations only), 27 for CHO (3 rations + 4 carbohydrate-based snacks), and 28 for PRO (3 rations + 4 protein-based snacks). A one-way ANOVA was used to determine differences in dietary intake across groups for each study. Data within a column not sharing the same superscript are different, $P < 0.05$.

Regardless of dietary treatment, CK concentrations increased in response to both LC and CE, and peaked 24-h PE, without returning to baseline levels during the study period (main effect of time, $P < 0.05$, Fig. 2A). Dietary treatment did not affect IL-6 responses to LC or CE; IL-6 concentrations were elevated 3-h and 24-h PE following CE, but not statistically changed following LC (mode × time, $P < 0.05$, Fig. 2B). Hepcidin was elevated at 3-h and 24-h PE following both LC and CE, independent of dietary treatment, and returned to baseline levels by 48-h (time main effect, $P < 0.05$, Fig. 2C). Ferritin concentrations were elevated 24-h PE and remained elevated throughout the 72-h PE study period, independent of dietary treatment or exercise mode (main effect of time, $P < 0.05$, Fig. 2D).

Study 2

Although sample size differed between the PRO ($n = 28$) and CHO ($n = 27$) compared to the CON ($n = 18$), volunteer characteristics across the three dietary treatment groups were not different at the start of the study period (Table 1). As intended and previously reported (Margolis et al. 2016), energy and macronutrient intake during the training exercise differed across experimental and control groups (Table 2). Energy intake in the CHO group was greater ($P < 0.05$) than CON and similar to PRO. Carbohydrate intake was greater ($P < 0.05$) in CHO compared to PRO and CON. Protein intake was greater ($P < 0.05$) in the PRO group compared to CHO and CON. Total energy expenditure was similar across groups and averaged 6155 ± 515 kcal day^{-1}, resulting in a mean loss of 2.7 ± 1.2 kg body mass and an energy deficit of 55%; the reductions in body weight and energy balance were not different across groups (Margolis et al. 2016).

Circulating CK was nearly six times greater (main effect of time, $P < 0.05$) POST versus PRE (Fig. 3A). IL-6 was greater (main effect of time, $P < 0.05$, Fig. 3B) POST versus PRE and hepcidin was greater (main effect of time, $P < 0.05$, Fig. 3C) POST versus PRE. Similarly, ferritin increased (time main effect, $P < 0.05$) with training (Fig. 3D). Dietary treatment did not significantly impact CK, IL-6, hepcidin, or ferritin responses to training.

Hepcidin concentrations after the 4-d training operation were associated ($P < 0.05$) with total daily energy expenditure ($r = 0.40$), energy intake ($r = -0.26$) and resulting energy balance ($r = -0.43$) (Fig. 4A–C). There were no significant associations between IL-6, hepcidin, and ferritin, or between changes in these variables from PRE to POST. There were no significant associations between carbohydrate and protein intake with IL-6, hepcidin, and the change in these variables during training.

Discussion

The inflammatory response to physical activity has been characterized in civilian (Roecker et al. 2005) and military populations (McClung et al. 2013), and may result in poor iron status by increasing hepatic expression of hepcidin (McClung et al. 2009b). The objective of this study was to clarify the impact of exercise mode and to explore the efficacy of energy and macronutrient interventions for attenuating the inflammatory responses to physical activities of relevance to the military. In sum, intensity-matched LC and traditional, nonweight-bearing CE caused comparable changes in IL-6, hepcidin and ferritin over a 72-h period. Consuming a combined carbohydrate and protein (essential amino acids) supplement during the exercise bout did not attenuate inflammation, and macronutrient manipulation did not affect their responses to multiday military training. However, the increase in hepcidin levels postmilitary training was positively associated with energy expenditure and negatively associated with energy balance. These data reinforce that the hepcidin response to highly demanding military training are, in part, dependent on nutritional status, in particular, energy status (McClung et al. 2009b, 2013; Yanovich et al. 2015).

The unaccustomed physical activities performed during military training, including sustained low-to-moderate intensity LC exercise (McClung et al. 2013; Nindl et al.

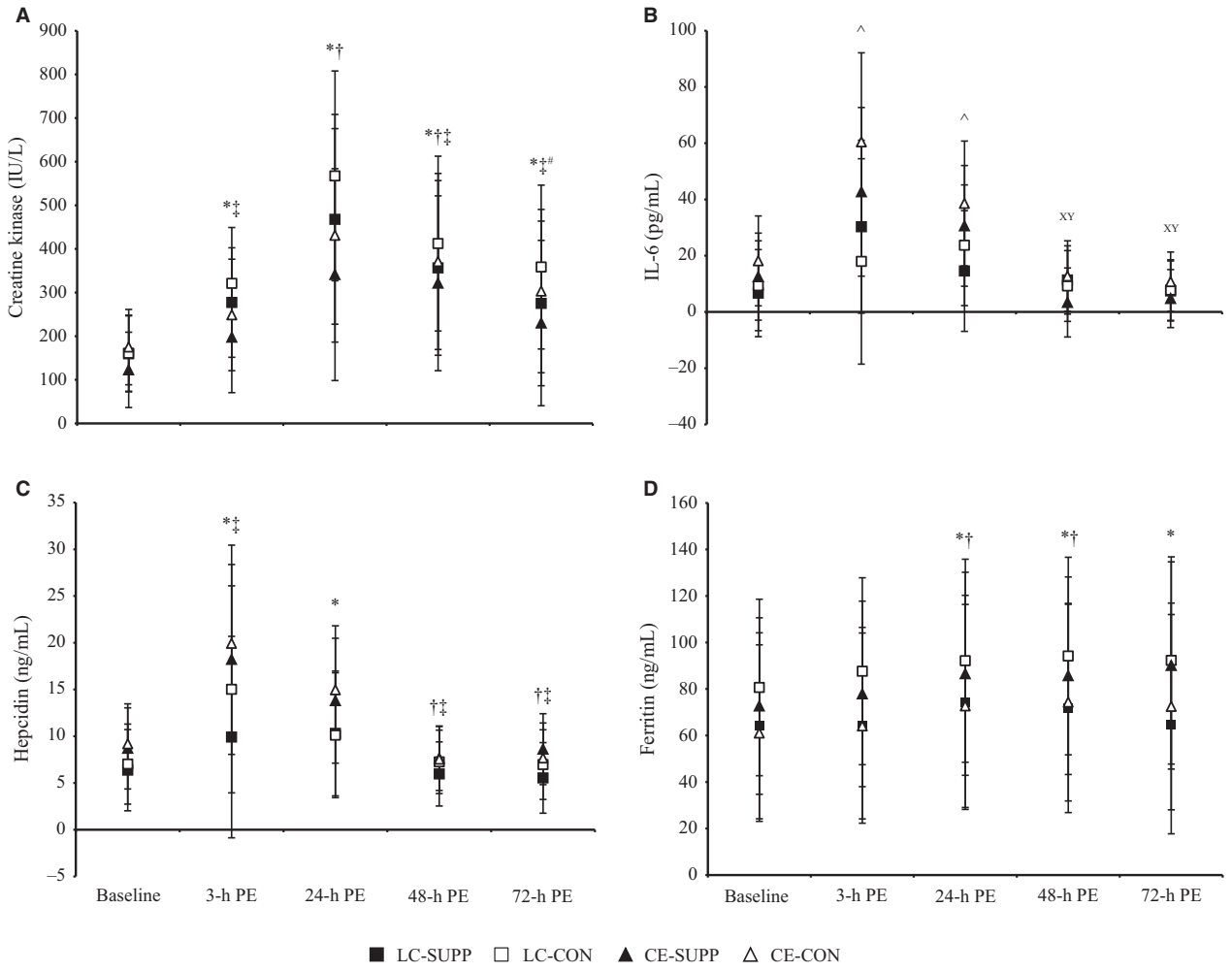

Figure 2. Inflammatory responses to load carriage and cycle ergometry exercise, with or without combined essential amino acid and carbohydrate supplementation. Data are mean ± SD, n = 10 per group. Repeated measures ANOVA was used to determine time, exercise mode, and dietary treatment effects and their interactions. Overall mean different from baseline*, 3-h PE†, 24-h PE‡, and 48-h PE#, main effects of time, $P < 0.05$. Overall mean for CE 3-h and 24-h PE^ different than baseline and overall mean for CE 48-h and 72-h different from CE 24-h PE^{xy}, mode-by-time, $P < 0.05$. PE, postexercise, CE, conventional endurance exercise; LC, load carriage; CON, control; and SUPP, essential amino acid + carbohydrate supplement.

2013), may contribute to long-term decrements in iron status (Karl et al. 2010; McClung et al. 2009a, 2009b; Yanovich et al. 2015). Like others (Nieman et al. 1998), we suspected that the eccentric forces generated during LC (i.e., weight-bearing exercise) would exceed CE, and produce more muscle damage and greater increases in IL-6 and hepcidin. However, with few exceptions, the effects of LC and CE exercise on CK, IL-6 and hepcidin were not different. We suspect that the variability in IL-6 contributed to our inability to detect a statistical increase in IL-6 after LC, because numerically, the magnitude of increase in IL-6 after LC was similar to the increase observed after CE. These data indicate that carrying additional weight during low-to-moderate intensity endurance exercise was not quantitatively more damaging and did not cause more inflammation than nonweight-bearing, traditional endurance exercise when the absolute intensity and work performed during the 90-min sessions were matched. These findings are supported by others (Ostrowski et al. 2000; Sim et al. 2013; Toft et al. 2002), including Sim et al. (2013), who demonstrated that increases in IL-6 and hepcidin levels 3-h after completing continuous low (45 min at 65% VO_{2peak}) or intermittent, high intensity (8 × 3-min intervals at 85% VO_{2peak}) running and CE were similar across modalities and intensities. Studies comparing longer duration (2.5-h), higher intensity (75% VO_{2max}) treadmill running and CE exercise bouts have reported similar findings (Nieman et al.

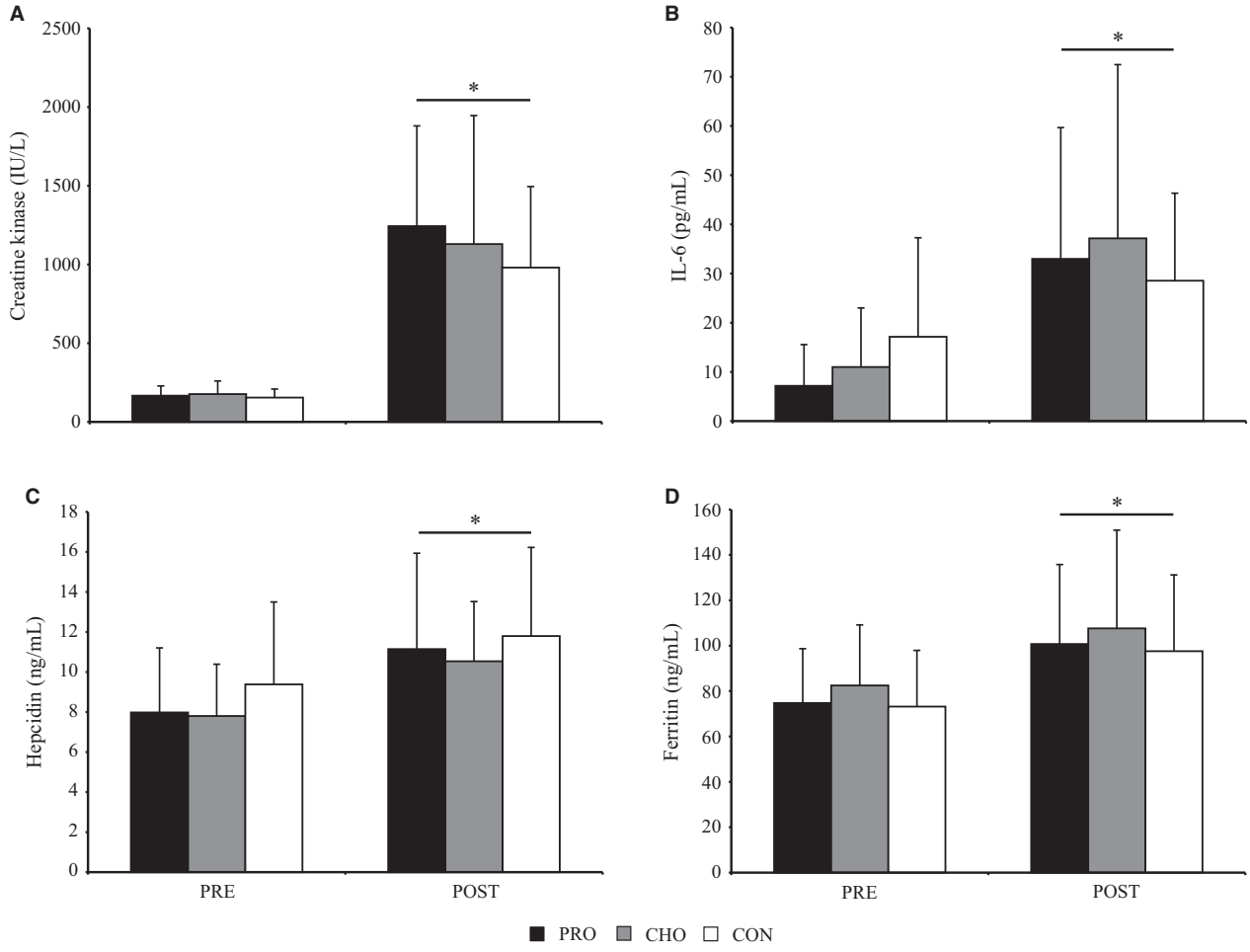

Figure 3. Effects of carbohydrate and protein supplementation on inflammation and associated outcomes before (PRE) and after (POST) a 4-day arctic military training operation. Data are mean ± SD for CON (control, $n = 18$), CHO (carbohydrate, $n = 27$), and PRO (protein, $n = 28$) dietary groups (G). A repeated measures ANOVA was used to determine time main effects and time by group interactions. Overall mean for postarctic military training (POST) different than baseline (PRE)*, $P < 0.0001$. CK, creatine kinase, and IL-6, interleukin-6.

1998). Our data confirm the transient effects of low-to-moderate intensity endurance exercise on IL-6 and hepcidin, and indicate that when the absolute intensities are matched, exercise mode may not augment the inflammatory and hepcidin response to endurance exercise.

Low carbohydrate availability and muscle glycogen depletion upregulate intramuscular gene expression (Keller et al. 2001; Steensberg et al. 2001) and circulating levels of IL-6 (Steensberg et al. 2000). The relationship between carbohydrate availability and muscle glycogen provided the basis for studies attempting to attenuate postendurance exercise inflammation using carbohydrate supplementation alone (Nieman et al. 1998; Cox et al. 2008; Kerasioti et al. 2013; Sim et al. 2012; Badenhorst et al. 2015) or combined with protein (Kerasioti et al. 2013; Nelson et al. 2013; Rowlands et al. 2008). In this study, a combined carbohydrate (46 g) and essential amino acid (10 g) supplement was provided during the 90-min LC and CE exercise bouts, and although the intervention was originally designed to maximize muscle recovery (Pasiakos et al. 2015), the levels of carbohydrate and protein provided were similar to other studies specifically designed to assess inflammation (Nelson et al. 2013; Sim et al. 2012). Sim et al. (2012) provided approximately 51 g of carbohydrate in a 6% solution at a dose of 3 mL kg^{-1} every 20-min during a 90-min treadmill run (75% VO_{2peak}) and found no differences in IL-6 and hepcidin 3-h and 24-h post exercise compared to a placebo. Nelson et al. (2013) compared a leucine-enriched protein (7.5 and 20 g), carbohydrate (89 g) and fat (22 g) supplement to an isocaloric carbohydrate (119 g) and fat (22 g) supplement provided once every 3-h after intense CE for six consecutive days and found no benefit of either supplement on postexercise cytokine expression. The lack of

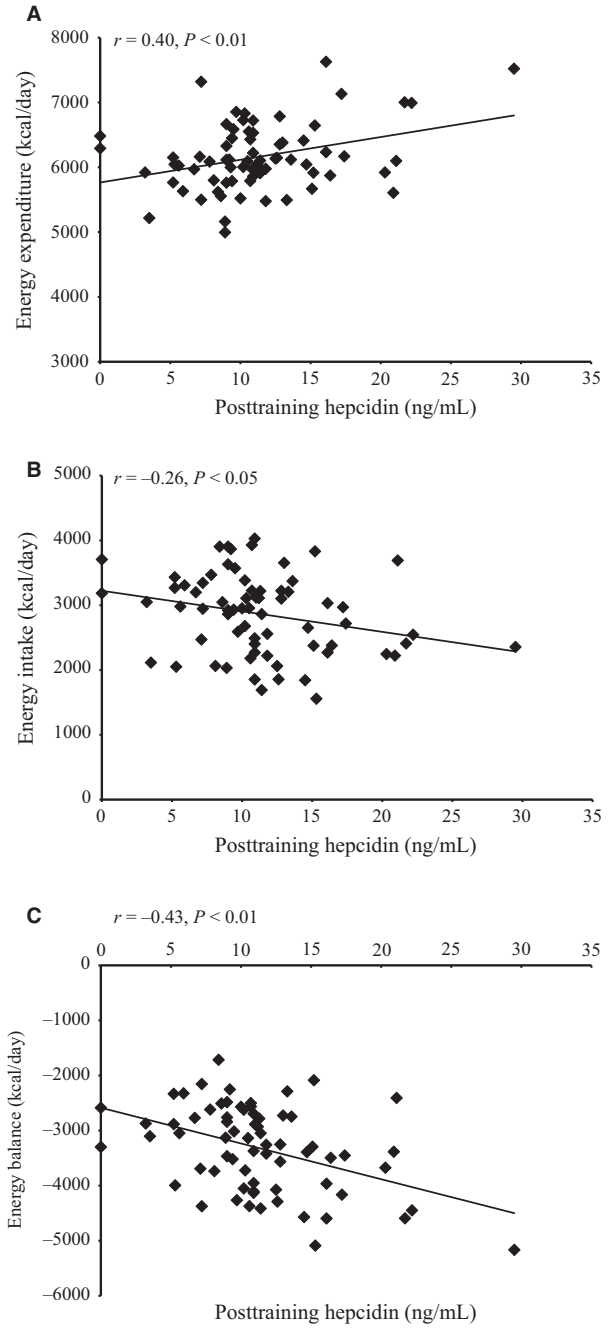

Figure 4. Relationships between energy expenditure (A), energy intake (B), energy balance (C), and hepcidin concentrations after (POST) completing a 4-day arctic military training operation using Pearson correlation coefficients.

any measureable anti-inflammatory benefit in this study conflicts with our hypothesis, and although we provided similar or lower amounts of carbohydrate and protein compared to previous studies (Sim et al. 2012; Nelson et al. 2013), we expected inflammation and subsequent hepcidin release to be greater for LC, providing an inflammatory state that may be sensitive to the anti-inflammatory potential of carbohydrate and protein. However, postexercise and recovery measures of inflammation and hepcidin were similar between the intensity-matched modes of exercise, an effect likely attributed to the highly controlled period of energy balance in which studies were conducted.

In study 2, the 4-day military training comprised of daily, sustained LC exercise produced high energy expenditure, severe energy deficit, muscle damage, and increased IL-6, hepcidin, and ferritin concentrations. The extent to which these parameters changed during the 51-km ski march was similar to a previous winter training study (McClung et al. 2013; Margolis et al. 2014). We hypothesized that the level of the energy deficit produced during training would diminish endogenous carbohydrate availability and exacerbate the inflammatory response (Cox et al. 2008). Consistent with our hypothesis, energy expenditure and negative energy balance explained a significant portion of the variance in hepcidin levels following the military training activity. As such, we expected that providing additional energy in the form of carbohydrate- and protein-based bars would effectively increase energy intake, spare endogenous carbohydrate availability, and attenuate increases in IL-6 and hepcidin. However, there were no differences in IL-6 and hepcidin between groups nor were associations observed between the types of macronutrient consumed and IL-6, hepcidin, and ferritin levels. However, there was an inverse relationship between the magnitude of energy imbalance and the hepcidin response to the ski march. Although others have assessed cytokine responses to dietary manipulations during military training (Kramer et al. 1997), no studies have directly assessed the potential anti-inflammatory effects of eating enough to preserve energy balance during short-term, metabolically demanding military training.

Experimental control and sample size are strengths of the present studies. Dietary intake and exercise intensities for LC and CE trials were highly controlled, and although the cross-sectional design may be considered a limitation, the control of diet- and exercise-related confounders diminish the negative effects of between group variability. Inability to fully quantify the volume and type of physical exercise performed during the 4-day ski march may also be a limitation. Finally, the designs of these studies do not allow us to characterize whether added energy intake would reduce the magnitude of inflammation.

In conclusion, these studies are the first to characterize the effects of a military endurance-type exercise mode (i.e., load carriage) performed once in a controlled setting and repeatedly during a strenuous, energy demanding,

real-world military training exercise. That findings are consistent with previous studies suggests that military endurance-type exercise is not, in and of itself, more inflammatory than metabolically matched conventional endurance exercise; independent of supplemental nutrition. Furthermore, consuming supplemental carbohydrate and protein during short-term training did not attenuate the inflammatory response when there was substantial energy deficit. That energy intake, expenditure, and balance were associated with elevations in hepcidin concentrations said the magnitude of hepcidin response was inversely related to energy deficit, suggesting that eating enough to more closely meet energy expenditure, might attenuate potential declines in iron status that result from repeated exposure to unaccustomed activities. Overall, these studies provide novel and practical information for future studies designed to improve military field feeding by manipulating policy and combat ration development to promote energy balance and attenuate the inflammatory response to military training.

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Conflict of Interest

The investigators adhered to the policies for protection of human subjects as prescribed in Army Regulation 70–25, and the research was conducted in adherence with the provisions of 32 CFR part 219. The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Army or the Department of Defense. Any citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement of approval of the products or services of these organizations.

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Effects of Supplemental Energy on Protein Balance during 4-d Arctic Military Training

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ABSTRACT

MARGOLIS, L. M., N. E. MURPHY, S. MARTINI, Y. GUNDERSEN, J. W. CASTELLANI, J. P. KARL, C. T. CARRIGAN, H.-K. TEIEN, E.-H. MADSLIEN, S. J. MONTAIN, and S. M. PASIAKOS. Effects of Supplemental Energy on Protein Balance during 4-d Arctic Military Training. *Med. Sci. Sports Exerc.*, Vol. 48, No. 8, pp. 1604–1612, 2016. Soldiers often experience negative energy balance during military operations that diminish whole-body protein retention, even when dietary protein is consumed within recommended levels (1.5–2.0 g·kg^{-1}·d^{-1}). **Purpose**: The objective of this study is to determine whether providing supplemental nutrition spares whole-body protein by attenuating the level of negative energy balance induced by military training and to assess whether protein balance is differentially influenced by the macronutrient source. **Methods**: Soldiers participating in 4-d arctic military training (AMT) (51-km ski march) were randomized to receive three combat rations (CON) (n = 18), three combat rations plus four 250-kcal protein-based bars (PRO, 20 g protein) (n = 28), or three combat rations plus four 250-kcal carbohydrate-based bars daily (CHO, 48 g carbohydrate) (n = 27). Energy expenditure ($D_2{}^{18}O$) and energy intake were measured daily. Nitrogen balance (NBAL) and protein turnover were determined at baseline (BL) and day 3 of AMT using 24-h urine and [^{15}N]-glycine. **Results**: Protein and carbohydrate intakes were highest ($P < 0.05$) for PRO (mean ± SD, 2.0 ± 0.3 g·kg^{-1}·d^{-1}) and CHO (5.8 ± 1.3 g·kg^{-1}·d^{-1}), but only CHO increased ($P < 0.05$) energy intake above CON. Energy expenditure (6155 ± 515 kcal·d^{-1}), energy balance (−3313 ± 776 kcal·d^{-1}), net protein balance (NET) (−0.24 ± 0.60 g·d^{-1}), and NBAL (−68.5 ± 94.6 mg·kg^{-1}·d^{-1}) during AMT were similar between groups. In the combined cohort, energy intake was associated ($P < 0.05$) with NET (r = 0.56) and NBAL (r = 0.69), and soldiers with the highest energy intake (3723 ± 359 kcal·d^{-1}, 2.11 ± 0.45 g protein·kg^{-1}·d^{-1}, 6.654 ± 1.16 g carbohydrate·kg^{-1}·d^{-1}) achieved net protein balance and NBAL during AMT. **Conclusion**: These data reinforce the importance of consuming sufficient energy during periods of high energy expenditure to mitigate the consequences of negative energy balance and attenuate whole-body protein loss. **Key Words:** ENERGY EXPENDITURE, MACRONUTRIENTS, ENERGY BALANCE, MILITARY, WHOLE-BODY PROTEIN TURNOVER

Energy balance, the nutritional state when energy intake matches energy expenditure, is a primary determinant of whole-body protein balance (5). Periods when energy expenditure exceed energy intake produce a state of negative energy balance. The level of negative energy balance may be detrimental to skeletal muscle mass because proteins are degraded to provide amino acids that can be used as substrates for oxidation and gluconeogenesis, resulting in increased nitrogen excretion and negative protein balance (3,4,35). Achieving energy balance during periods of high energy expenditures can be challenging, particularly in situations where meals are served at discrete times and/or eating is secondary to the activity (30). Increasing protein intake during periods of modest negative energy balance is an effective nutritional countermeasure to attenuate protein loss and protect muscle mass (1,17). The benefits of high-protein diets are largely independent of body size, because consuming twice and three times the recommended dietary allowance for protein (0.8 g·kg^{-1}·d^{-1}) equally preserves protein balance and muscle mass in normal weight individuals exposed to sustained, moderate negative energy balance (21 d, 40% energy deficit; percentage of energy required to achieve energy balance based on the difference between energy expenditure and energy intake) (21).

Military personnel commonly experience negative energy balance during training and combat operations. Negative energy balance is largely driven by sustained periods of low-to-moderate physical activity that result in daily energy expenditures that are difficult to match with energy intake,

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because food supply and time availability to eat or prepare a meal are often limited (16,19). As such, protein recommendations for these scenarios range from 1.5 to 2.0 $g \cdot kg^{-1} \cdot d^{-1}$ (23). Whether current military operational protein recommendations are actually sufficient, particularly if degree of negative energy balance is severe (>40% energy deficit), is not clear. Evidence justifying the recommendations of higher protein diets during military operations was generated from controlled feeding studies that used energy deficits that were generally ≤40% of the energy needed to match expenditure (21,26). Energy deficits during "real-world" military operations may exceed 40% of the energy needed to achieve energy balance. For example, energy expenditures of approximately 6800 $kcal \cdot d^{-1}$ were recently observed in Norwegian Soldiers participating in a 3-d arctic military training (AMT) exercise. Despite being provided nearly 5100 $kcal \cdot d^{-1}$, the soldiers only consumed 50% (3400 $kcal \cdot d^{-1}$) of the energy expended (15). Interestingly, dietary protein intake was within current recommendations (1.7 $g \cdot kg^{-1} \cdot d^{-1}$), but whole-body protein balance decreased during the training operation, suggesting that protein intake under these conditions should have been higher. The possibility exists that attenuating negative energy balance to more manageable energy deficits (<40%) by consuming more carbohydrate, a critical fuel source during sustained operations, could reduce the reliance on endogenous protein and spare whole-body protein balance to a similar extent as increasing protein intake.

The objective of this study was to determine whether supplementing standard combat rations with protein- or carbohydrate-based snack bars would sufficiently increase total energy intake and attenuate negative energy balance during short-term challenging military training, such that whole-body protein balance is maintained when compared with consuming combat rations alone. We hypothesized that calorically equivalent protein- and carbohydrate-based supplemental nutrition would similarly diminish the level of negative energy balance by increasing total energy intake, but that whole-body protein balance would be better maintained when protein is the primary form of supplemental nutrition.

MATERIALS AND METHODS

Participants/experimental design. Norwegian army soldiers stationed in Skjold, Norway, participating in a 4-d AMT operation, were recruited to participate in this randomized controlled trial. After providing informed, written consent, participants ($n = 71$ males, $n = 2$ females) were block randomized by body mass to one of three dietary treatment groups, each receiving three Norwegian arctic combat rations, either alone (control, CON) or supplemented with four whey protein-based (PRO) or four carbohydrate-based (CHO) snack products. Baseline (BL) (study days −1 and 0) whole-body protein turnover, nitrogen balance (NBAL), and body mass and composition were measured before starting the 4-d AMT (see Figure, Supplemental Digital Content 1, study design, http://links.lww.com/MSS/A677). Beginning on day 1, participants performed cross-country skiing in 50/10-min work-to-rest ratios while carrying an approximately 45-kg pack for a total distance of approximately 13 $km \cdot d^{-1}$. The total distance covered was about 51 km. Energy expenditure and energy and macronutrient intake were assessed daily. Whole-body protein turnover and NBAL were reassessed on day 3 of AMT, whereas change in body mass was determined at the conclusion of training (POST) (day 5).

This study was approved by the Institutional Review Board of the US Army Research Institute of Environmental Medicine (Natick, MA) and the Regional Committees for Medical and Health Research Ethics (REK sør-øst, Oslo, Norway; www.clinicaltrials.gov NCT02327208). It is also important to note that the 4-d AMT was not developed for this study; rather, the exercise is an annual mandatory training operation for the second battalion stationed at the garrison in Skjold. Our study did not alter the training exercise, which was conducted in January 2015.

Anthropometrics and body composition. Vertical height was measured at BL to the nearest 0.1 cm using a stadiometer (Seca; Creative Health Products, Plymouth, MI). Seminude (underwear only) body mass was measured using a calibrated digital scale (Befour model PS6600; Befour Inc., Saukville, WI) at BL and POST to the nearest 0.1 kg. Body composition was determined at baseline from skinfold thickness measurements of the chest, triceps, and subscapular for men, and the triceps, suprailiac, and abdomen for women, by a trained technician using Lange calipers (Beta Technology, Santa Cruz, CA) (12,13). Body composition data were used to characterize participants at BL as well as used for estimates of resting metabolic rate (RMR) and in regression modeling, described below, to correct for influence of fat-free mass (FFM) on energy expenditure. This predictive model was then used to estimate daily energy expenditure for the entire study cohort.

Energy intake, expenditure, and components of energy balance. Beginning on day −1 and through the duration of the study, participants were provided three Norwegian arctic combat rations to consume daily as per local command policy. No outside food was permitted for the study participants. Three combat rations provided approximately 3487 $kcal \cdot d^{-1}$, 141 $g \cdot d^{-1}$ protein, 435 $g \cdot d^{-1}$ carbohydrate, and 126 $g \cdot d^{-1}$ fat if entirely consumed. Beginning on day 1 of the AMT, participants assigned to PRO and CHO were provided with four snack bars per day to be consumed in addition to the provided rations. The four whey-based PRO bars provided 1062 $kcal \cdot d^{-1}$, 85 $g \cdot d^{-1}$ protein, 102 $g \cdot d^{-1}$ carbohydrate, and 35 $g \cdot d^{-1}$ fat, whereas four CHO bars provided 1058 $kcal \cdot d^{-1}$, 11 $g \cdot d^{-1}$ protein, 189 $g \cdot d^{-1}$ carbohydrate, and 29 $g \cdot d^{-1}$ fat. The snack bars were manufactured by the Combat Feeding Directorate at the Natick Soldier Systems Center (Natick, MA) and designed to be isocaloric and similar in serving size, taste, and textural qualities.

The primary objective and practical application of this investigation was to determine whether the incorporation of

supplement nutrition in the form of protein- or carbohydrate-based snack bars would augment field feeding practices during real-world military training operations by increasing total energy intake, thereby attenuating the negative energy balance caused by high energy expenditures. There were no specific requirements for participants to consume all ration items and snack bars provided to them; rather, participants were instructed to consume the three combat rations as they normally would during training and to consume snack bars between meals. Participants were instructed to only eat the bars they received and not trade or share bars with other soldiers participating in AMT.

To determine the amount of ration components and snack bars consumed daily, participants were provided with food logs that contained a list of all the items for each provided ration. Before AMT, participants were trained to record the percent of each item consumed using the provided logs. This technique has been used effectively by our laboratory in previous investigations to characterize combat ration intake during military operations (2,15,16). Food logs also included a 10-cm visual analog scale asking participants to rate their level of hunger, with 0 cm being "not hungry at all" and 10 cm being "very hungry." Food logs were collected daily, with trained registered dietitians verifying items consumed with each participant. The amount of each ration item consumed was subtracted from the known initial amount provided to calculate energy, protein, carbohydrate, and fat intake. Nutritional composition of all combat ration items included in this study was confirmed by chemical analysis to verify the accuracy of our dietary analyses (Covance Laboratories, Inc., Madison, WI).

Energy expenditure was assessed in a subset of participants using the doubly labeled water (DLW) technique. After an overnight fast and before dosing of DLW, a subset of participants (n = 14 PRO, n = 14 CHO, n = 14 CON) provided a baseline urine sample to correct for background abundance of ^{18}O and ^{2}H. After ingestion of DLW (0.23 g of $H_2{}^{18}O$ per total body water (TBW) (kg) and 0.15 g of $^{2}H_2O$ per TBW (kg); Sigma-Aldrich, St. Louis, MO), participants fasted for 4 h to minimize disturbance of isotopic enrichment. At 4 and 6 h postdose, participants provided urine samples to determine peak enrichment. On subsequent study days, daily morning voids were collected for determination of isotopic elimination rates, calculated by linear regression from the rate of disappearance in the urine over AMT. Enrichments of ^{2}H and ^{18}O were assessed using isotope ratio mass spectrometry (Finnigan Mat 252; Thermo Fisher Scientific, Waltham, MA). Determination of CO_2 production to calculate energy expenditure was determined according to Schoeller et al. (29):

$$rCO_2\left(\text{mol}\cdot\text{d}^{-1}\right) = (N/2.078)(1.01K_O - 1.04K_H) - 0.0246rH_2O_f$$

where N is TBW; K_O and K_H are ^{18}O and ^{2}H isotope disappearance rates, respectively; and rH_2O_f is the rate of fractionated evaporated water loss, estimated to be $10.5N\,(1.01K_O - 1.04K_H)$. Energy expenditure was calculated using the energy equivalent of CO_2 for a respiratory quotient of 0.86.

To account for ^{2}H and ^{18}O abundance in local drinking water and background shift during the ski march, three participants were dosed with a placebo (tap water) rather than DLW to serve as the control. Drinking water from the garrison and on the ski march was collected and analyzed to correct for background abundance.

To account for the influence of FFM on energy expenditure between groups, regression modeling, with FFM as a covariate, was conducted (27). The predictive equation generated was then used to determine estimated energy expenditure of participants not dosed with DLW. The equation was constructed from the constant and β of FFM determined by regression model.

$$\text{energy expenditure model}\left(\text{kcal}\cdot\text{d}^{-1}\right) = 1291 + (69.1\text{FFM})$$

Energy balance was calculated by subtracting mean energy intake from mean total daily energy expenditure during AMT. Furthermore, to determine the contribution of physical activity to total daily energy expenditure, RMR was estimated using baseline measures of FFM (6):

$$\text{RMR}\left(\text{kcal}\cdot\text{d}^{-1}\right) = 370 + (21.6\text{FFM})$$

Diet-induced thermogenesis was estimated as 10% total daily energy expenditure (38), with activity-induced energy expenditure calculated as total daily energy expenditure minus RMR and diet-induced thermogenesis (39). Physical activity level (PAL) was defined as the ratio of total daily energy expenditure to RMR (37).

Whole-body protein turnover. The end-product method was used to assess 24-h whole-body protein turnover at BL and during AMT to determine the influence of energy balance, as well as energy and macronutrient intake on whole-body protein kinetics between groups (PRO, CHO, and CON) and over time (36). This method has previously been shown to be effective in estimating whole-body protein turnover in free living participants (8). In addition, assessing total urinary nitrogen enrichment accounts for 80% to 85% of total nitrogen excretion, thereby providing a reasonable estimate of whole-body protein balance (11,32). Before ingestion of a single bolus (4 $\text{mg}\cdot\text{kg}^{-1}$) of [$^{15}N$]-glycine (Cambridge Isotope Laboratories, Andover, MA), participants provided a spot urine to correct for background isotope enrichment and to serve as the starting point of the 24-h urine collection time. The 24-h collection ended with the first void of the following morning. Urine containers from 12 participants were damaged or lost during AMT; as such, 61 total BL and AMT measures were included in the final analyses.

Total nitrogen enrichment was used to determine whole-body protein turnover to maintain consistency with our previous work (15) and to minimize any bias of enrichment

partitioning between the ammonia and the urea nitrogen pools (8). Enrichment of tracer to tracee (tr:T) for [^{15}N]-nitrogen was determined using isotope ratio mass spectrometry (Metabolic Solutions Inc., Nashua, NH). Whole-body protein flux (Q), protein synthesis (PS), protein breakdown (PN), and net protein balance (NET) were calculated as follows:

$$Q\ (\mathrm{gN{\cdot}kg^{-1}{\cdot}d^{-1}}) = d/\text{corrected tr:T}/24\ \times\ \text{body mass}$$

$$\mathrm{PS}\ (\mathrm{g{\cdot}kg^{-1}{\cdot}d^{-1}}) = Q - (E/24 \times \text{body mass})\ \times\ 6.25$$

$$\mathrm{PB}\ (\mathrm{g{\cdot}kg^{-1}{\cdot}d^{-1}}) = Q - (I/24\ \times\ \text{body mass})\ \times\ 6.25$$

$$\mathrm{NET}\ (\mathrm{g{\cdot}kg^{-1}{\cdot}d^{-1}})\ =\ \mathrm{PS} - \mathrm{PB}$$

where d is the [^{15}N] oral dose (g glycine × 0.1972); E is the 24-h urinary nitrogen excretion; I is the 24-h nitrogen intake; and 6.25 is the conversion of nitrogen to protein. All samples were run in duplicate. Quality control samples (ammonia sulfate) at three different enrichments were run at the beginning and end of the batch run. During the batch run, a quality control sample was run after every 10 samples to check performance of the instrument. ^{15}N measurements were reported against atmospheric nitrogen (^{15}N/^{14}N ratio of 0.0036765), with a delta range of 7 to 10 delta per mil at BL and 70 to 750 delta per mil for enriched urines at AMT.

NBAL. Nitrogen excretion and NBAL were determined from the 24-h urine collection used to assess whole-body protein turnover. Nitrogen content was determined from a single pooled 24-h urine sample using pyrochemiluminescence (Antek 9000; Antek Instruments, Houston, TX). NBAL was calculated as the difference of nitrogen intake minus urinary nitrogen excretion plus miscellaneous (estimated as 5 mg·kg^{-1}) and fecal (estimated as 2 mg·kg^{-1}) nitrogen losses (9). To confirm complete 24-h urine collections, urinary creatinine was measured using the Jaffe reaction (UniCel DXC 600 Pro; Beckman Coulter, Brea, CA). For incomplete 24-h urine collections during AMT (n = 20, due to weather and logistical constraints), baseline 24-h urinary creatinine was used to correct nitrogen excretion (31). To verify whether it was appropriate to include these data in our report, statistical analyses were performed and measures of NBAL and NET during AMT were not different between data sets (i.e., all urine samples vs urine samples excluding incomplete 24-h collections). Therefore, all 24-h urine collections and their resulting outcomes were included in the final analyses.

Statistical analysis. Participants were randomized 3/1 (intervention/control) to capture differences between PRO and CHO groups during AMT, which were likely to have smaller between-group differences compared with CON. Normality was confirmed using Shapiro–Wilk tests for dependent variables. One-way ANOVA was used to assess between-group (PRO, CHO, and CON) differences for baseline characteristics, energy expenditure, energy and macronutrient intake, and energy balance. Differences between energy expenditure and energy intake were determined using an independent t-test. A χ^2 test was conducted to assess between-group differences for categorical dietary intake data. Repeated-measures ANOVA was used to determine the main effects of time (BL and AMT or POST), group, and time–group interactions for body mass, hunger, and whole-body protein flux, synthesis, and breakdown. An ANCOVA was used to assess AMT NBAL and NET by group adjusting for BL NBAL and NET.

To further examine associations between dietary intake and NBAL and whole-body protein turnover, the full cohort was combined and exploratory analyses conducted irrespective of the diet group. Linear regression was used to examine associations between energy, protein, carbohydrate, and fat intakes (expressed as percentage energy intake), and NBAL, flux, PS, PB, and NET during AMT. Regression analysis showed that energy intake was a positive predictor of NBAL and NET during AMT. Associations between energy intake and NBAL and NET were further examined by separating energy intake into quartiles and expressed as a categorical variable. In this model, differences in energy expenditure, energy and macronutrient intake, energy balance, and change in body mass between quartiles were assessed using one-way ANOVA. An ANCOVA was used to assess AMT NBAL and NET by energy intake quartile, adjusting for BL NBAL and NET. Bonferroni adjustment was used for *post hoc* analysis for significant main effects. Data were analyzed using IBM SPSS Statistics for Windows (version 22.0; IBM Corp., Armonk, NY). Significance was set at $P < 0.05$, and data are presented as mean ± SD.

RESULTS

Participant characteristics. Although sample size differed between the PRO (n = 28) and the CHO (n = 27) compared with the CON (n = 18), there were no group differences for any baseline characteristic, particularly age (PRO, 20 ± 1; CHO, 20 ± 1; CON, 19 ± 2), weight (PRO, 77.8 ± 9.1; CHO, 78.2 ± 8.8; CON, 77.7 ± 6.6), and FFM (PRO, 71.4 ± 8.6; CHO, 69.7 ± 7.3; CON, 69.7 ± 7.9) (see Table, Supplemental Digital Content 2, participant descriptions, http://links.lww.com/MSS/A678).

Energy and macronutrient intake. During AMT, participants in PRO, CHO, and CON consumed 62% ± 13%, 69% ± 14%, and 72% ± 12%, respectively, of the total energy provided. The percentage of energy intake attributed to the energy provided in the combat rations was lower ($P < 0.05$) for PRO (56% ± 15%) than CON (72% ± 12%, Table 1). There was no difference in the percentage energy intake from the rations provided for CHO (64% ± 14%) compared with PRO and CON. Participants in the PRO group consumed less ($P < 0.05$) of all ration items compared with CON, expect for condiments, with the greatest difference observed for consumption of snacks, dried fruit, and bread (Table 2). Participants in the PRO and CHO groups consumed 82% ± 19% and 85% ± 21%, respectively, of the provided energy

TABLE 1. Energy and macronutrient intake during AMT.

	PRO	CHO	CON
Ration			
Energy (kcal·d^{-1})	1953 ± 518^{a} (1001–3099)	2227 ± 493^{a,b} (1387–2918)	2506 ± 409^{b,c} (1557–3111)
Protein (g·d^{-1})	78 ± 19^{a} (38–117)	88 ± 20^{a,b} (50–113)	100 ± 15^{b,c} (65–124)
Carbohydrate (g·d^{-1})	238 ± 70^{a} (87–401)	274 ± 62^{a,b} (163–368)	312 ± 47^{b,c} (193–385)
Fat (g·d^{-1})	73 ± 20^{a} (37–110)	82 ± 21^{a,b} (45–116)	91 ± 20^{b,c} (57–117)
Supplements			
Energy (kcal·d^{-1})	872 ± 202^{a} (259–1128)	904 ± 224^{a} (282–1140)	—
Protein (g·d^{-1})	70 ± 15^{a} (24–90)	10 ± 3^{b} (3–20)	—
Carbohydrate (g·d^{-1})	83 ± 20^{a} (21–108)	160 ± 40^{b} (48–204)	—
Fat (g·d^{-1})	29 ± 7^{a} (9–37)	25 ± 6^{b} (9–32)	—
Total (absolute)			
Energy (kcal·d^{-1})	2825 ± 599^{a,b} (1691–4029)	3131 ± 633^{a} (1843–3940)	2506 ± 410^{b,c} (1557–3111)
Protein (g·d^{-1})	148 ± 25^{a} (96–191)	98 ± 22^{b} (58–130)	100 ± 15^{b} (65–124)
Carbohydrate (g·d^{-1})	321 ± 77^{a} (171–490)	434 ± 86^{b} (253–543)	312 ± 47^{a} (193–385)
Fat (g·d^{-1})	102 ± 23^{a} (59–141)	107 ± 24^{a} (56–146)	91 ± 20^{a} (57–117)
Total (relative)			
Energy (kcal·kg^{-1}·d^{-1})	37.3 ± 7.8^{a,b} (22.2–50.2)	42.2 ± 9.9^{a} (23.9–57.6)	33.6 ± 6.2^{b,c} (19.1–43.2)
Protein (g·kg^{-1}·d^{-1})	2.0 ± 0.3^{a} (1.3–2.6)	1.3 ± 0.3^{b} (0.7–1.9)	1.3 ± 0.2^{b} (0.8–1.7)
Carbohydrate (g·kg^{-1}·d^{-1})	4.2 ± 1.0^{a} 3(2.2–6.1)	5.8 ± 1.3^{b} (3.3–8.0)	4.2 ± 0.7^{a} (2.4–5.3)
Fat (g·kg^{-1}·d^{-1})	1.3 ± 0.3^{a} (0.8–1.8)	1.4 ± 0.4^{a} (0.7–2.0)	1.2 ± 0.3^{a} (0.7–1.7)

The values are presented as mean ± SD and range (min–max).
Values not sharing the same superscript are different, $P < 0.05$.

from treatment snack bars, with no difference between groups (Table 1). Provision of protein and carbohydrate snack bars during AMT was successful in altering macronutrient intake between groups, but despite PRO and CHO receiving an additional approximately 1000 kcal·d^{-1}, only CHO achieved a significantly higher ($P < 0.05$) total daily energy intake compared with CON, with no difference between PRO and CHO, or PRO and CON. Mean hunger increased ($P < 0.05$) from 5.4 ± 1.7 at BL to 6.4 ± 1.3 during AMT, with no difference between groups.

Energy balance. Total daily energy expenditure of all participants during AMT was 6155 ± 515 kcal·d^{-1}, with no differences between groups (Table 3). Estimated RMR accounted for 31% (1890 ± 161 kcal·d^{-1}) of total daily energy expenditure, with thermic effect of feeding estimated to contribute 10% (615 ± 51 kcal·d^{-1}) of total daily energy expenditure; activity-induced energy expenditure accounted for the remaining 59% (3649 ± 302 kcal·d^{-1}) of total energy expenditure. High daily activity-induced energy expenditures resulted in a PAL of 3.3 ± 0.1 for all groups. There were no differences between dietary treatment groups for any energy expenditure-related variable.

TABLE 3. Energy balance and expenditure.

	PRO	CHO	CON
Energy balance (kcal·d^{-1})	−3402 ± 687	−3050 ± 888	−3595 ± 606
Energy expenditure (kcal·d^{-1})[a]	6167 ± 592	6181 ± 505	6096 ± 412
RMR (kcal·d^{-1})	1894 ± 185	1898 ± 158	1871 ± 129
Thermic effect of feeding (kcal·d^{-1})	617 ± 59	618 ± 50	610 ± 41
Activity-induced energy expenditure (kcal·d^{-1})	3657 ± 348	3665 ± 297	3615 ± 242
PAL	3.29 ± 0.03	3.29 ± 0.03	3.29 ± 0.02

The values are presented as mean ± SD.
Means were not different between groups, $P > 0.05$.
[a]Adjusted energy expenditure for FFM using regression modeling.

Energy expenditure exceeded ($P < 0.05$) energy intake similarly across dietary treatment groups, because the negative energy balance for CON was not different from the deficits observed for PRO and CHO, averaging 54% (3313 ± 776 kcal·d^{-1}) of the total energy requirements for all participants (Table 3). As a result, loss of body mass was similar for PRO (2.6 ± 1.2 kg), CHO (2.9 ± 1.3 kg), and CON (2.7 ± 1.1 kg).

Whole-body protein turnover and NBAL. For calculations of whole-body protein turnover and NBAL on day 3 of AMT, dietary protein and nitrogen intake were highest ($P < 0.05$) for PRO (155 ± 40 g·d^{-1}, 25 ± 6 g·d^{-1}) compared with CHO (101 ± 29 g·d^{-1}, 16 ± 5 g·d^{-1}) and CON (114 ± 22 g·d^{-1}, 18 ± 3 g·d^{-1}). Within groups, daily protein and nitrogen intakes were similar ($P > 0.05$). The AMT resulted in a downregulation ($P < 0.05$) of whole-body protein flux (−0.19 ± 0.35 gN·kg^{-1}·d^{-1}), PS (−1.62 ± 2.41 g·kg^{-1}·d^{-1}), and PB (−1.47 ± 2.49 g·kg^{-1}·d^{-1}) compared with baseline, regardless of the dietary treatment group (Fig. 1A–C). NET was negative during AMT for PRO (−0.15 ± 0.66 g·kg^{-1}·d^{-1}), CHO (−0.24 ± 0.64 g·kg^{-1}·d^{-1}), and CON (−0.38 ± 0.46 g·kg^{-1}·d^{-1}) with no differences between groups. Similarly, NBAL was negative (PRO, −55.4 ± 106.8; CHO, −70.0 ± 101.4; CON, −85.1 ± 66.5 mg·kg^{-1}·d^{-1}) during AMT, but not affected by the dietary treatment group.

Given that no treatment effects were observed and that energy and macronutrient intake varied considerably across groups, linear regression analysis was used to evaluate associations between energy, protein, carbohydrate, and fat intake on NBAL and NET. Energy intake was positively ($P < 0.05$) associated with NBAL ($r = 0.59$, $r^2 = 0.34$) and NET ($r = 0.56$, $r^2 = 0.32$), whereas energy balance was ($P < 0.05$) associated with NBAL ($r = 0.51$, $r^2 = 0.26$) and NET ($r = 0.50$, $r^2 = 0.25$). Because of the stronger association for energy intake, data were used to separate participants into quartiles (quartile 1,

TABLE 2. Percent ration items consumed (%).

	Energy (kcal·d^{-1})*	PRO	CHO	CON
Entreés	517 (450–543)	88^{a}	88^{a}	94^{b}
Snacks	152 (72–314)	51^{a}	62^{b}	74^{c}
Dried fruit	156 (93–157)	47^{a}	51^{a,b}	64^{b}
Candy	77 (5–261)	40^{a}	40^{a}	52^{b}
Bread	205 (N/A)‡	38^{a}	48^{a}	68^{b}
Spreads	47 (46–49)	28^{a}	29^{a}	33^{b}
Sides	132 (N/A)	18^{a}	18^{a}	29^{b}
Condiments	20 (N/A)	10^{a}	34^{b}	12^{a}
Powdered beverages	36 (4–130)	18^{a}	19^{a}	17^{b}

Values not sharing the same superscript are different, $P < 0.05$.
*Mean (range) energy content of rations component.
‡No range.

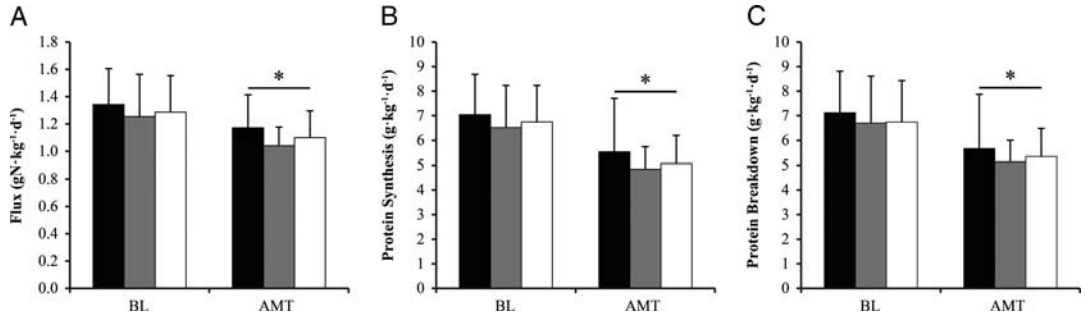

FIGURE 1—Determination of whole-body protein flux (A), synthesis (B), and breakdown (C) from BL to AMT between PRO (■), CHO (■), CON (□). Values presented as mean ± SD. *Main effect of time AMT different from BL, $P < 0.05$.

<2356 kcal·d^{-1}; quartile 2, 2356–2862 kcal·d^{-1}; quartile 3, 2863–3303 kcal·d^{-1}; quartile 4, >3303 kcal·d^{-1}). Participants in quartile 4 consumed more energy, resulting in a lower negative energy balance compared with quartiles 3, 2, and 1 ($P < 0.05$, Table 4). Participants in quartile 4 also tended ($P = 0.06$) to lose less body mass, particularly when compared with quartile 1. NBAL and NET were also higher ($P < 0.05$) in quartile 4 during AMT than quartile 1 (Fig. 2). No other differences were observed across quartiles.

DISCUSSION

The primary finding of this study was that during a challenging AMT, which produced energy expenditures as high as 6000 kcal·d^{-1}, increasing energy intake, regardless of the macronutrient source, attenuated negative energy balance and spared whole-body protein. High daily energy expenditures were the product of activity-induced energy expenditures (3650 kcal·d^{-1}). Whole-body protein losses were only reduced in those that consumed enough energy to reduce negative energy balance to levels comparable with those typically used to elicit moderate weight loss (approximately 40% energy deficit). Although supplementing combat rations with eat-on-the-move protein- or carbohydrate-based snack bars was an effective approach in altering macronutrient intake, eating the bars did not independently attenuate negative energy balance or decrements in protein balance. These findings highlight the difficulty of optimizing military field feeding, as participants in PRO and CHO compensated for the extra energy consumed in the snack products by consuming less of their combat rations.

The dietary intervention largely failed to attenuate the severity of negative energy balance, despite higher energy intake for those assigned to CHO compared with CON. As such, there was no apparent benefit of providing supplemental protein or carbohydrate on NBAL and NET during the 4-d AMT. Energy balance, although highly variable (−1700 to −5200 kcal·d^{-1}), was associated with NBAL and NET. In fact, whole-body protein loss was negated for the participants who consumed the most energy, an observation consistent with previous works establishing the relation between energy balance and protein use (18,33,41). This finding emphasizes that during periods of high-level physical activity, attention to food discipline so as to better match energy intake and energy expenditure, to reduce the level of negative energy balance, is warranted.

Although participants with the highest energy intake achieved protein balance, they remained in a severe state of negative energy balance (−2400 kcal·d^{-1}, 39% energy deficit), suggesting that at this level of negative energy balance, macronutrient intake may have had influence in mitigating protein losses. In agreement with our previous study (15), consuming 1.7 g·kg^{-1}·d^{-1} or more of dietary protein failed to independently modulate NBAL and NET, when negative energy balance exceeded 3000 kcal·d^{-1} (>50% energy deficit). However, consistent with our hypothesis, attenuating negative energy balance limited whole-body protein loss with elevated protein and carbohydrate intakes. In fact, 14 of the 15 participants in

TABLE 4. Energy balance and macronutrient intake by energy intake quartile.

	Quartile 1 (n = 15)	Quartile 2 (n = 15)	Quartile 3 (n = 15)	Quartile 4 (n = 15)
Energy intake (kcal·d^{-1})	1783 ± 437^{a}	2658 ± 149^{b}	3076 ± 136^{c}	3723 ± 360^{d}
Energy expenditure (kcal·d^{-1})	5920 ± 483^{a}	6375 ± 637^{a}	6276 ± 427^{a}	6133 ± 359^{a}
Energy balance (kcal·d^{-1})	−4138 ± 696^{a}	−3740 ± 700^{a,b}	−3229 ± 489^{b}	−2410 ± 582^{c}
△Body mass*	−3.17 ± 1.20^{a}	−3.11 ± 1.38^{a}	−2.59 ± 1.17^{a}	−1.95 ± 1.33^{a},†
Protein (g·kg^{-1}·d^{-1})	1.16 ± 0.36^{a}	1.68 ± 0.47^{b}	1.74 ± 0.27^{b}	2.11 ± 0.45^{c}
Carbohydrate (g·kg^{-1}·d^{-1})	3.26 ± 0.97^{a}	4.13 ± 0.59^{a,b}	4.97 ± 0.94^{b}	6.54 ± 0.1.16^{c}
Fat (g·kg^{-1}·d^{-1})	0.77 ± 0.25^{a}	1.13 ± 0.21^{b}	1.34 ± 0.19^{c}	1.66 ± 0.19^{d}

The values are presented as mean ± SD.
Values not sharing the same superscript are different, $P < 0.05$.
*Delta body mass calculated as POST − BL.
†Tendency to being different from quartile 1, $P = 0.06$.

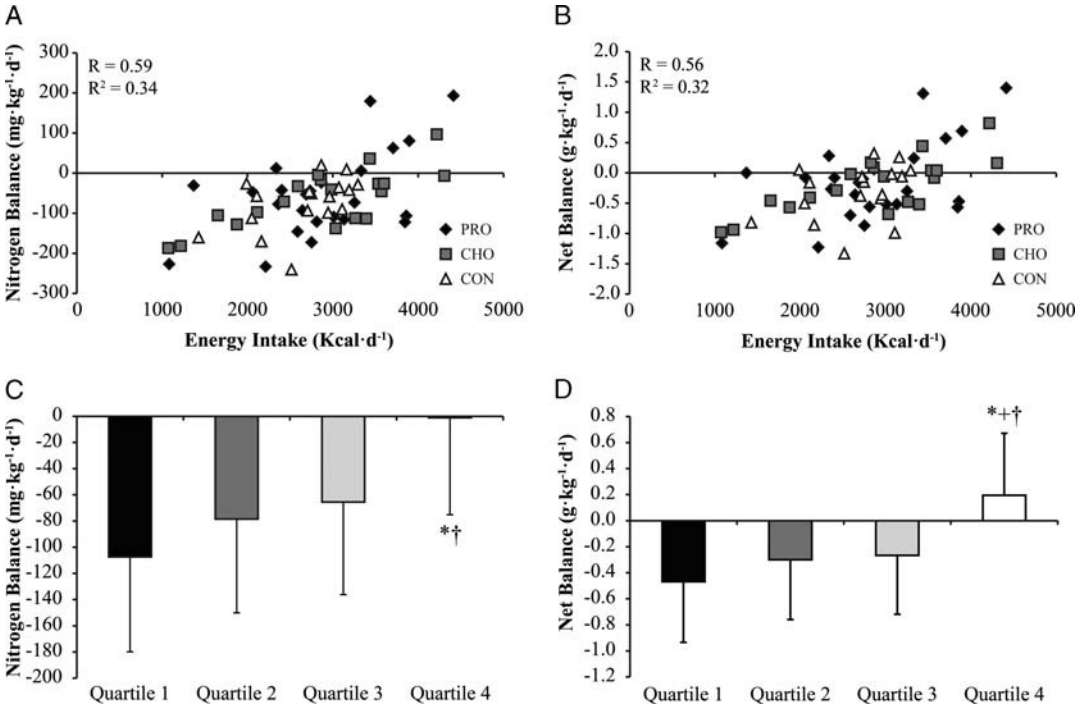

FIGURE 2—Correlation of energy intake for PRO (♦), CHO (■), and CON (Δ) to NBAL (A) and NET (B). Comparison for NBAL (C) and NET (D) between energy quartiles. Values presented as mean ± SD. *Different from quartile 1, $P < 0.05$. †Different from quartile 2, $P < 0.05$. +Different from quartile 3, $P < 0.05$.

quartile 4, where protein balance was maintained, were assigned to PRO ($n = 7$) and CHO ($n = 7$). Those soldiers not only consumed more food but also consumed protein at or slightly above the upper limit of current military operational protein recommendations (>2.0 $g \cdot kg^{-1} \cdot d^{-1}$) (20). Likewise, carbohydrate intake for those who consumed the most energy was about 6.5 $g \cdot kg^{-1} \cdot d^{-1}$, a level within current recommendations (6–10 $g \cdot kg^{-1} \cdot d^{-1}$) for endurance-type exercise (28). These data suggest that achieving a more manageable energy deficit during demanding military operations by consuming both protein (2.0 $g \cdot kg^{-1} \cdot d^{-1}$) and carbohydrate (6.5 $g \cdot kg^{-1} \cdot d^{-1}$) within recommended levels could positively affect skeletal muscle mass and performance.

Whole-body protein turnover was downregulated during AMT. Typically, during acute negative energy balance (≤3 day), whole-body protein flux and breakdown are upregulated to liberate amino acids for energy production (5,14,18). Downregulations in whole-body protein turnover generally occur with sustained (≥14 d) negative energy balance, because adaptation occurs and amino acids are no longer heavily relied on as an energy source (22,40). Measuring whole-body protein turnover over a 24-h period (10) that included high levels of physical activity (14), in both fasted and fed states (14,18), is likely the reason why our turnover data differs from past studies. Our findings show that during periods of severe negative energy balance caused primarily by exercise-induced energy expenditure, there is a downregulation in whole-body protein turnover, representing an adaptive response to spare endogenous protein.

Providing energy-dense supplemental nutrition during sustained (8 wk), metabolically challenging (5000 $kcal \cdot d^{-1}$) military operations has been an effective countermeasure, attenuating negative energy balance and reducing total body mass loss from 5.0 to 1.6 kg (7). In the current investigation, despite strong compliance with the supplemental nutrition intervention, the treatment failed to attenuate negative energy balance, decrements in NBAL and NET, and losses in body mass compared with CON. The lack of an effect can be explained by the fact that snack bars were consumed at the expense of some of the food in the rations. Participants in the PRO and CHO groups consumed about 500 and 300 $kcal \cdot d^{-1}$ less from their rations compared with CON. Diminished ration consumption and wide ranges in energy and macronutrient consumption within groups can be attributed to *ad libitum* intake during the training operation.

We recognize that not controlling dietary intake during this field feeding study limits our ability to assess the effects of increased energy intake from protein or carbohydrate, but *ad libitum* intake more accurately depicts how soldiers actually eat during military operations. That the soldiers chose to eat less when provided with our snack items raises questions as how or what approach should be used to increase voluntary energy intake. Drinks rather than bars might minimize reductions in ration intake by reducing feeling of fullness (34), but powdered carbohydrate drinks were the least consumed food product in the rations provided. Providing additional food or augmenting current ration items to provide energy in other forms may allow soldiers to eat as they train,

minimize the effect on overall ration intake, and improve energy intake to support performance and function during sustained operations (24,25).

In conclusion, the current investigation reinforces the importance of eating during challenging military training operations, because protein balance was maintained in those soldiers who ate enough to reduce the level of negative energy balance to an energy deficit of <40% of the total daily energy requirements. Those soldiers who maintained protein balance in the present investigation consumed protein at the upper end of the recommendations (2.0 $g \cdot kg^{-1} \cdot d^{-1}$), suggesting a benefit of higher protein intake, as long the deficits, as a percentage of energy requirements, are less than 40%. In addition, consumption of 6.5 $g \cdot kg^{-1} \cdot d^{-1}$ carbohydrate likely also contributed to the maintenance of whole-body protein balance by providing a readily available substrate for exercise metabolism. Although benefits were observed with increased energy intake, attempting to increase energy intake with supplemental snack bars was ineffective. Future studies are warranted to identify effective military field feeding strategies that maintain recommended intakes of protein and carbohydrate, attenuate negative energy balance, sustain exercise metabolism, and spare whole-body protein.

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The investigators adhered to the policies for protection of human subjects as prescribed in Army Regulation 70–25, and the research was conducted in adherence with the provisions of 32 CFR part 219. The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Army or the Department of Defense. Any citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement of approval of the products or services of these organizations. The results of the present study do not constitute endorsement by the American College of Sports Medicine.

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RESEARCH ARTICLE | *Microbiome and Host Interactions*

Changes in intestinal microbiota composition and metabolism coincide with increased intestinal permeability in young adults under prolonged physiological stress

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Karl JP, Margolis LM, Madslien EH, Murphy NE, Castellani JW, Gundersen Y, Hoke AV, Levangie MW, Kumar R, Chakraborty N, Gautam A, Hammamieh R, Martini S, Montain SJ, Pasiakos SM. Changes in intestinal microbiota composition and metabolism coincide with increased intestinal permeability in young adults under prolonged physiological stress. *Am J Physiol Gastrointest Liver Physiol* 312: G559–G571, 2017. First published March 23, 2017; doi:10.1152/ajpgi.00066.2017.—The magnitude, temporal dynamics, and physiological effects of intestinal microbiome responses to physiological stress are poorly characterized. This study used a systems biology approach and a multiple-stressor military training environment to determine the effects of physiological stress on intestinal microbiota composition and metabolic activity, as well as intestinal permeability (IP). Soldiers ($n = 73$) were provided three rations per day with or without protein- or carbohydrate-based supplements during a 4-day cross-country ski-march (STRESS). IP was measured before and during STRESS. Blood and stool samples were collected before and after STRESS to measure inflammation, stool microbiota, and stool and plasma global metabolite profiles. IP increased $62 \pm 57\%$ (mean ± SD, $P < 0.001$) during STRESS independent of diet group and was associated with increased inflammation. Intestinal microbiota responses were characterized by increased α-diversity and changes in the relative abundance of >50% of identified genera, including increased abundance of less dominant taxa at the expense of more dominant taxa such as *Bacteroides*. Changes in intestinal microbiota composition were linked to 23% of metabolites that were significantly altered in stool after STRESS. Together, pre-STRESS Actinobacteria relative abundance and changes in serum IL-6 and stool cysteine concentrations accounted for 84% of the variability in the change in IP. Findings demonstrate that a multiple-stressor military training environment induced increases in IP that were associated with alterations in markers of inflammation and with intestinal microbiota composition and metabolism. Associations between IP, the pre-STRESS microbiota, and microbiota metabolites suggest that targeting the intestinal microbiota could provide novel strategies for preserving IP during physiological stress.

NEW & NOTEWORTHY Military training, a unique model for studying temporal dynamics of intestinal barrier and intestinal microbiota responses to stress, resulted in increased intestinal permeability concomitant with changes in intestinal microbiota composition and metabolism. Prestress intestinal microbiota composition and changes in fecal concentrations of metabolites linked to the microbiota were associated with increased intestinal permeability. Findings suggest that targeting the intestinal microbiota could provide novel strategies for mitigating increases in intestinal permeability during stress.

microbiology; gut barrier; exercise; energy metabolism; metabolomics

THE INTESTINAL BARRIER is a selective physical and immunological barrier that facilitates fluid and nutrient absorption while deterring translocation of potentially harmful luminal antigens into circulation (3). Disruption or dysfunction of the intestinal barrier increases intestinal permeability (IP), initiating a cycle in which translocation of luminal compounds (e.g., bacterial cell wall LPS) can induce immune and inflammatory responses that exacerbate intestinal barrier damage and further increase IP (3, 15, 54). Sequelae of increased IP and subsequent inflammation can include gastrointestinal distress (54), impaired nutrient absorption and metabolism (35), increased susceptibility to illness and infection (53), decrements in cognitive function and physical performance (12), and, if chronic, increased disease risk (19, 53).

The intestinal microbiota and its metabolites are integral mediators of intestinal barrier function and IP, capable of both perturbing and enhancing intestinal barrier integrity by modulating immune responses, oxidative stress, inflammation, vagal signaling, and nutrient availability (40). Intestinal microbiota composition and activity are malleable, influenced by the availability of undigested dietary components (13, 40) and the intestinal environment (e.g., pH, motility, inflammation, and immune activity) (48). Dietary ratios of fiber, carbohydrate, protein, and fat are also important, as low-fiber, high-protein, and high-fat diets have been reported to increase intestinal inflammation and IP by altering ratios of microbes and metabolites that modulate inflammation (13, 14, 41). Severe physical stress (12, 15, 54), psychological stress (34), sleep deprivation and circadian disruption (17, 50), and environmental stressors

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(9, 24) have also been independently associated with altered intestinal microbiota composition and increased IP. However, current understanding of the role of the intestinal microbiota in mediating effects of physical, psychological, and environmental stressors on the intestinal barrier is largely limited to information derived from animal models, which may not fully represent the human condition (12, 34).

Military training environments offer the opportunity for novel insights into the magnitude, temporal dynamics, and health effects of stress responses within the human intestinal microbiome, as military personnel commonly endure combinations of prolonged physical exertion, psychological stress, sleep deprivation, and environmental extremes during training and combat (31, 51). Transient and chronic gastrointestinal distress (46), suboptimal micronutrient status (21, 36), and cognitive decrements (31) have been reported in military personnel during training and combat. Although underlying etiologies are multifactorial, all are possible sequelae of increased IP, suggesting that intestinal barrier dysfunction and the intestinal microbiota may play a role. Recently, Li et al. reported that gastrointestinal distress during combat training was linked to stress, anxiety, inflammation, and increased intestinal and blood-brain barrier permeability (29, 30). Phua et al. observed changes in urinary concentrations of several metabolites potentially derived from the intestinal microbiota and an association of these changes with gastrointestinal symptomology and IP (44). Although they speculated that changes in intestinal microbiota composition may have contributed to these findings, the authors did not assess microbiota composition.

The present study used a physically demanding military training exercise as a model for elucidating the effects of physiological and metabolic stress on IP and intestinal microbiota composition and activity and to identify associations between dietary intake, IP, inflammation, and the intestinal microbiota. The data were collected during a trial designed to determine the extent to which dietary carbohydrate and protein supplementation spare whole body protein and attenuate decrements in physiological status during military training (32, 43). We hypothesized that the multiple-stressor environment, which was expected to induce negative energy balance and body weight loss, would adversely affect intestinal microbiota composition (e.g., decrease diversity, increase abundance of proinflammatory taxa, and decrease abundance of putatively beneficial taxa) and increase IP. We further hypothesized that supplemental protein would exacerbate these decrements by promoting the generation of potentially harmful bacterially derived metabolites, whereas carbohydrate supplementation would attenuate these decrements by reducing the magnitude of negative energy balance.

METHODS

Participants and experimental design. Seventy-three Norwegian Army soldiers (71 men and 2 women) participating in a 4-day arctic military training exercise consented to participate in this randomized, controlled trial in January 2015 (32, 43). All soldiers >18 yr of age participating in the training were eligible for the study, which was approved by the Institutional Review Board at the US Army Research Institute of Environmental Medicine and the Regional Committees for Medical and Health Research Ethics (REK sør-øst, Oslo, Norway). Investigators adhered to the policies for protection of human subjects as prescribed in 32 CFR Part 219, US Department of Defense Instruction 3216.02 (Protection of Human Subjects and Adherence to Ethical Standards in DoD-Supported Research) and Army Regulation 70-25. The trial was registered on www.clinicaltrials.gov as NCT02327208.

Study staff block-randomized volunteers by body weight to a control (CNTRL, $n = 18$), protein-supplemented (PRO, $n = 28$), or carbohydrate-supplemented (CHO, $n = 27$) group in a 1:3 (control-to-intervention) ratio. All volunteers were provided with three Norwegian arctic rations per day to consume during the 4-day training exercise. The PRO group was also provided with four whey protein-based snack bars per day, while the CHO group was provided with four carbohydrate-based snack bars per day. Bars were similar in appearance, taste, and texture, enabling investigators, study staff, and volunteers to remain blind to the macronutrient composition. The training consisted of a 51-km cross-country ski-march, during which volunteers skied in 50:10-min work-to-rest ratios while carrying a ~45-kg pack (STRESS). Stool samples were collected over the 2 days before STRESS and on the night of or day after completion of STRESS in a self-selected subset of volunteers. Twenty-four-hour urine collections were completed on the day before STRESS and on *day 3* of STRESS. Blood samples were collected on the morning before and the morning after STRESS. Primary study objectives were to determine the effects of macronutrient supplementation on whole body protein balance, body mass, and physiological status during military training and are reported elsewhere (32, 43). This report details secondary study objectives of determining the impact of a multiple-stressor military training environment on IP and intestinal microbiota composition and activity.

Volunteers began consuming provided rations 2 days before training and the intervention snack bars on *day 1* of STRESS. Three Norwegian field rations provide 14.6 MJ of energy, 141 g of protein, 435 g of carbohydrate, and 126 g of fat. The four protein-based snack bars provided an additional 4.4 MJ of energy, 85 g of whey protein, 102 g of carbohydrate, 35 g of fat, and <1 g of fiber, while the four carbohydrate-based snack bars provided an additional 4.4 MJ of energy, 11 g of whey protein, 189 g of carbohydrate, 29 g of fat, and 1 g of fiber. All snack bars were manufactured by a third party that did not participate in data collection (Combat Feeding Directorate, Natick Soldier Systems Center, Natick, MA). Investigators, study staff, and volunteers were blind to the macronutrient composition of the bars. Volunteers were asked to consume the rations and bars as they normally would during training and to consume only foods and caloric beverages provided by the study team. All volunteers were provided with ration-specific food logs, which were collected and reviewed daily by study staff and used to calculate actual intakes (Table 1).

IP assay. IP was assessed by quantifying the urinary excretion of orally ingested sugar substitutes (29, 38). Fasted volunteers consumed a solution of 2 g of sucralose and 4 g of mannitol dissolved in ~180 ml of water and then collected all urine produced over the subsequent 24 h. Sucralose is not degraded by the colonic microbiota, is excreted in proportion to paracellular permeability, and is a common marker for whole-gut IP (38). In contrast, mannitol is used for small bowel permeability measurements (3) but is degraded by the colonic microbiota, which prevents its use for IP measurements >5 h. Mannitol results are presented solely for comparison with a previous study conducted in a military training environment (29). Sucralose and mannitol concentrations were measured by HPLC (model 1100, Agilent, Santa Clara, CA) as previously described (1, 33). For calculation of fractional excretion, the measured concentration of each probe was multiplied by the total volume of urine collected, and the product was divided by the dose administered. Logistical constraints and adverse weather precluded more frequent urine collections and prevented procurement of complete post-STRESS urine collections from 24 volunteers.

Blood biochemistries. After an overnight fast, blood was collected by antecubital venipuncture, separated into serum or plasma, and immediately frozen. Samples were then shipped on dry ice to the US

Table 1. *Volunteer characteristics, energy expenditure, and dietary intake*

	CNTRL (n = 18)	CHO (n = 27)	PRO (n = 28)
Age, yr	19 ± 2	20 ± 1	20 ± 1
BMI, kg/m^2	23.6 ± 1.8	24.1 ± 2.3	23.3 ± 2.1
Energy expenditure, MJ/day	25.5 ± 1.7	25.8 ± 2.1	25.8 ± 2.5
Energy intake, MJ/day	10.5 ± 1.7 (6.5–13.0)	13.1 ± 2.6* (7.7–16.4)	11.8 ± 2.5 (7.1–16.8)
Carbohydrate, g/day	312 ± 47 (193–385)	434 ± 86* (253–543)	321 ± 77† (171–490)
Protein, g/day	100 ± 15 (65–124)	98 ± 22 (58–130)	148 ± 25*† (96–191)
Fat, g/day	91 ± 20 (57–117)	107 ± 24 (56–146)	102 ± 23 (59–141)
Fiber, g/day	25 ± 4 (16–32)	25 ± 6 (13–33)	22 ± 5† (12–33)

Values are means ± SD and range. Volunteers received 3 rations/day (CNTRL), 3 rations/day and 4 carbohydrate-based snack bars/day (CHO), or 3 rations/day and 4 protein-based snack bars/day (PRO). 1 CNTRL and 2 PRO subjects were excluded because of incomplete food logs. BMI, body mass index. [Adapted from Margolis et al. (32) and Pasiakos et al. (43).] *$P < 0.05$ vs. CNTRL (1-way ANOVA). †$P < 0.05$ vs. CHO (1-way ANOVA).

Army Research Institute of Environmental Medicine, where they were stored at −80°C until they were shipped to Pennington Biomedical Research Center (Baton Rouge, LA) or Metabolon (Durham, NC) for analysis. Plasma LPS was measured by ELISA (Cusabio, College Park, MD), serum IL-6 by multianalyte profiling (MILLIPLEX, Millipore, Billerica, MA), serum high-sensitivity C-reactive protein (CRP) by a chemiluminescent immunometric assay (Immulite 2000, Siemens, Malvern, PA), and serum creatine kinase (a marker of muscle damage) by an automated chemistry analyzer (model DXC 600 Pro, Beckman Coulter, Brea, CA).

Stool microbiota composition. Stool sample collection was optional to encourage maximal participation for primary study outcomes. A self-selected subset of 38 volunteers provided stool samples; 26 of these volunteers provided both pre- and post-STRESS samples.

Stool samples were collected into provided collection containers, immediately placed on ice, and frozen in ~500-mg aliquots within 12 h of collection. Samples were shipped on dry ice to the US Army Research Institute of Environmental Medicine, where they were stored at −80°C. Samples were then shipped to Metabolon for metabolomics analysis and to the US Army Center for Health and Environmental Research for intestinal microbiota composition analysis.

Samples were selected for DNA extraction in random order, and DNA was extracted using the PowerFecal DNA Isolation kit (MO BIO Laboratories, Qiagen, Carlsbad, CA). Primers designed to amplify the V3–V4 region of the 16S rRNA gene were employed for PCR amplification (22) according to the Illumina 16S Metagenomic Sequencing Library Preparation manual (catalog no. 15044223 Rev B, Illumina, San Diego, CA). A limited-cycle PCR generated a single amplicon of ~460 bp to which Illumina sequencing adapters and dual-index barcodes were added. Paired 300-bp reads and MiSeq v.3 reagents were used to generate full-length reads of the V3 and V4 regions in a single run on the Illumina MiSeq platform.

Sequencing data were processed using Quantitative Insights Into Microbial Ecology (QIIME) v.1.9.1 (8). Read quality assessment, filtering, barcode trimming, and chimera detection were performed on demultiplexed sequences using USEARCH (16). Operational taxonomic units (OTUs) were assigned by clustering sequence reads at 97% similarity. The most abundant sequences with a minimum sequence length of 150 bp were aligned against the Greengenes database core set v.gg_13_15 (37) using PyNAST (7). Taxonomic assignment was completed using the RDP classifier v.2.2 (55).

Stool and plasma metabolomics. Stool and plasma aliquots from soldiers providing both pre- and post-STRESS stool samples were submitted for global metabolite profiling (Metabolon). Samples were analyzed using two separate reverse-phase (RP)/ultra-high-performance liquid chromatography (UPLC)-tandem mass spectrometry (MS/MS) methods with positive ion mode electrospray ionization (ESI), RP/UPLC-MS/MS with negative ion mode ESI, and hydrophilic interaction chromatography (HILIC)/UPLC-MS/MS with negative ion mode ESI.

Several recovery standards were added before the first step in the extraction process and were analyzed with the experimental samples for quality control. All analysis methods utilized an ACQUITY UPLC (Waters, Milford, MA) and a Thermo Scientific Q-Exactive high-resolution/accurate MS interfaced with a heated ESI-II source and Orbitrap mass analyzer operated at 35,000 mass resolution. Sample extracts were dried and reconstituted in solvents compatible with each of the four methods. Each reconstitution solvent also contained a series of standards at fixed concentrations to ensure injection and chromatographic consistency. One aliquot was analyzed using acidic positive ion conditions chromatographically optimized for more hydrophilic compounds. In this method, the extract was gradient-eluted from a C-18 column (Waters UPLC BEH C-18, 2.1 mm × 100 mm, 1.7 μm) using water and methanol containing 0.05% perfluoropentanoic acid and 0.1% formic acid. Another aliquot was also analyzed using acidic positive ion conditions chromatographically optimized for more hydrophobic compounds. In this method, the extract was gradient-eluted from the same C-18 column using methanol, acetonitrile, water, 0.05% perfluoropentanoic acid, and 0.01% formic acid and operated at an overall higher organic content. Another aliquot was analyzed using basic negative ion optimized conditions and a separate dedicated C-18 column. The basic extracts were gradient-eluted from the column using methanol and water, but with 6.5 mM ammonium bicarbonate at pH 8. The fourth aliquot was analyzed via negative ionization following elution from a HILIC column (Waters UPLC BEH Amide 2.1 mm × 150 mm, 1.7 μm) using a gradient consisting of water and acetonitrile with 10 mM ammonium formate at pH 10.8. The MS analysis alternated between MS and data-dependent MS^n scans using dynamic exclusion. The scan range varied slightly between methods but covered 70–1,000 *m/z*.

Raw data were extracted, peaks were identified, and data were quality control-processed using Metabolon's proprietary hardware and software. Compounds were identified by comparison with a library that is maintained by Metabolon and contains entries of purified standards or recurrent unknown entities. Biochemical identifications were based on three criteria: retention index within a narrow retention index window of the proposed identification, accurate mass match to the library ±10 ppm, and the MS/MS forward and reverse scores between the experimental data and authentic standards. The MS/MS scores were based on a comparison of the ions present in the experimental spectrum with the ions present in the library spectrum. Peaks were quantified using area under the curve.

Bioinformatics. Analyses were completed using R v.3.3.1, Multiexperiment Viewer v.4.9.0, SPSS v.21, and XLSTAT v.2015. We obtained an average of 140,762 ± 103,480 16S rDNA sequences per stool sample, which clustered into 2,015 OTUs at 97% sequence identity. OTUs could be assigned to 12 phyla and 83 genera. α-Diversity (Shannon and Chao1 indexes and observed OTUs) was calculated using the *phyloseq* R bioconductor package, and β-diversity was calculated using Bray-Curtis distances. Prior to statistical analysis of sequencing data, phylum-, genus-, and OTU-level relative abundances were calculated by dividing the number of reads for each taxon by the total number of reads in the sample. Ordination and cluster analyses were conducted on OTU-level relative abundances, whereas differential analyses were conducted on phylum- and genus-level relative abundances. For differential analyses, any OTUs that could not be assigned to the genus level were grouped at the next-lowest level of classification possible (e.g., family or order).

Relative abundances were arcsine square-root-transformed before differential analysis to stabilize variance and better approximate normality. Prior to analysis of stool and plasma metabolites, any missing values were imputed using the minimum observed value for each compound, normalized to set the median equal to 1, and $\log_{10}$-transformed.

Ordinations were conducted by principal coordinates analysis (PCoA) of the OTU Bray-Curtis dissimilarity matrix, principal components analysis (PCA) of metabolite data, and hierarchical complete-linkage clustering of Euclidean distances (OTU and metabolite data). Supervised classification of pre- and post-STRESS samples was conducted using random forest analysis, and the mean decrease accuracy was used to identify taxa driving classification. To examine associations between stool microbiota composition and global metabolite profiles, metabolite PCA ordinations were compared with OTU PCoA ordinations using Procrustes analysis implemented in the R package *vegan*.

A knowledge-based approach was used to better identify microbially derived metabolites by predicting changes in stool metabolite profiles based on changes in stool microbiota composition. For these analyses, PICRUSt v.1.0.0 was first used to predict metagenome functional content from 16S rDNA data (26). Final metagenome functional predictions were performed by multiplying normalized OTU abundance by each predicted functional profile. Differences in predicted metagenomic profiles were examined by comparing KEGG orthologs between pre- and post-STRESS samples and PCA. Changes in metagenome functional counts over time were examined following Trimmed Mean of *M* component normalization by fitting linear models using moderated standard errors and the empirical Bayes model. Metabolites predicted to derive from significantly altered KEGG orthologs ($P \leq 0.05$) were annotated using HMDB v.2.5, KEGG v.80.0 (compounds, pathways, orthologs, and reactions), SMPDB v.2.0, and FOODB v.1.0. These metabolites were then compared with the list of metabolites in stool that increased or decreased over time ($P < 0.10$). Overlapping metabolites were considered indicative of functional relationships between changes in the microbiome and the metabolome.

Statistical analysis. Sample size calculations were based on primary study outcomes, which are reported elsewhere (32, 43). Statistical analyses were completed using SPSS v.21 and R v.3.3.1. Data were assessed for normality before analysis and transformed if necessary to meet model assumptions. When transformation was not successful, nonparametric tests were used. Repeated-measures ANOVA was used to test effects of STRESS and diet and their interaction on study outcomes. Pair-wise comparisons of pre- and post-STRESS genus relative abundances were conducted using Wilcoxon's signed rank test, and between-group comparisons of changes in genus relative abundances were conducted using the Kruskal-Wallis test. Spearman's rank correlation (ρ), Pearson's correlation (r), multiple linear regression, and linear mixed models were used to examine associations among variables. Relationships between surcalose excretion and LPS, IL-6, and CRP concentrations and ordinations of stool microbiota composition and stool/plasma metabolites were also assessed using linear mixed models. All mixed models included subject as a random factor and time as a continuous covariate. Sucralose excretion or LPS, IL-6, or CRP concentration was entered as dependent variable, and scores for the first three principal components of the ordinations were included as independent variables. Finally, backward stepwise regression was used to identify the strongest predictors of changes in IP. Independent variables included in the regression model were those that were significantly correlated with changes in sucralose excretion and included dietary parameters (protein intake), change scores for inflammation markers (IL-6 and CRP), pre-STRESS stool microbiota characteristics (Shannon diversity and Actinobacteria and Proteobacteria relative abundances), and change scores for stool metabolites linked to changes in microbiota composition changes (cysteine and arginine). Changes in Shannon diversity and pre-STRESS *Sutterella* relative abundance were also considered in place of pre-STRESS Shannon diversity and Proteobacteria relative abundance, respectively.

The false discovery rate for all tests including taxa or metabolite data was controlled by adjusting P values using the Benjamini-Hochberg procedure. Adjusted P values are presented as Q values. Values are means ± SD unless otherwise noted. Statistical significance was set at $P \leq 0.05$ or $Q \leq 0.10$.

RESULTS

Macronutrient intakes varied across study groups as planned (Table 1). Specifically, mean protein intake was higher in PRO than CNTRL and CHO ($P < 0.05$), mean carbohydrate intake was higher in CHO than CNTRL and PRO ($P < 0.05$), and fat intake did not differ between groups. Energy intake was higher in CHO than CNTRL and PRO ($P < 0.05$; Table 1). Energy expenditure was high, averaging 25.7 ± 2.2 MJ/day and did not differ between groups (32). The high energy expenditure resulted in a 55% energy deficit and 2.7 ± 1.2 kg loss of body mass, which also did not differ between groups (32, 43). Serum creatine kinase, IL-6, and CRP concentrations are reported elsewhere (43). All increased during STRESS independent of diet group, indicating that muscle damage and inflammation were induced during STRESS.

The volunteers who chose to provide stool samples were men and did not differ in age ($P = 0.59$), body mass index ($P = 0.47$), or body mass loss ($P = 0.98$) or change in intestinal permeability ($P = 0.42$), energy intake ($P = 0.51$), macronutrient intake ($P \geq 0.11$), or energy expenditure ($P = 0.94$) during STRESS relative to volunteers who chose not to provide stool samples.

IP, plasma LPS, and inflammation. Sucralose excretion increased 62 ± 57% during STRESS independent of diet (main effect of time, $P < 0.001$; Fig. 1*A*), suggesting increased IP, and was correlated with changes in creatine kinase ($r = 0.34$, $P = 0.02$), CRP ($\rho = 0.36$, $P = 0.01$), IL-6 (Fig. 1*B*), and protein intake ($\rho = -0.31$, $P = 0.03$). Mannitol excretion also increased during STRESS independent of diet [28 ± 8% (pre-STRESS) vs. 33 ± 13% (post-STRESS), main effect of time, $P = 0.01$]. Plasma LPS concentrations did not differ from pre- to post-STRESS ($P = 0.79$; Fig. 1*C*). However, soldiers with increased LPS concentrations demonstrated a trend to greater increases in IL-6 concentration than those with no change or a decrease in LPS concentration (Fig. 1*D*).

Stool microbiota composition. The Shannon α-diversity index increased during STRESS independent of diet (main effect of time, $P = 0.04$), whereas the Chao1 index (main effect of time, $P = 0.42$) and total observed OTUs (main effect of time, $P = 0.45$) were not affected by STRESS or diet, indicating an increase in the evenness, but not the richness, of the stool microbiota (Fig. 2*A*). PCoA (Fig. 2*B*) and cluster analysis (Fig. 2*C*) demonstrated an effect of STRESS on the microbiota independent of diet. Random forest analysis differentiated pre- and post-STRESS samples with 100% accuracy. The top 10 taxa contributing to the high prediction accuracy were *Peptostreptococcus, Christensenella, Faecalibacterium, Staphylococcus*, unassigned taxa within the Mogiobacteriaceae, Christensenellaceae, and Planococcaceae families, and unassigned taxa within the CW040 and RF39 orders (see Supplemental Table S1 in Supplemental Material for this article available online at the Journal website). At the phylum level, decreases in Bacteroidetes and increases in Firmicutes and several other

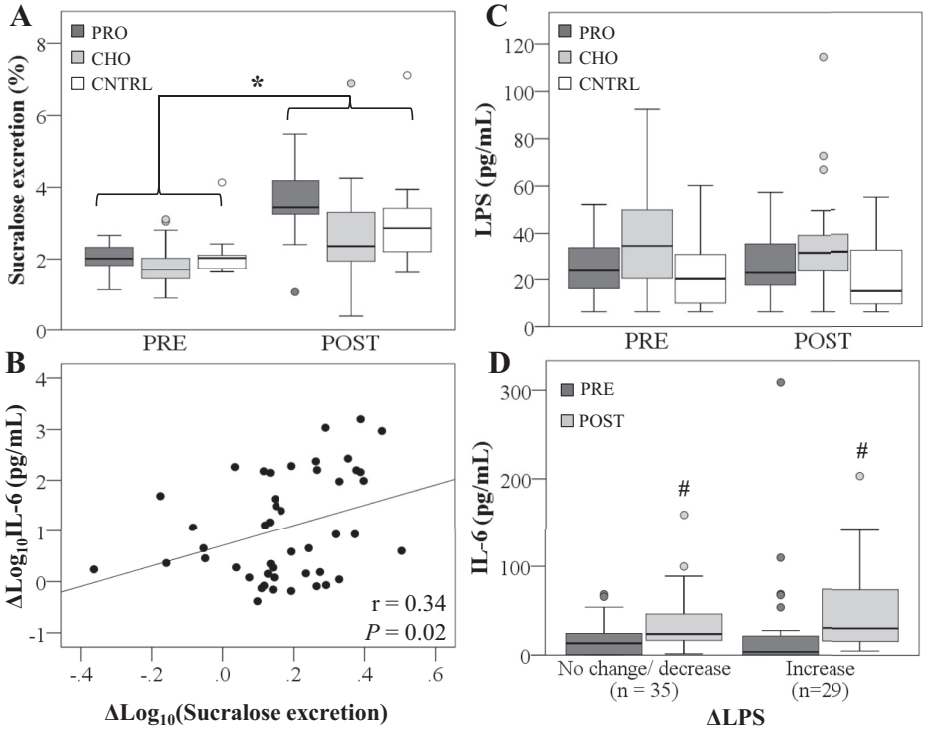

Fig. 1. Intestinal permeability (IP), plasma LPS, and inflammation during military training. *A* and *C*: intestinal permeability (IP) measured by 24-h urine collection following ingestion of 2 g of sucralose ($n = 49$) and plasma LPS concentrations ($n = 67$) before (PRE) and after (POST) military training. Boxes, median and interquartile range; whiskers, 1.5 times the interquartile range, or minimum and maximum if no observations within that range; circles, data points >1.5 times the interquartile range. *$P < 0.001$ (repeated-measures ANOVA, main effect of time). *B*: correlation of changes in IP with changes in serum IL-6 concentrations (Pearson's correlation, $n = 46$). *D*: a trend for larger increases in serum IL-6 in soldiers experiencing increases in plasma LPS during training than in those experiencing a decrease or no change in plasma LPS ($P = 0.07$, repeated-measures ANOVA, time × ΔLPS interaction). #$P < 0.05$ vs. PRE. CHO, carbohydrate-supplement group; CNTRL, control group (rations only); PRO, protein-supplement group.

phyla were observed ($Q < 0.10$; Fig. 2*D*). At the genus level, changes in the relative abundance of 48 of 83 identified genera were observed ($Q < 0.10$; see Supplemental Table S1). Changes in genus relative abundances did not differ by diet group ($Q > 0.75$ for all).

Stool and plasma metabolites. A total of 694 compounds were identified in stool. PCA (Fig. 3*A*) and cluster analysis (Fig. 3*B*) of these compounds did not suggest an effect of time point or diet. However, random forest analysis correctly differentiated pre- and post-STRESS stool samples with 84% accuracy (Fig. 3*C*), and 274 compounds demonstrated statistically significant changes ($Q < 0.10$). Of these, 81%, including several metabolites of amino acid, fatty acid, carbohydrate, and energy metabolism, decreased during STRESS (see Supplemental Table S2). Secondary bile acids and amino acid metabolites (Fig. 4) known to be solely or partially derived from microbial metabolism were generally decreased as well or unchanged, with the notable exception of *p*-cresol, a microbial metabolite of tyrosine fermentation, which was increased in stool post-STRESS.

A total of 737 compounds were identified in plasma; of these, 478 demonstrated statistically significant changes during STRESS ($Q < 0.10$). Changes primarily reflected increases in host energy metabolism, lipolysis, fatty acid oxidation, branched-chain amino acid catabolism, and steroid metabolism (data not shown). However, changes in plasma concentrations of several metabolites known to be partially or fully derived from microbial metabolism were also observed. Specifically, mean concentrations of phenylalanine and tyrosine metabolites, including *p*-cresol sulfate (+48%), *p*-cresol glucuronide (+79%), phenylacetate (+44%), phenyllactate (+42%), phenylacetylglutamine (+24%), and 3-(4-hydroxyphenyl)lactate (+40%), were increased (Fig. 4). In contrast, mean concentrations of the benzoate metabolites 2-hydroxyhippurate (−22%), 3-hyroxyhippurate (−61%), and 4-hyroxyhippurate (−35%) were decreased ($Q < 0.10$). Mean concentrations of secondary bile acids in plasma demonstrated more variable responses: glycolithocolate sulfate (+21%), glycohyocholate (+6%), taurolithocholate 3-sulfate (+89%), and taurocholenate sulfate (+56%) concentrations increased, while deoxycholate (−66%), ursodeoxycholate (−63%), and isoursodeoxycholate (−51%) concentrations decreased ($Q < 0.10$).

Associations between stool microbiota composition, stool and plasma metabolites, IP, and inflammation. Changes in sucralose excretion were inversely associated with pre-STRESS Shannon diversity ($\rho = -0.43$, $P = 0.05$) and Actinobacteria relative abundance ($\rho = -0.53$, $Q = 0.09$) and positively correlated with pre-STRESS Proteobacteria ($\rho = 0.64$, $Q = 0.02$) and *Sutterella* ($\rho = 0.68$, $Q = 0.09$) relative abundance (Fig. 5; see Supplemental Table S1) and changes in Shannon diversity ($\rho = 0.58$, $P = 0.02$). No statistically significant correlations between the pre-STRESS relative abundance of any taxa or the change in relative abundance of any taxa and changes in LPS, IL-6, or CRP concentration were detected. Additionally, no association between these variables and scores extracted from the first three principal components of the stool microbiota PCoA was detected.

Procrustes analysis demonstrated a significant association between the ordinations of stool metabolites and stool microbiota composition ($M^2 = 0.76$, Monte Carlo $P = 0.001$; Fig. 6*A*), indicating an association between stool metabolites and the stool microbiota. Additionally, prediction models linked changes in stool microbiota composition to 69 of the metabolites found to be altered in stool (see Supplemental Table S3). These models were supported by Procrustes analysis on ordinations of the significantly altered taxa and these metabolites ($M^2 = 0.72$, Monte Carlo $P = 0.001$). Of the 69 metabolites, amino acid and nucleotide metabolites comprised the majority

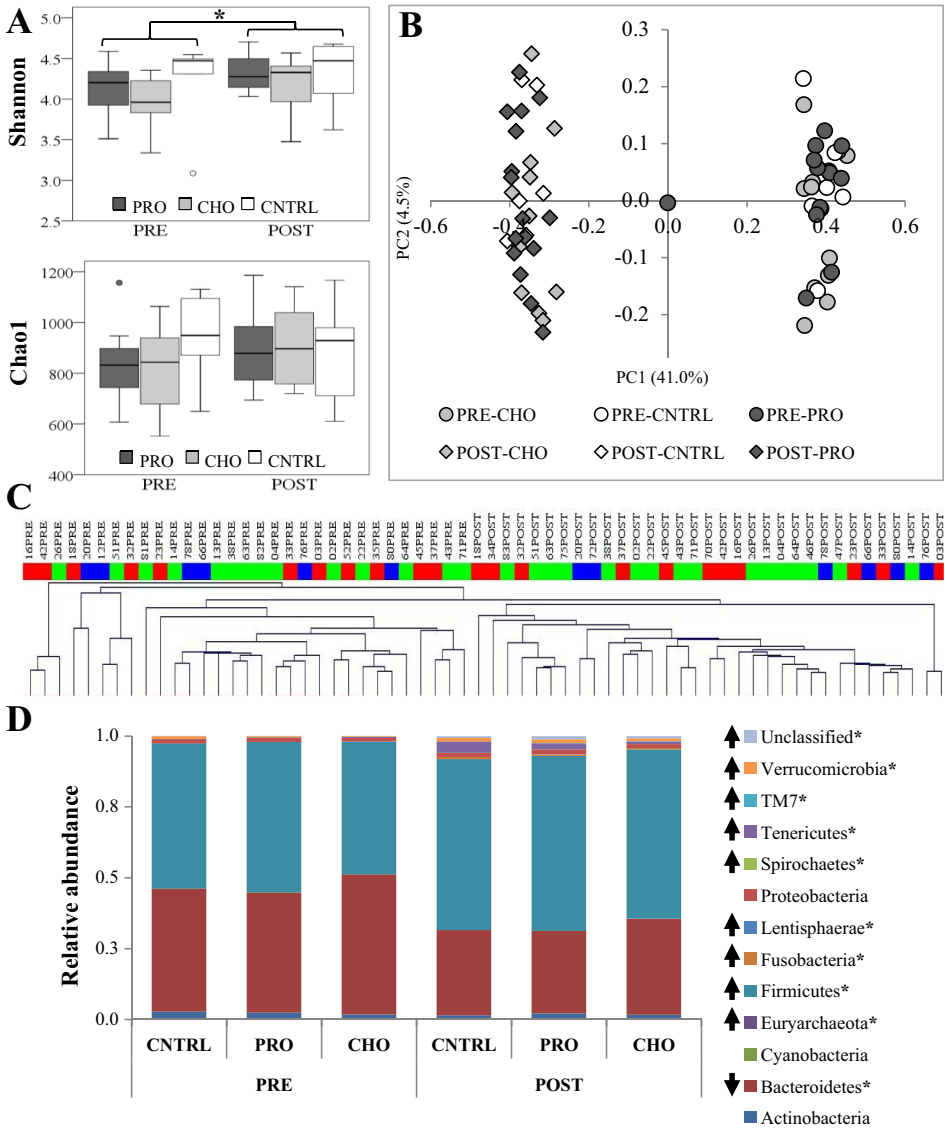

Fig. 2. Military training elicits changes in intestinal microbiota composition. *A*: α-diversity before (PRE) and after (POST) military training. Boxes, median and interquartile range; whiskers, 1.5 times the interquartile range, or minimum and maximum if no observations within that range; circles, data points >1.5 times the interquartile range. *P = 0.04 (repeated-measures ANOVA, main effect of time). *B*: principal coordinates (PC) analysis of Bray-Curtis dissimilarity matrix indicates that composition of the stool microbiota community was more strongly influenced by training environment than by individual variability or diet group. Data points represent the stool microbiota community of a single individual. Points closer together are more similar. *C*: hierarchical complete-linkage clustering of Euclidean distances of operational taxonomic unit (OTU) relative abundances measured in stool collected before and after military training (n = 38). Colored bars are data points representing the stool microbiota composition of an individual. Branches (lines) within the same node (points where branches split) reflect similarity in composition of the stool microbiota community. Clustering of branches by time point indicates that composition of the stool microbiota community was more strongly influenced by training environment than by individual variability or diet group. *D*: phylum-level shifts in gut microbiota composition. Bars, mean relative abundances. Arrows indicate direction of change in relative abundance from PRE to POST. *P < 0.05 (repeated-measures ANOVA, main effect of time). CHO, carbohydrate-supplement group (n = 9); CNTRL, control group (rations only, n = 5); PRO, protein-supplement group (n = 12).

and were generally lower post-STRESS than pre-STRESS (Q < 0.10). Changes in two of these metabolites, arginine and cysteine, were correlated with changes in sucralose excretion during STRESS (Table 2). Changes in the concentrations of another 14 metabolites were also inversely correlated with changes in sucralose excretion (Table 2). Scores on the first principal component from the ordination of stool metabolite data were associated with sucralose excretion ($\beta \pm SE$ = -0.05 ± 0.01, P = 0.01), indicating that the effect of STRESS on stool microbiota was associated with IP.

Procrustes analysis also demonstrated a significant association between the ordinations of plasma metabolites and stool microbiota composition (M^2 = 0.49, Monte Carlo P = 0.001; Fig. 6*B*), indicating an association between plasma metabolites and the stool microbiota. Furthermore, plasma concentrations of 30 of the 69 metabolites that linked the stool microbiota to the stool metabolome in prediction models were altered (Fig. 6*C*; see Supplemental Table S3). However, plasma metabolite changes were not correlated with changes in sucralose excretion or IL-6 or CRP concentration.

Backward stepwise regression was used to identify the strongest predictors of changes in IP. The final model comprising pre-STRESS Actinobacteria relative abundance, change in serum IL-6 concentrations, and changes in stool cysteine concentrations explained 84% of the variability in the change in sucralose excretion (Table 3). Collectively, these findings demonstrate an association between intestinal microbiota composition, stool metabolite concentrations, and changes in IP.

DISCUSSION

The magnitude, temporal dynamics, and physiological effects of intestinal microbiome responses to stress are poorly characterized. Our findings demonstrate that a multiple-stressor environment characterized by high physical exertion, suboptimal energy intake, muscle damage, and inflammation adversely affects intestinal barrier integrity concomitant with alterations in intestinal microbiota composition and metabolism. Associations between increased IP, the pre-STRESS

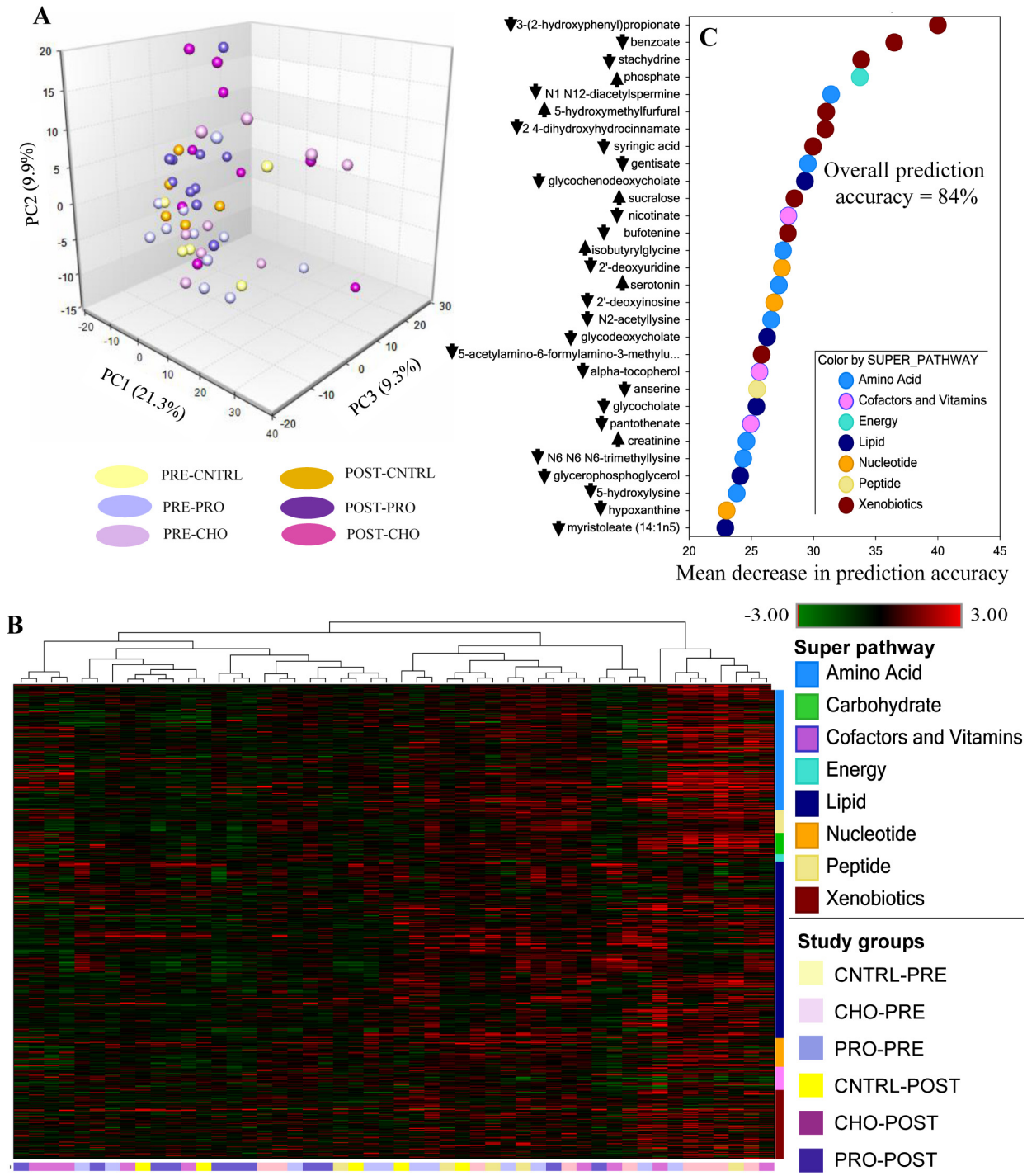

Fig. 3. Stool metabolomics before (PRE) and after (POST) military training. *A*–*C*: principal components (PC), hierarchical complete-linkage clustering of Euclidean distances, and random forest analyses of stool metabolites (n = 25). *A*: individual data points represent metabolite composition within a single individual. Points closer together are more similar. *B*: columns are individuals and rows are metabolites shaded by abundance within a sample. Branches (lines) within the same node (points where branches split) reflect similarity in metabolite composition. Stool metabolites did not demonstrate a distinct clustering pattern. *C*: top-30 metabolites with the strongest influence on prediction accuracy of the random forest analysis presented in order of importance (*top* to *bottom*). Random forest analysis used individual metabolite profiles to predict whether the samples were from pre- or posttraining. Mean decrease in prediction accuracy is the mean decrease in the percentage of observations classified correctly when that metabolite is assigned a random value. Arrows indicate direction of metabolite change from pre- to posttraining. CHO, carbohydrate-supplement group; CNTRL, control group (rations only); PRO, protein-supplement group.

microbiota, and stool metabolites associated with the microbiota suggest that targeting the intestinal microbiota could provide novel strategies for maintaining intestinal barrier integrity during physiological stress.

The increase in IP in association with increased inflammation (Fig. 1) is consistent with the only other study to our knowledge that has assessed IP in military personnel during training (29). In these environments, intense or prolonged

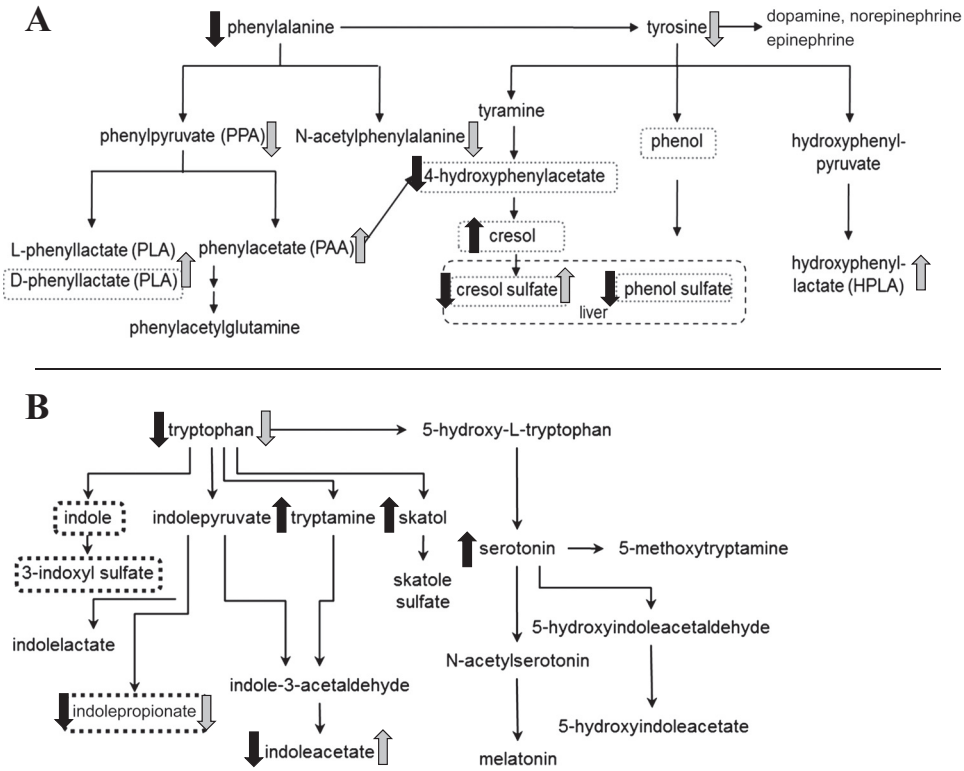

Fig. 4. Qualitative changes in phenylalanine and tyrosine (*A*) and tryptophan (*B*) metabolites in stool and plasma during military training. Arrows indicate direction of change in stool (black) and plasma (gray) from pre- to posttraining (repeated-measures ANOVA, main effect of time, $Q < 0.10$). Metabolites circled by dashed line are compounds known to be wholly or partially derived from microbial metabolism. Compounds without arrows were either unchanged ($Q > 0.10$) or not detected.

exercise may reduce splanchnic perfusion, which can trigger intestinal hypoxia, inflammation, and oxidative stress, which collectively degrade intestinal barrier integrity and increase IP (15, 24, 54). Stress-induced muscle damage may also contribute to inflammation, potentiating increases in IP by inducing tight junction dysfunction (15). Ultimately, the increase in IP is thought to result in mild endotoxemia and inflammation and contribute to gastrointestinal distress in endurance athletes (4,

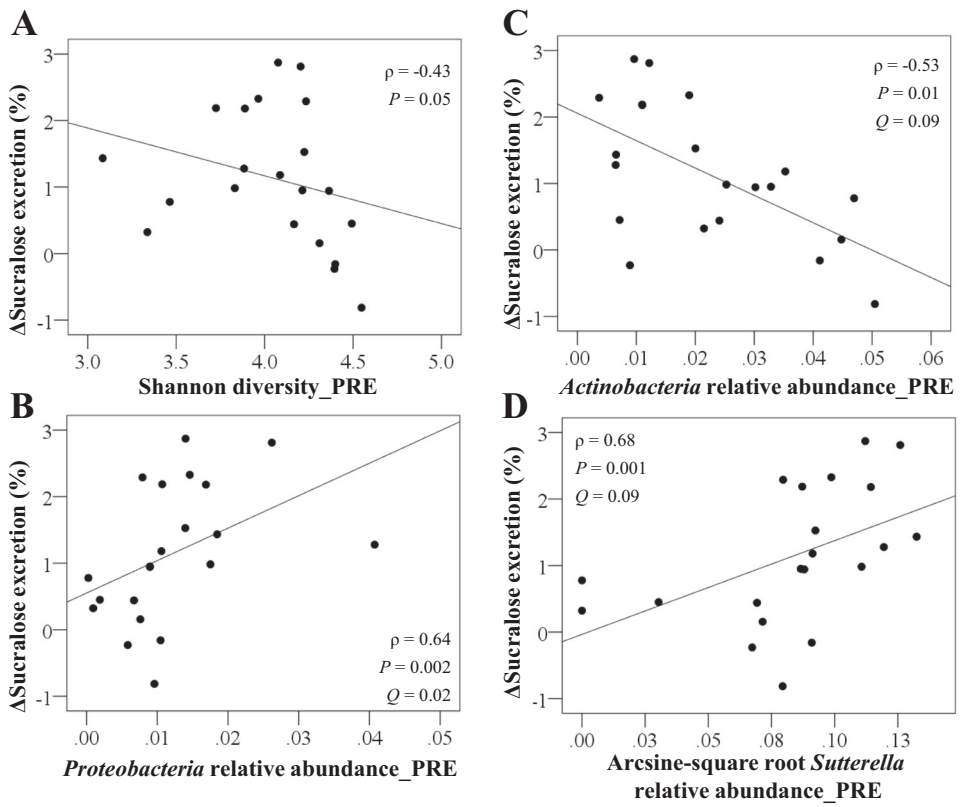

Fig. 5. Factors associated with increased IP during military training (*A–D*). IP was measured by 24-h urine collection following ingestion of 2 g of sucralose [Spearman's correlation (ρ), $n = 21$]. *P* values for correlations with taxa were adjusted using the Benjamini-Hochberg correction (*Q*).

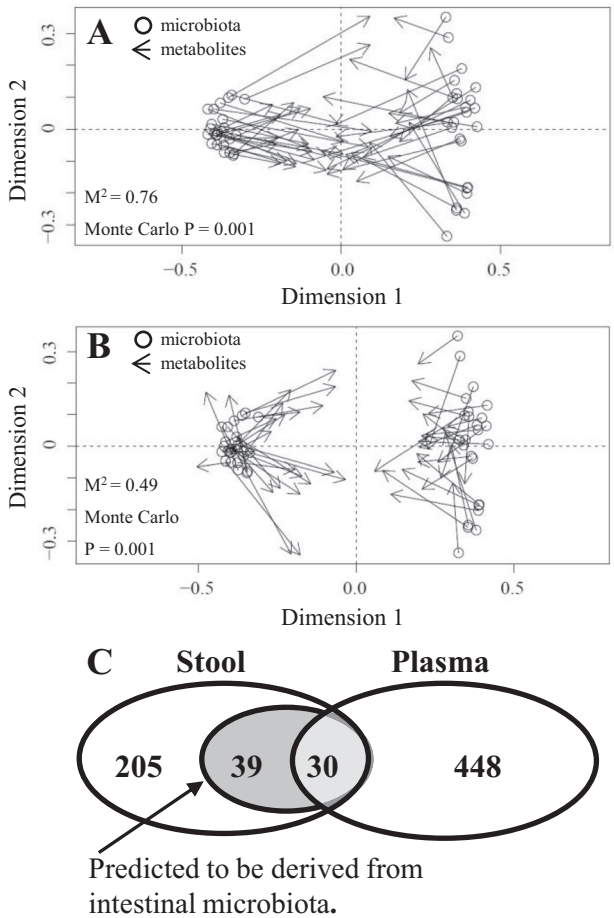

Fig. 6. Stool microbiota composition is associated with stool metabolite and plasma metabolite concentrations. Procrustes analysis of stool microbiota data ordinated using principal coordinates analysis of Bray-Curtis distances and stool (*A*) and plasma (*B*) metabolite profiles ordinated using principal components analysis. The first 3 components of each ordination were extracted and analyzed using Procrustes rotation, which attempts to rotate ordinations to maximal similarity. Circles, stool microbiota community of a single individual before or after military training; arrowheads, stool or plasma metabolite profile of a single individual before or after military training. Vectors connect microbiota composition with metabolite profiles of the same individual for each time point. Longer vectors indicate greater intraindividual dissimilarity. The fit of each Procrustes rotation over the first 3 dimensions is reported as the M^2 value. *P* values were calculated after 1,000 permutations. Results indicate similar clustering patterns between stool microbiota composition and stool metabolites and between stool microbiota composition and plasma metabolites. *C*: Venn diagram of stool and plasma metabolites that were significantly altered during military training ($Q \leq 0.10$). Prediction models linked changes in stool microbiota composition to 69 of the metabolites found to be altered in stool, 30 of which were also significantly altered in plasma.

15, 20, 24) and possibly military personnel (29). Although gastrointestinal symptoms were not assessed in the present study, in the study of Li et al. (29), 70% of soldiers participating in a 6-wk combat training course reported gastrointestinal distress symptomology (i.e., abdominal pain, diarrhea, and constipation); these symptoms were more frequent in soldiers with the largest increases in IP and were associated with psychological decrements. Gastrointestinal distress, to include infectious diarrhea, is historically the leading nonbattle injury encountered in deployed military personnel, representing a significant burden to military health care and operational readiness (45–47). Identification of mediators of intestinal barrier responses to severe stress and development of strategies to target those mediators may therefore have substantial benefit for military personnel.

Our findings suggest that the intestinal microbiota may be one mediator of IP responses to severe physiological stress and that targeting the microbiota before stress exposure may be one strategy for maintaining IP. In particular, increasing microbiota diversity and Actinobacteria relative abundance and decreasing Proteobacteria and *Sutterella* relative abundances before stress exposure may be effective in lieu of the observed associations with changes in IP during stress (Fig. 5). Greater microbiota diversity is generally considered indicative of a healthy intestinal ecosystem, having been frequently associated with lower chronic disease risk (11, 19). Similarly, species within the Actinobacteria phylum, including those belonging to the *Bifidobacterium* and *Collinsella* genera, have favorable anti-inflammatory and immunomodulatory effects that may protect the intestinal barrier during stress (2, 42). *Bifidobacterium* strains are included in multistrain probiotics that have demonstrated some, although weak, efficacy for favorably impacting IP in athletes (25, 49). Use of prebiotics such as oligofructose to increase *Bifidobacterium* relative abundance has also been shown to promote intestinal barrier integrity in animal models (6). In contrast, Proteobacteria are endotoxin producers that have been linked to inflammatory bowel diseases and subclinical inflammation (19, 27). *Sutterella*, a genus within the Proteobacteria phylum, has been shown to promote inflammatory bowel disease by inhibiting immunoglobulin A secretion (39). As such, although findings are correlative and the study design precludes determination of causality, the observed associations between the pre-STRESS microbiota and changes in IP during STRESS are plausible and provide potential targets for further study.

To our knowledge, this study is the first to examine intestinal microbiota responses during military training and expands knowledge regarding the temporal effects of exercise and psychological stress on the microbiome, which is largely limited to animal studies at present (12, 34). Human studies have demonstrated that drastic changes in diet impact intestinal microbiota composition (13, 41) by altering the availability of metabolic substrates for intestinal microbes (23). In contrast to these earlier reports, our findings demonstrate alterations in microbiota composition that most likely were not solely attributable to diet and were more pronounced than those commonly reported in human diet studies (Fig. 2). Although potential mechanisms were not directly assessed, changes in immune activity, intestinal inflammation and oxidative stress, and altered hypothalamic-pituitary-adrenal axis and vagal signaling have been postulated as mechanisms through which physical and psychological stress modulate the microbiome (12, 34).

The increase in Shannon α-diversity and the numerous genus-level changes in relative abundance demonstrated that changes in microbiota composition were broadly characterized by an increase in abundance of less dominant taxa at the expense of more dominant taxa such as *Bacteroides* (Fig. 2). This included increased relative abundances of several potentially deleterious and infectious taxa (e.g., *Peptostreptococcus, Staphylococcus, Peptoniphilus, Acidaminococcus*, and *Fusobacterium*) and decreased relative abundances of several taxa thought to deter pathogen invasion, reduce inflammation, and promote immunity (e.g., *Bacteroides, Faecalibacterium, Col-*

Table 2. *Stool metabolites associated with changes in intestinal permeability during military training*

Super Pathway	Sub Pathway	Biochemical Name	ρ	*P* Value	*Q* Value
Amino acid	Leucine, isoleucine and valine metabolism	3-Methylglutaconate	−0.75	0.001	0.05
	Methionine, cysteine, *S*-adenosylmethionine, and taurine metabolism	*N*-acetyltaurine	−0.73	0.001	0.06
		L-Cysteine	−0.70	0.003	0.07
		Taurine	−0.68	0.004	0.08
		N-acetylmethionine sulfoxide	−0.67	0.005	0.09
	Polyamine metabolism	*N*-acetylputrescine*	−0.78	<0.001	0.05
	Urea cycle; arginine and proline metabolism	L-Arginine	−0.70	0.002	0.06
Carbohydrate	Amino sugar metabolism	Glucuronate	−0.68	0.004	0.08
Cofactors and vitamins	Nicotinate and nicotinamide metabolism	Nicotinate ribonucleoside	−0.69	0.003	0.07
Lipid	Endocannabinoid	Linoleoyl ethanolamide	−0.75	0.001	0.05
		Oleoyl ethanolamide	−0.71	0.002	0.06
	Mevalonate metabolism	Mevalonate	−0.71	0.002	0.06
	Phospholipid metabolism	Trimethylamine *N*-oxide	−0.71	0.002	0.06
	Secondary bile acid metabolism	7-Ketodeoxycholate	−0.86	<0.001	0.01
		12-Dehydrocholate	−0.71	0.002	0.06
Xenobiotics	Xanthine metabolism	1-Methylxanthine	−0.76	0.001	0.05

Data represent Spearman's correlation (ρ) of change in metabolite vs. change in sucralose excretion (Post − Pre). *P* values were adjusted using Benjamini-Hochberg correction (*Q* value). *Significantly increased from pre- to posttraining; all other metabolites decreased ($Q < 0.10$; see also Supplemental Table S2).

linsella, and *Roseburia*). As such, an increase in the ratio of less-abundant, potentially harmful taxa to beneficial taxa may explain the unexpected observation that greater increases in diversity during training were correlated with larger increases in IP. However, several alternative explanations exist. Individuals with the lowest pre-STRESS Shannon diversity also demonstrated the largest increases in diversity during STRESS ($r = -0.60$, $P = 0.001$). Therefore, the association between increased diversity and increased IP may be attributable to lower pre-STRESS diversity. Alternately, higher stool microbiota diversity has been correlated with longer intestinal transit time and higher urinary concentrations of potentially harmful degradation products of bacterial protein metabolism (48). In this study, stool and plasma concentrations of protein degradation products did not uniformly change, although they were more commonly decreased in stool and increased in plasma (Fig. 4; see Supplemental Tables S2 and S3). Whether these observations reflect changes in transit time could not be determined from the collected data. Nonetheless, no protein degradation metabolite was independently associated with increased IP or inflammation. This observation contrasts with reports that bacterial protein metabolites induce intestinal barrier damage and inflammation in vitro (56) and suggests that the positive association between protein intake and increases in IP during training was not mediated by bacterial metabolism of diet-derived amino acids.

Table 3. *Model predicting changes in intestinal permeability during military training (STRESS)*

	β ± SE	Standardized β	*P* Value
Actinobacteria relative abundance (pre-STRESS)	−45.0 ± 8.5	−0.59	<0.001
$\Delta\log_{10}$ IL-6 (pg/ml)	0.4 ± 0.6	0.43	0.003
$\Delta\log_{10}$ stool cysteine	−2.4 ± 0.6	−0.43	<0.001
Intercept	1.4 ± 0.3		<0.001
Adjusted $R^2 = 0.84$			<0.001

Dependent variable is change in sucralose excretion (Post − Pre) measured from 24-h urine collection following ingestion of 2 g of sucralose and expressed as percentage of ingested dose ($n = 15$).

Decreased concentrations of several stool metabolites were associated with increased IP (Table 2). Metabolites included two amino acids, arginine and cysteine, which were predicted to be associated with changes in microbiota composition and are plausible modulators of IP on the basis of known physiological functions. Specifically, arginine is a precursor to polyamines required for intestinal mucosal growth and repair and for nitric oxide, a potent vasodilator that may protect intestinal barrier integrity by improving splanchnic perfusion, deterring pathogen invasion, and modulating inflammation (28, 54). It has been reported that arginine supplementation preserves intestinal barrier integrity in various animal stress and intestinal injury models (2), although the effects in humans are less clear (5). Cysteine is an essential component of glutathione, an antioxidant tripeptide critical to maintaining a favorable redox balance in the intestine (10). Phua et al. (44) recently reported that increases in urinary concentrations of a glutathione metabolite, possibly reflecting increased oxidative stress, were associated with gastrointestinal symptomology during military training. Our findings also suggest that interactions between the intestinal microbiota and dietary fat metabolism may impact IP (Table 2). 7-Ketodeoxycholate and 12-dehydrocholate are secondary bile acids derived from bacterial metabolism of bile acids secreted in response to dietary fat intake. Secondary bile acids are recognized as important signaling molecules with functions that are thought to include promotion of gut barrier integrity (52). Collectively, these findings suggest that changes in intestinal microbiota composition and metabolism may impact IP during physiological stress by modulating the availability of amino acid precursors critical to moderating inflammation and oxidative stress and of secondary bile acids.

Study strengths include the provision of diets of known composition providing a range of macronutrient intakes and the integration of physiological, stool microbiota composition, and metabolomics data. However, results should be interpreted in the context of the study design and several limitations. The physically demanding environment coupled with the physiological demands imposed by undereating may have masked some associations and limited generalizability of the findings

but provides unique and novel insights into the temporal dynamics of host-microbiome interactions during prolonged physical stress. While psychological and sleep deprivation stresses were likely also present, we did not quantify those responses. Study participants were predominantly young men, and findings may not be generalizable to older populations or women. Limitations include the correlative nature of associations between outcomes from which causality cannot be determined, despite evidence of plausibility, and limited statistical power for some analyses, especially those including between-group comparisons resulting from only a subset of the full cohort participating in stool collections. The method for measuring plasma LPS concentrations is also a limitation, as it did not quantify endotoxin activity, which is known to vary between LPS forms (18). Nonetheless, the weak association between changes in plasma LPS and IL-6 concentrations is consistent with the well-established proinflammatory effects of the compound (18). Inclusion of metagenomic or transcriptomic analysis of stool samples would have strengthened findings and complemented the metabolomics analysis by allowing more accurate functional predictions of microbiota function. Reliance on stool for measurements of microbiota composition and metabolites is also a limitation, as the composition of the stool may be more reflective of the distal colon than the entirety of the gastrointestinal tract. However, plasma metabolite measurements were included to better capture bacterial metabolism along the full gastrointestinal tract. Finally, logistical constraints prevented more frequent measurements, which would have provided additional insight into temporal dynamics.

In this study a systems biology approach was used to confirm the hypothesis that a multiple-stressor environment can induce increases in IP that are associated with inflammation, as well as intestinal microbiota composition and metabolism. Furthermore, these findings extend the current evidence base by demonstrating that such environments can induce rapid and pronounced changes in the intestinal microbiota and suggest that the pre-STRESS intestinal microbiota and changes in microbial metabolism may be important for mediating intestinal barrier responses to stress. As such, targeting the intestinal microbiota could provide novel strategies for mitigating increases in IP and associated sequelae induced by physically and psychologically demanding environments.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

DISCLAIMERS

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AUTHOR CONTRIBUTIONS

J.P.K., L.M.M., E.H.M., J.W.C., Y.G., A.V.H., M.W.L., R.K., N.C., A.G., R.H., S.M., S.J.M., and S.M.P. conceived and designed research; J.P.K., L.M.M., E.H.M., N.E.M., J.W.C., Y.G., A.V.H., M.W.L., S.M., and S.J.M. performed experiments; J.P.K., A.V.H., M.W.L., and R.K. analyzed data; J.P.K., R.K., N.C., and A.G. interpreted results of experiments; J.P.K. prepared figures; J.P.K. drafted manuscript; J.P.K., L.M.M., E.H.M., N.E.M., J.W.C., Y.G., A.V.H., M.W.L., R.K., N.C., A.G., R.H., S.M., S.J.M., and S.M.P. edited and revised manuscript; J.P.K., L.M.M., E.H.M., N.E.M., J.W.C., Y.G., A.V.H., M.W.L., R.K., N.C., A.G., R.H., S.M., S.J.M., and S.M.P. approved final version of manuscript.

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Research Article

Performance Nutrition Dining Facility Intervention Improves Special Operations Soldiers' Diet Quality and Meal Satisfaction

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ABSTRACT

Objective: To assess the impact of the *Special Operations Forces Human Performance Program* dining facility (DFAC) intervention on patron diet quality and meal satisfaction.

Design: Nonrandomized, controlled time series study using digital food photography and surveys pre-post intervention (0, 4, 8, and 12 months).

Setting: Two Fort Bragg, NC military installation DFACs.

Participants: Volunteers (n = 688 total; n = 573 complete dataset) were US Army active duty soldiers.

Intervention: The DFAC intervention included food choice architecture, new performance-optimizing food recipes to increase nutrient density, revised menus to offer more performance foods daily, and nutrition labeling to influence food choice.

Main Outcome Measures: Daily DFAC nutrient intake and Healthy Eating Index (HEI) 2010 scores.

Analysis: Descriptive and ANOVA statistical analyses were performed between control and intervention groups and from baseline to 4, 8, and 12 months postintervention ($\alpha = .05$; 80% power).

Results: The intervention resulted in a higher posttest HEI score (60.1 ± 8.8 points; +3.4%; $P = .005$) and DFAC satisfaction compared with control (49.0 ± 10.4 points; $P > .05$). Improved intervention HEI scores were attributed to changes in citrus and melon fruit (+46%), red and orange vegetables (+35%), whole grains (+181%), legumes (65%), yogurt (+45%), oils (−26%), and solid fat (−18%) consumption (all $P < .05$).

Conclusions and Implications: These data illustrate that the *Special Operations Forces Human Performance Program* military DFAC nutrition intervention was feasible to implement and was associated with diet quality improvements. Access to high-quality ingredients and recipes may improve soldier meal quality and acceptance in other settings and warrants further investigation.

Key Words: diet quality, dining facility, Healthy Eating Index, military (*J Nutr Educ Behav.* 2018; 50:993−1004.)

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INTRODUCTION

Cafeteria and dining hall interventions that successfully promote change in healthy eating habits typically incorporate some form of informational or educational strategy and incentives to purchase healthy food items.[1−4] The efficacy of these types of interventions was documented by higher sales and improved consumption[1,5] of those items after incentives were removed.[1,3,5,6] Labeling foods using symbols,[7,8] color coding,[9] or caloric content[10] to identify healthy options at the point of choice (POC) was deemed to be effective in facilitating healthy food choices in *ad libitum* dining environments.

Prior research examining diet quality of military service members supported that dietary intakes and eating behaviors are often less than desirable, particularly falling short in the recommended intake of fruit, vegetables, and whole grains while exceeding recommendations for total and saturated

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fats, refined grains, and added sugars.[11–14] Modifications within the military dining facility (DFAC) have improved dietary intakes.[15,16] Promoting performance-optimizing food choices[17,18] in a dining facility environment may encourage more military personnel to adopt new eating behaviors consistent with a healthy lifestyle. Optimizing nutrition is considered a critical component of the US Army Special Operations Command (USASOC) *Human Performance Program* (HPP), the US Army's Performance Triad, and the Chairman of the Joint Chiefs of Staff Total Force Fitness Framework.[19–21]

Performance-based menu standards and guidelines[21] were developed jointly by the USASOC Performance Dietitian Working Group consisting of Army, Navy, and Air Force personnel. These standards were developed using the US Olympic Training Center menu standards,[22] which incorporated *Dietary Guidelines for Americans* 2010 (DGA)[23] as the foundation of the menu and were adjusted as necessary to ensure the revised menu would meet the Joint Subsistence Policy Board Department of Defense 2010 Menu Standards.[24] Subsequently, USASOC dietitians designed a performance nutrition intervention incorporating the new performance-based menu standards and guidelines, complementing the classroom nutrition education program that reinforced choosing high-quality foods within the DFAC. The USASOC students received up to 6 hours of nutrition education depending on their specific program of instruction. The DFAC revisions included food choice architecture involving strategic food placement to increase visibility of higher-quality foods, new performance-optimizing food recipes to increase nutrient density, revised menus to offer more performance foods daily, and population-specific POC labeling to influence food choice.

The study purpose was to assess the effectiveness of the USASOC HPP DFAC nutrition intervention to improve patron diet quality and dining satisfaction compared with a control DFAC without exposure to the experimental intervention.

METHODS

Study Design

The evaluation was conducted using a nonrandomized, controlled trial time series assessment of diet quality and diner satisfaction at 4 data collection time points (Figure 1). The study was executed at 2 separate Fort Bragg, NC military DFACs that served 500–800 soldiers/meal. The US Army John F. Kennedy Special Warfare Center and School DFAC was chosen by USASOC as the test site for the HPP intervention. The 82nd Airborne DFAC was chosen as the control site because of the similar location and specialized training. Both DFACs met Army food service program standards[25] and were open for breakfast, lunch, and dinner at a flat meal rate, with hot entrée and short-order lines as well as self-serve side dishes, a salad bar, a dessert bar, and beverage stations.

The HPP intervention was designed by USASOC registered dietitians with new high-quality, nutrient-dense foods offered and/or incorporated into recipes (Greek yogurt, walnuts, kale, quinoa, 100% whole-wheat products, etc), along with the reduction or elimination of saturated fats in food preparation, at each meal of the 21-day menu cycle. Food choice architecture included the placement of fruits, vegetables, whole grains, and other high-quality items in high-visibility areas whereas nutrient-poor items were more inconvenient to acquire. For instance, diced fruit were placed on the salad bar and dessert lines, vegetables were first on the entrée line, whereas high-fat foods were placed at the end of the serving line. Food choices were labeled with the Fresh-Lean-Clean-Perform color-coded concept at the POC to promote quick decisions on the serving lines (Figure 2).

Data were collected before the new HPP DFAC intervention implementation (baseline), at 4 months when the majority of new foods and menus were in place, at 8 months when the POC labels were operational, and at 12 months to examine whether changes persisted over time. Nutrition education (5 hours) was provided before the baseline assessment. The control DFAC was included to account for seasonal changes in food preferences or availability, or other situations that might act independently of the HPP intervention to change patron food selection choices and/or diner satisfaction.

The Institutional Review Board of the US Army Research Institute of Environmental Medicine approved the study and written informed consent was obtained from all volunteers after an informational briefing.

Participant Population

The HPP intervention DFAC served US Army Special Operations Forces soldiers and Special Forces Qualification Course students preparing to become Army elite soldiers (Green Beret, Ranger, etc), who had already served as conventional soldiers and were board-selected for the extensive training program. The control DFAC served soldiers from the 82nd Airborne Division who were also trained in specialized operations; however, they did not receive the same extensive training as Special Operations, but received more so than the conventional soldier and were 1 step closer to the elite soldier status. New participants were recruited at each data collection iteration. Participants were included if on active duty status and aged $\geq$18 years. No screening exclusion criteria were set; however, participants who did not consume at least 1 breakfast, lunch, and dinner were excluded from analysis. Participants were not monetarily compensated for study participation.

Number of Participants and Sample Size Estimations

The researchers hypothesized that the control group would not exhibit a significant change in diet quality or satisfaction pre- to posttest, whereas the HPP DFAC menu and POC labeling enhancements might be associated with improved dietary intake and Heathy Eating Index (HEI)-2010 scores in the intervention group. Statistical Software for the Social Sciences (SPSS) (version 3.0.1, SamplePower, IBM, Chicago, IL; 2013) was used to estimate sample size; power was set at 80% with α at .05 (2-tailed

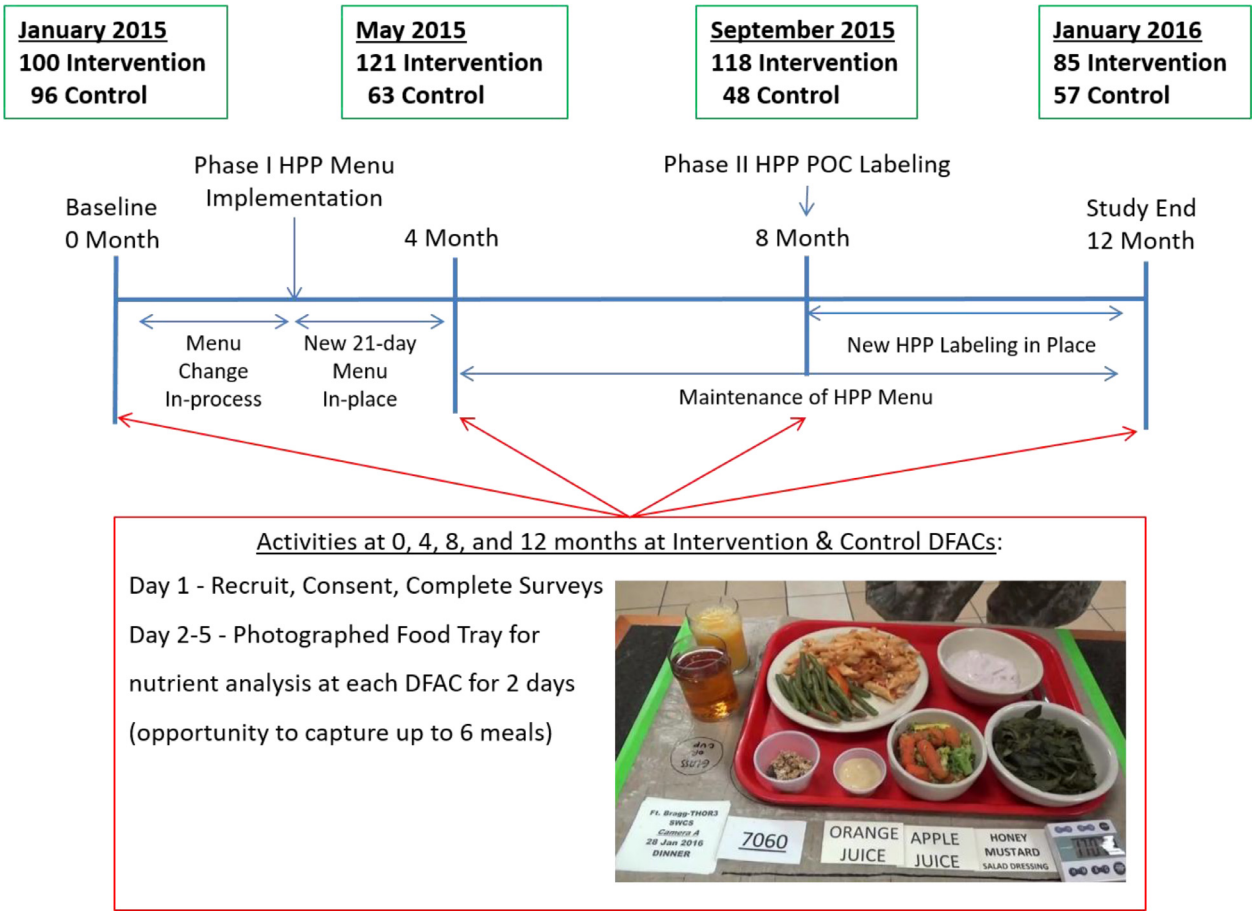

Figure 1. *Human Performance Program* (HPP) dining facility (DFAC) intervention implementation and evaluation time line. POC indicates point of choice.

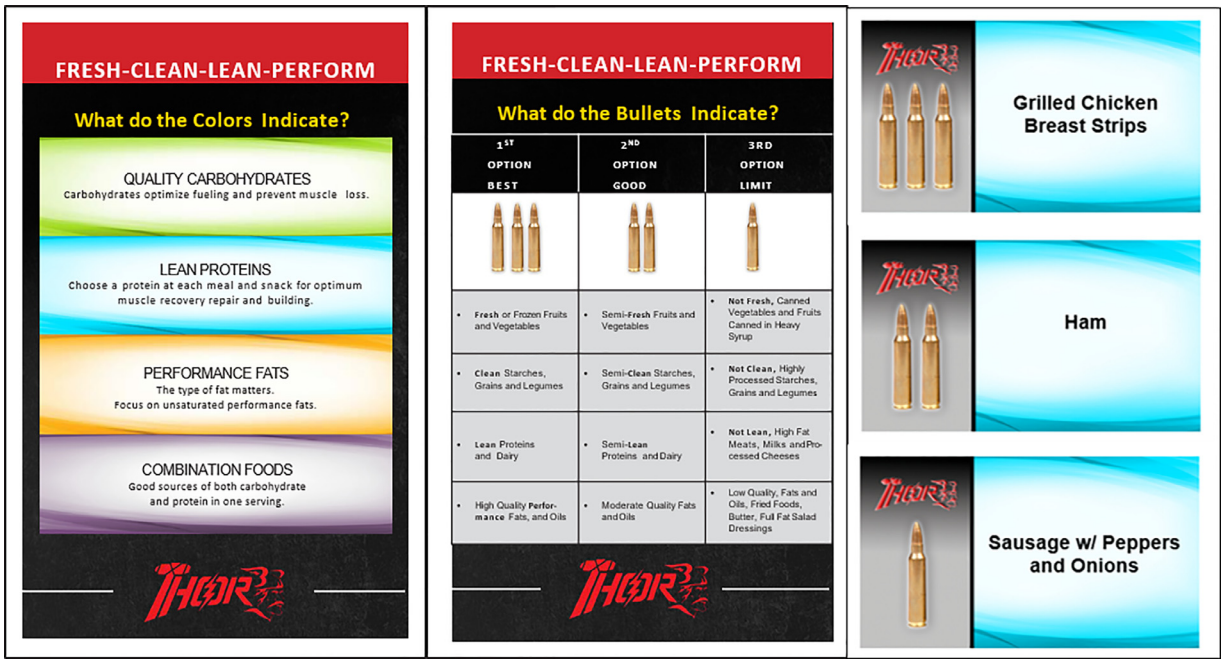

Figure 2. Fresh-clean-lean-perform point of choice food labeling for the *Human Performance Program* dining facility intervention.

analysis). The HEI scores between groups at posttest were anticipated as a 15-point mean score difference with a 30-point SD (effect size of 0.5) requiring 64 participants/group.

Instruments

Demographic survey. A paper demographic questionnaire was administered the day of recruitment to capture age, self-reported height and weight, ethnic and racial background, highest education level, military rank, the number of meals and snacks, and the most common location of meals and snacks.

Food photography. Food photography is an expedient and reliable tool to assess the nutrient intake of patrons in a fast-paced eating environment, such as a military dining facility.[16,26] Previous studies with adults found this procedure to be highly reliable and valid.[26–28] Before participant meal photography, standardized reference portions of food items were weighed and recorded by the lead Pennington Biomedical Research Center collaborator to assist in determining the weight of the food items on each tray compared with the actual weight of each standardized reference portion. A total of 643 food reference standards were collected over the study period. At each meal, food and beverages selected by study participants before and after eating were photographed using digital video cameras. Camera angles and distance were standardized to allow the apparent size of all foods to remain consistent across patrons and standardized food reference photographs.[26] Participant-specific deidentified code numbers were used on food trays before and after eating to link participant survey responses with nutritional intakes.

Digital photographs of reference portions, the patron food selection, and patron plate waste were entered into a computer application designed to estimate food portion sizes (Food Photo 2.0, Pennington Biomedical Research Center, Baton Rouge, LA; 2008). Two trained Pennington research associates used the software to view all photos simultaneously and estimate each food portion in the photographs to a tenth of a gram, with a third investigator available to adjudicate inconsistent estimates. Dietary intake was calculated as the difference in each participants' food and beverage items chosen before eating (pre-meal) and the food and beverages not consumed (post-meal) and did not include snacks consumed outside the DFAC. Recipes and food intake estimates were entered into a data entry grid in the computer software application for food composition analysis using the Food and Nutrient Database for Dietary Studies (version 2.0).[29]

Dining facility customer satisfaction survey. A 17-item DFAC satisfaction paper survey was used to assess patrons' opinions about DFAC food service operations related to DFAC food sensory qualities (3 questions about taste, texture, temperature, and appearance); availability of healthy and performance-based main dishes (6 questions), side dishes and dessert options (4 questions), as well as the usefulness of the new POC food labeling (4 questions). The DFAC satisfaction survey items were rated on a 7-point Likert scale ranging from strongly agree to strongly disagree. The survey was pretested with the sample population and used regularly by DFAC staff. It was not formally validated, but the Cronbach α and split-half α for the 17 items were .95 and .96, respectively.

Outcome Measures and Data analysis

The primary outcomes of the study were change in dietary intake and diet quality scores. Secondary outcomes of change were DFAC customer satisfaction and assessment of food plate cost.

Dietary intake. Dietary intake was compared between DFACs (intervention and control), by meals, and within each DFAC over the 4 time periods. Macronutrients were converted to kilograms per body weight. The mean number of food servings consumed daily from each DFAC was compared with the 2015 US DGA[30] (based on a mean daily intervention intake of 2,200 kcal). Dietary intake from food photography was reported as cups of total fruit, citrus and melon fruit, and fruit juice; total vegetables and dark green vegetables; total red and orange vegetables; total starch vegetables and vegetable legumes; total dairy, milk, and yogurt; ounces of total grains, whole grains, and refined grains; total protein, meat/protein/eggs, seafood, soy/nuts/seeds, and protein legumes; and grams of oils, solid fats, and added sugars.

Healthy Eating Index scores. Diet quality was assessed using the HEI-2010 score, a tool to measure diet quality as it relates to the 2010 US DGA and is based on nutrients per 1,000 cal.[31] The HEI-2010 was calculated from the average of 3 daily DFAC meals using the digital food photography dietary intake data. The HEI-2010 evaluated 12 components: total fruit, whole fruit, total vegetables, greens and beans, seafood and plant protein, total protein, whole grains, refined grains, dairy, fatty acids, sodium, and empty calories. Component scores ranged from 5 to 20 points, with a maximum total score of 100 points. Diet quality was classified as poor quality (≤50 points), needs improvement (51–80 points), and good quality (81–100 points); the US National HEI score average over the past 10 years ranged from 48 to 57 points.[31–33]

Plate cost. Plate cost was determined by the actual cost spent to produce the meal divided by the number of customers fed. Intervention cost feasibility was based on the standard basic daily food allowance of $13.11 to produce 3 meals on a daily basis.

Statistical analysis. Descriptive demographic analysis is reported as mean ± SD or frequency and percentage, depending on the scale of measurement. Body mass index (kg/m^2) was calculated from self-reported height and weight. Independent-sample *t* tests and nonparametric chi-square with contingency coefficient analyses were used to assess whether baseline demographic, snacking frequency, and meal location differences existed between the control

Table 1. Military Dining Facility Participant Demographic Descriptive Data Obtained at Baseline Data Collection (n = 573)

Variables	Intervention (n = 380)	Control (n = 193)	*P* Between Dining Facilities
Current age, y (mean [SD])	27.7 (5.8)	22.4 (3.2)	<.001
BMI, kg/m^2 (mean [SD])[a]	26.3 (2.7)	25.3 (3.0)	<.001
Military service, y (mean [SD])	5.4 (5.9)	2.4 (2.8)	<.001
Males, n (%)	380 (100)	185 (96)	ns
Race, n (%)			<.001
Caucasian	344 (90)	144 (75)	
African American/black	13 (3)	27 (14)	
Asian	6 (2)	4 (2)	
Other	17 (5)	18 (9)	
Ethnicity (Hispanic), n (%)	31 (8)	40 (20)	<.001
Education, n (%)			<.001
Up to high school/General Equivalency Diploma	46 (12)	107 (55)	
Some college to AAS	169 (45)	71 (37)	
Bachelor's degree	148 (39)	13 (7)	
Graduate degree	17 (4)	2 (1)	
Rank, n (%)			<.001
Junior Enlisted (E1-E4)	205 (54)	1,747 (91)	
Non-Commissioned Officer (E5-E7)	62 (16)	18 (9)	
Warrant Officer Candidate (WOC)	56 (15)	0	
Warrant Officer (WO1–CW4)	34 (9)	0	
Company-Grade Officer (O1–O3)	21 (6)	0	

AAS indicates Associates degree; BMI, body mass index (calculated from self-reported height and weight); E1–E4, Private, Private First Class, and Specialist; E5–E7, Sergeant, Staff Sergeant, and Sergeant First Class); ns, not significant; WO1–CW4, Warrant Officer grades 1–3 and Chief Warrant Officer; O1–O3, Second Lieutenant, First Lieutenant, and Captain.
[a]BMI was calculated from self-reported height and weight.
Notes: Data were combined for all 4 study iterations. Independent sample *t* tests and nonparametric chi-square and contingency coefficient analyses were used to assess whether baseline differences existed between the control and intervention groups.

and intervention groups as well as changes in HEI diet quality from pre- to postintervention. One-way ANOVA was conducted to assess changes in dietary intake and HEI-2010 over the 4 time points (0, 4, 8, and 12 months) for each DFAC instead of repeated measures because new participants were recruited for each data collection. Tukey *post hoc* analysis was performed to identify where statistical differences resided within a DFAC and between the distinct time points. However, data are reported as baseline (pretest) and consolidated mean values for 4-, 8-, and 12-month data (posttest) for ease of viewing and comparison. Data for the control DFAC were consolidated for 0, 4, 8, and 12 months for tabular data representation owing to nonsignificant results over time.

RESULTS

Subject Demographics

A total of 688 soldiers were enrolled in the intervention (n = 428) and control (n = 260) DFACs over 4 iterations; however, only 573 soldiers consumed at least 1 breakfast, lunch, and dinner meal and had complete demographic data (380 intervention and 193 control participants). Participants missing food photography data were not assessed in the data. Table 1 depicts demographic data stratified by DFAC. The overall sample was predominantly male, mean age 25.9 ± 5.7 years, mean body mass index 26.0 ± 2.8 kg/m^2, and mean 4.4 ± 5.1 years of active duty service. There were significant yet expected demographic differences between intervention and control participants. Of the overall sample, 83% of participants reported consuming breakfast, 92% lunch, and 96% dinner at least 5 times/wk. Significantly more intervention participants reported consuming an early morning snack (after physical training conducted before breakfast) compared with the control group (29% vs 10%; $P < .001$), as well as a morning snack (28% vs 15%; $P = .001$), lunch (96% vs 83%; $P < .001$), and an afternoon snack (42%

Table 2. Healthy Eating Index-2010 (HEI-2010) Score Comparison Between Intervention and Control Groups (n = 573)

HEI-2010 Component and Total Score	Control (0–12 Mo) (n = 193) (Mean [SD])	Intervention (0 Mo) (n = 96) (Mean [SD])	Intervention (4–12 Mo) (n = 284) (Mean [SD])	Change	% Change	Pre-Post *P*
HEI 5-point category						
Total fruit	2.8 (1.9)	2.9 (1.6)	3.3 (1.7)	+0.4	+13	ns
Whole fruit**	1.5 (1.8)	1.8 (1.6)	2.3 (1.9)	+0.5	+26	.02
Total vegetables**	3.5 (1.3)	4.3 (1.0)	4.0 (1.2)	−0.3	−7	.02
Greens and beans**	1.7 (1.8)	3.7 (1.6)	3.3 (1.9)	−0.4	−11	ns
Total protein*	3.4 (1.4)	3.0 (1.3)	4.0 (1.2)	+1.0	+35	<.001
Seafood and plants Protein**	1.7 (1.8)	2.5 (1.9)	3.0 (1.8)	+0.5	+21	.01
HEI point category						
Dairy	6.7 (3.1)	7.2 (3.0)	6.4 (3.0)	−0.8	−11	.03
Whole grains**	1.4 (2.0)[a]	1.1 (1.6)	3.0 (2.7)	+1.9	+181	<.001
Refined grains**	7.4 (2.7)	8.7 (1.8)	8.8 (2.0)	−0.1	0	ns
Fatty acids**	5.9 (2.5)	7.6 (2.3)	6.9 (2.5)	−0.7	−9	.02
Sodium**	2.7 (2.6)	1.1 (1.6)	1.3 (1.8)	+0.3	+26	ns
HEI 20-point category						
Empty calories**	14.0 (4.8)[b]	17.3 (3.3)	17.9 (2.5)	+0.6	+3.4	ns
HEI 100-point total score						
Total HEI score**	49.1 (10.4)	56.7 (8.8)	60.1 (9.0)	+3.4	+6	.002

ns indicates not significant.
[a]Control pre-post change: −0.6 points whole grains ($P = .05$); [b]Control pre-post change: +1.9 points empty calories ($P = .01$). One-way ANOVA was conducted to assess changes over 4 time periods with Tukey *post hoc* analysis. Significant changes were identified pretest to 4 months in the intervention dining facility HEI scores, which were maintained over time (no significant differences in 4- to 12-month assessments). Thus, data are reported as baseline (pretest) and the consolidated mean values for 4-, 8-, and 12-month data (posttest); *$P < .05$; **$P < .001$ between control and intervention patrons at baseline.

vs 31%; $P = .01$). Meals were most commonly consumed at a military DFAC, whereas snacks were commonly obtained from the participant's home or barracks regardless of snack regularity.

Healthy Eating Index-2010 Scores and Dietary Intake

Table 2 depicts the component and total HEI-2010 scores between intervention and control groups. Significant changes were identified from pretest to 4 months in the intervention DFAC HEI scores, which were maintained over time (no significant differences in 4- to 12-month assessments). The total HEI-2010 score for intervention participants increased 3 points to reach 60 points over 4–12 months ($P = .002$), compared with the control group, which remained consistent over the 4 time points (range, 48.0–50.7 point; $P > .05$). Intervention participants had increases in whole fruits, total protein, and seafood and plant protein, and decreases in total vegetables, dairy, and fatty acids, limiting the overall total HEI-2010 score improvement.

The HEI-2010 quality categories (poor, needs improvement, and good-quality diet) were significantly different between groups. A significant transition in diet quality occurred for intervention patron at all 3 meals (not reported in Table 2): 28% poor diet quality pretest to 14% at posttest, 71% needs improvement pretest to 81% posttest, and 1% good-quality diet for both pre- and posttest ($P < .001$). The distribution of HEI-2010 score categories for the control group was nonsignificant (51% to 53% poor quality and 41% to 49% needs improvement) and no control patrons fell within the good-quality diet category.

Dietary intake classified by food group servings provided the detail needed to understand why the HEI-2010 scores changed after the HPP DFAC intervention. These are noted in Table 3 along with the 2015 US DGA[30] as a reference for recommended dietary intakes. Intervention patrons exhibited a significant decrease in caloric density (kilocalories per kilogram body weight) whereas nutrient density increased in several areas: citrus and melon fruits, fruit juice, red and orange vegetables, legumes as vegetable and protein sources, whole grains, and yogurt. Although improvements were noted, the mean intervention patron intake did not meet 2015 DGA recommendations for

Table 3. Dietary Intake Comparison Among the Intervention Group, Control Group, and 2015 *Dietary Guidelines for Americans* (DGA) (n = 573)

Variables	2015 DGA	Control (0–12 Mo) (n = 193) (Mean [SD])	Intervention (0 Mo) (n = 96) (Mean [SD])	Intervention (4–12 Mo) (n = 284) (Mean [SD])	Pre-Post *P*
Total calories, kcal	2,200	2,017 (836)	2,210 (644)	2,013 (730)	.02
Kcal/kg BW	25	26.3 (11.8)	26.8 (8.3)	24.0 (8.8)	.007
Protein (g/kg BW)**	0.8-1	1.2 (0.5)	1.5 (0.5)	1.4 (0.5)	ns
Carbohydrate (g//kg BW)*	3-12	3.1 (1.5)	2.9 (1.1)	2.7 (1.2)	ns
Fat (g/kg BW)*	<1	1.1 (0.5)	1.1 (0.38)	0.9 (0.4)	<.001
Total fruit (cups)*	2	1.4 (1.4)	1.5 (1.3)	1.7 (1.5)	ns
Citrus and melon fruit (cups)*	N/A	0.6 (0.8)	0.5 (0.6)	0.7 (0.9)	.006
Fruit juice (cups)**	N/A	0.3 (0.6)	0.3 (0.6)	0.5 (0.8)	.03
Total vegetables (cups)**	3	1.8 (1.1)	3.1 (1.5)	2.4 (1.4)	<.001
Dark green vegetables (cups)**	0.3	0.2 (0.3)	0.6 (0.4)	0.4 (0.4)	<.001
Total red orange vegetables (cups)**	0.9	0.3 (0.3)	0.6 (0.5)	0.8 (0.7)	ns
Total starch vegetables (cups)*	0.9	0.8 (0.6)	1.0 (0.7)	0.6 (0.7)	<.001
Legumes, vegetables (cups)**	0.3	0.1 (0.1)	0.1 (0.1)	0.2 (0.3)	<.001
Total dairy (cups)	3	2.4 (1.9)	2.7 (1.7)	2.1 (1.6)	.002
Milk (cups)	N/A	1.5 (1.6)	1.8 (1.5)	1.0 (1.2)	<.001
Yogurt (cups)	N/A	0.1 (0.3)	0.2 (0.3)	0.3 (0.4)	.007
Total grains (oz)	7	5.0 (2.8)	4.1 (2.2)	3.9 (2.5)	ns
Whole grains (oz)	3.5	0.5 (0.7)	0.4 (0.6)	1.1 (0.1)	<.001
Refined grains (oz)	3.5	4.5 (2.7)	3.7 (2.2)	2.9 (2.0)	<.001
Total protein (oz)**	6	7.1 (3.2)	10.4 (3.5)	10.4 (4.1)	ns
Meat, poultry, eggs (oz)**	4.0	4.9 (2.6)	6.7 (2.5)	6.5 (3.3)	ns
Seafood (oz)**	1.3	0.5 (1.0)	1.2 (1.8)	1.2 (1.8)	ns
Soy, nuts, and seeds (oz)**	0.7	0.4 (0.9)	0.9 (1.7)	0.9 (1.4)	ns
Legumes, protein (oz)**	N/A	0.2 (0.6)	0.3 (0.5)	0.9 (1.0)	<.001
Oils (g)	29	24.0 (15.0)	30.6 (15.1)	22.7 (15.9)	<.001
Solid fats (g)**	16	34.5 (18.1)	31.6 (14.9)	25.8 (12.2)	.001
Added sugar (g)**	32	15.1 (11.3)	12.6 (8.5)	10.9 (8.0)	ns

BW indicates self-reported body weight; N/A, not applicable; ns, not significant.
*P <.05; **P <.01 differences between control and intervention at baseline.
Notes: One-way ANOVA was conducted to assess changes over 4 time periods with Tukey *post hoc* analysis. Data are reported as baseline (pretest) and the consolidated mean values for 4-, 8-, and 12-month data (posttest).

fruits, vegetables, dairy, grains (particularly whole grains), or solid fats, all of which contributed to overall assessment of a needs improvement diet quality.

There were pre- to posttest changes in several control group food servings without a significant impact on the HEI scores (not reported in Table 3): citrus and melon fruit (−0.4 cups; −47%), fruit juice (+0.2 cups; +231%), legumes as protein sources (+0.3 oz; +677%), whole grains (−0.3 oz; −41%), cheese (+0.3 oz; +53%), and oils (+4.5 g; +22%) (all P < .05).

Opinions on Dining Satisfaction

Intervention patrons' opinions about DFAC food service significantly improved on 11 of 17 food-related customer satisfaction items postintervention, whereas the control patrons' satisfaction remained consistent over the 12-month study (Table 4).

Plate Costs

The plate cost of the HPP intervention increased to a temporary maximum of $14.20/d during the first 6

Table 4. Dining Facility Customer Satisfaction Ratings Between Intervention and Control Groups (n = 573)

DFAC Customer Satisfaction	Control (0–12 Mo) (n = 193) (% Agree)	Intervention (0 Mo) (n = 96) (% Agree)	Intervention (4–12 Mo) (n = 284) (% Agree)	Intervention Pre-Post *P*
Appearance of the food is pleasing	70	61	67	.47
Flavor and taste of the food are good	60	64	71	.31
Choices available are adequate	51	48	64	.03
Availability of healthy foods is adequate	61	48	70	<.001
Availability of performance foods is adequate	58	40	61	<.001
Portion sizes are appropriate*	35	43	56	.49
Availability of fresh fruit is adequate	62	41	61	.02
The salad bar offers a variety of fresh vegetables*	71	53	64	.002
The main dishes served are healthy and performance-based	55	32	67	<.001
The side dishes are served without added fat (eg, butter)	50	42	56	.008
Healthy and performance-based dessert choices are available	45	17	42	<.001
Temperature of food (eg, hot food is hot) is just right	44	20	42	.002
Vegetarian food choices are available	62	60	78	<.001
I find the DFAC nutrition labels easy to use*	65	38	40	.14
Nutrition labels provide knowledge to make performance-based choices*	63	39	47	.02
I use the DFAC nutrition labels to choose healthy foods*	46	37	33	.20
I use the DFAC nutrition labels to choose performance foods*	46	36	36	.22

DFAC indicates dining facility.
*$P < .02$ between control and intervention patrons at baseline.
Notes: One-way ANOVA was conducted to assess changes over 4 time periods with Tukey *post hoc* analysis. Data are reported as baseline (pretest) and the consolidated mean values for 4-, 8-, and 12-month data (posttest).

months of implementation followed by cost stabilization to $12.05 to $12.95/d between 8 and 12 months meeting the standard maximum allowance of $13.11.

> Incorporating high-quality food ingredients and recipes within the military dining facility menu cycle may improve diet quality of military patrons.

DISCUSSION

The study purpose was to assess the effectiveness of the USASOC HPP DFAC nutrition intervention to improve patron diet quality and dining satisfaction compared with a control DFAC. This study demonstrated the effectiveness of the USASOC HPP performance menu guidelines to improve diet quality without compromising meal satisfaction. The primary finding was that the DFAC intervention produced modest but persistent increases in patron diet quality regardless of season, because dietary intake improvements were maintained 4–12 months postintervention. The intervention patrons had a diet quality score 11 points higher than that of the control DFAC patrons. The control DFAC currently operates within the Department of Defense foodservice guidelines and its patrons' HEI closely mirrored that of the US National HEI, ranging from 48 to 57 points.[31–33] The improved intervention patrons' HEI-2010 scores may be attributed to choosing new intervention foods rich in protein, fiber, vitamins, minerals, and phytonutrients while reducing refined grains, starchy vegetables, oils, and solid fat. Previous military DFAC studies found similar soldier food selections improvements after recipe and menu modifications, but not as comprehensive as in this study.[16,34] Crombie et al[16] assessed dietary intake at 1 lunch meal at 5 military DFACs with increased availability of fruits, vegetables, whole grains, and reduced foods high in dietary fat and sugar compared with 5 military DFACs with no

intervention. They found a resultant patron decrease in dietary intake of total calories, total and saturated fat, and refined grains but no significant improvement in fruit and vegetable intake. Similarly, Belanger and Kwon[34] assessed dietary intake at a lunch meal after increasing the availability of fruits, vegetables, whole grains, lean proteins, and lower-fat dessert and short-order bar items and found a decrease in total calories, total fat, saturated fat, and sodium intake. Although a positive shift was noted in the current study patrons' diet quality, the majority of patrons still exhibited an HEI score requiring improvement, with deficiencies noted in several beneficial nutrient-dense food groups (ie, whole grains, dairy, and dark green and red or orange vegetables).

Before the intervention onset, several leaders expressed a concern that changing DFAC food ingredients, recipes, and the menu cycle would have a negative impact on patron dining satisfaction. On the contrary, intervention patrons' satisfaction for 11 of 17 ratings increased significantly over the course of the 12-month study. The DFAC satisfaction in conjunction with soldiers' improved diet quality confirm intervention feasibility and effectiveness. Past research within the military DFAC setting either demonstrated increased satisfaction with portion size, overall food choice, and flavor after recipe modifications[16] or reported no difference in customer satisfaction outcomes.[34]

> Implementing food choice architecture within the military dining environment may positively influence food choice without detriment to meal satisfaction.

Food choice architecture and POC labeling within the DFAC may positively influence food choice; however, research on the effectiveness of POC labeling to promote healthy food choices yielded mixed results. Food choice architecture in a Finland military study found that providing healthier food items within the military environment decreased the selection of higher-fat and sugar-containing foods, although it did not influence fruit and vegetable consumption.[15] Sproul et al[35] found that 60% of military patrons acknowledged observing the promotional labels, but 79% of those were not influenced by the labels when making food selections. Arsenault et al[9] identified 47% of military patrons' self-reported use of POC traffic light–style color-coded food labels, which was associated with lower fat intake. Two studies conducted by Thorndike et al[36,37] in a hospital cafeteria setting demonstrated that the use of the traffic light color-coded food labels along with food choice architecture promoted increased sales of healthier green-coded beverage options whereas sales of red-coded items decreased. Christoph et al[38] identified through a college dining hall study that only 20% of college students used the POC nutrition labels for food selection although 46% were aware of the presence of the food labels. Vyth et al[39] found in a Danish worksite study that although foods sales did not increase after nutrition labels were implemented at POC, patrons who reported using the food labels also reported an intent to eat healthier and pay attention to the labels. In the current study, POC labeling was introduced just before the 8-month data collection and continued through to the 12-month evaluation. At the 12-month study end, nearly twice the intervention patrons agreed that the POC labeling provided knowledge to make better selections of performance-based foods, yet the HEI-2010 remained essentially unchanged (+1 point). The POC signage was positioned within the DFAC without staff or patron training regarding how to use the labeling system to maximize dietary choice. Thus, inadequate POC marketing might have partly contributed to the lack of additional positive effects on diet quality. The current results may also be interpreted to indicate that HPP food-choice architecture creates greater dietary change than does the POC labels.

Initially, intervention soldiers were expected to have higher HEI-2010 scores compared with control soldiers because of covariates of age (eg, higher education, more years of military service, and higher military ranks), greater interest in personal health within the Special Operations Forces, and nutrition education. The USASOC HPP performance nutrition program incorporated up to 6 hours of nutrition education provided by a registered dietitian nutritionist (RDN). Education is specific to performance optimization and injury mitigation and was administered before the HPP DFAC intervention was implemented. Nutrition education about the value of food in health and resilience was found to contribute to healthy food choice decisions.[40,41] This education component is unique to Special Operations Forces and was not provided to the control garrison soldiers. This may have contributed to higher than expected baseline intervention HEI-2010 scores between intervention and control patrons and to the magnitude of change that occurred after the new foods were introduced into the HPP intervention DFAC.

Research supports that diet quality increases as the food costs and monetary value of the diet (dollars per day) increase, owing to the costs associated with fresh, lean, and performance-based foods (eg, nuts, beans, fruits, vegetables, whole grains, and lean proteins).[40,42–44] The HPP DFAC intervention further enhanced access and availability to foods that were colorful, flavorful, fresh, and seasonal. It also incorporated new seasonings (for enhanced flavor) and used cost-saving strategies such as bulk purchase and a reduction in prepackaged foods. These changes were implemented across all meals and the subsections of the DFAC including short-order grill, hot entrées, side dishes, salads, fruits, desserts, condiments, and beverages. Food sales of specific items were not captured at the register within these military DFACs because patrons pay a flat rate upon entry. Instead, plate cost is calculated based on the production and waste costs for the number of patrons consuming the meal. Initially, elevated plate costs were associated with new food catalog items, minimum bulk and special requirements by the prime food vendor, difficulty forecasting the use of new foods without

historical consumption estimates, and unfamiliarity of DFAC staff with using and preparing new ingredients and recipes. Despite these challenges, the HPP DFAC intervention successfully met their authorized daily food allowance throughout the program sustainment assessment period. The net effect of the intervention was a higher-quality food plate delivered at an equivalent total cost, which supported Drewnowski's[43] claim that nutrient-dense diets are attainable when following principles of nutrition economics, contrary to other researchers who depicted a higher food cost to achieve a higher diet quality score.[42,44] Expansion of the HPP DFAC program to larger ones within the Department of Defense is feasible, it but would require modifications partially owing to differences in basic daily food allowance.

Certain methods used within the study set it apart from previous dining facility research. The current study used food photography to quantify daily nutrient intakes from 3 meals for enhanced accuracy of actual dietary consumption. Most military diet quality studies used only 1 meal to assess diet quality[16,34] and relied on self-reported food intake data through dietary recall[45] or food-frequency questionnaires.[13,15,46–48] In addition, inclusion of a control group accounted for potential confounding seasonal biases that could have influenced food choices.

> The Registered Dietitian Nutritionists' unique skill set may assist in shaping nutrition opinions, enhancing diet quality, and improving dining satisfaction.

Several study limitations exist. The study followed a time-series research design with new soldiers participating at each data collection time point, thereby introducing potential biases in the group differences observed. When feasible, future assessment of DFAC interventions would benefit from a repeated-measures longitudinal design in which the same soldiers were examined at subsequent time points and covariates were controlled for during analysis. Demographic and customer satisfaction survey data relied on self-reported information and were not formally validated. Another potential confounder was that nutrition education was introduced before the HPP DFAC intervention. Educational strategies to promote performance-based eating behavior effectiveness should be independently examined. Logistical constraints resulted in delayed implementation of POC labeling; thus, intervention patrons did not receive ample exposure to the POC labeling, preventing a clear-cut assessment of this program subcomponent. The HEI data did not include food intake consumed outside the DFAC. The study was not designed to measure the impact of food choice on military performance over time; future research should examine the impact of optimal fueling on military readiness and important health metrics over a longer period than a few months. Furthermore, the findings may not be generalizable to the larger military population or the civilian sector.

IMPLICATIONS FOR RESEARCH AND PRACTICE

The HPP performance dietitians were successful in making high-quality food choices accessible to soldiers, which was positively associated with nutrition-related behaviors. This program supported the USASOC HPP goals as well as the US Army's Performance Triad and the Chairman of the Joint Chiefs of Staff Total Force Fitness Framework.[19,20] Adoption of this HPP DFAC program across all USASOC DFACs may result in overall improved diet quality for this special military population; however, for military-wide adoption, adjustments in the basic daily food allowance and/or modifications to the menu may be needed to ensure economic feasibility. Military leaders can capitalize on the expertise and unique skill set of RDNs to shape positive opinions about nutrition, enhance diet quality, and improve dining satisfaction within the military environment. In addition, RDN expertise is needed to translate evidence-based performance nutrition research and reassess current regulations and policies related to soldier fueling.

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United States Army
Medical Research & Materiel Command

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Evaluation of a Dining Facility Intervention on U.S. Army Special Operations Soldiers' Meal Quality, Dining Satisfaction, and Cost Effectiveness

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The study purpose was to test the initial effectiveness of this USASOC HPP (Human Performance Program) intervention and whether changes were sustained over time. Dietary intakes were assessed using food photography before and after three main meals over 2 days. Daily nutrient content and Healthy Eating Index (HEI) scores were computed. Descriptive and independent t-test analyses were performed pre to post HPP implementation (α=0.05; 80% power). A total of 688 Soldiers participated(98% male; mean age of 25.6±2.9 yrs). At 12-months, USASOC patrons exhibited a significantly higher sodium-adjusted HEI score (70.3±8.7 points; +4.6 pts; p=0.005) compared to the control DFAC (avg of 56.3 ±10.8 points over 12-months). Specific improvements in USASOC patron intake were +0.6 cups fruit (2.5 cups/d), +0.8 oz whole grains (5.9 oz/d), +1.9 oz protein (14.9 oz/d; 2.0g/kg BW), -0.3 oz refined grains (4.3 oz/d), and -0.13g/kg fat (1.24g/kg BW) ($p<0.05$). These data illustrate that education, introduction of healthy food options, and revised cooking practices are effective interventions for improving Warfighter meal quality.

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BACKGROUND

The Joint Chiefs of Staff and the Army Surgeon General consider nutrition as one of the pillars of health and readiness. Soldiers' eating behaviors are generally less than ideal with suboptimal consumption of fruits, vegetables, and other nutrient-rich foods. As approximately 74% of non-deployed military personnel consume at least one meal per day in military dining facilities (DFAC), interventions that encourage performance-based food choices and nutritious eating behaviors in this dining environment have the potential to positively influence eating patterns of large numbers of military personnel. The USASOC Human Performance Program (HPP) dietitians developed an intervention for implementation in one of their DFACs that included education, a shift to a performance-based menu and a population-specific point-of service labeling system in an attempt to improve their Soldiers' eating behaviors. The aim of this investigation was to test the feasibility, effectiveness, and sustainability of this intervention approach.

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EXECUTIVE SUMMARY

The USASOC Human Performance Program (HPP) dietitians developed a comprehensive strategy designed to improve Special Operations Forces (SOF) Warfighter eating behaviors through nutrition education and dining hall food changes that included a novel performance-based menu and point-of-service labeling system. The study purpose was to test the initial effectiveness of this HPP intervention and whether changes were sustained over time. Comparison to a best practice Army DFAC was included to control for seasonal changes in eating behavior. Diet quality through food photography, food cost relative to the plate cost, DFAC satisfaction, satiety and eating rate, and food service management practices were assessed at baseline, and 4, 8 and 12 months after initiating the intervention. Daily nutrient content and Healthy Eating Index (HEI) scores were computed. Descriptive, pre to post t-test, and ANOVA statistical analysis from baseline to 4, 8, and 12-month post-intervention were performed (α=0.05; 80% power). A total of 688 (98% male; mean age of 25.6±2.9 yrs) Soldiers participated. At 12-months, USASOC patrons exhibited a significantly higher sodium-adjusted HEI score (70.3±8.7 points; +4.6 pts; p=0.005) compared to the control DFAC (avg of 56.3 ±10.8 points over 12-months). The HPP nutrition program exceeded the national HEI score (ranging 48-57 pts). The improved HEI scores for USASOC patrons were attributed to significant increases of 0.4 cups/d fruit, 0.4 cups/d red/orange vegetables, 0.8 cups/d whole grains, 0.9 cups/d protein-based legumes, and 0.6 oz/d protein (p<0.05). USASOC patrons also exhibited significant reductions of 0.5 cups/d refined grains, 0.6 cups/d starchy vegetables, 0.9 cups/d milk (although 0.2 cups/d increase in yogurt), 11 g/d of oil, and 6 g/d of solid fat (p<0.05). These data illustrate that education, introduction of healthy food options, and revised cooking practices are effective interventions for improving Warfighter meal quality.

INTRODUCTION

A search of PubMed (performed 25 Sep 2014) and the Defense Technical Information Center (DTIC; performed 30 Sep 2014) was conducted using the keywords: Dining Facility (or Dining Hall); Special Forces (or Military); Healthy Eating Index 2010 (or HEI 2010); Menu Planning; Healthy Eating; and Performance. These searches identified a gap with limited research supporting military dining facility nutrition interventions.

The worksite environment is an ideal medium for promoting change in health habits, particularly in relation to nutrition.[1] Interventions in civilian worksite and university cafeterias have been effective in changing eating behaviors.[2] Successful interventions incorporate some form of informational or educational strategy[2], and incentives for purchase of healthy food items have resulted in higher sales[3-7] and improved intakes[7,8] of those items after incentives were removed.[5-8] Labeling foods based on energy density[9], as healthy choices[10,11], or suggesting alternatives within the nutrition labeling format at point-of-service[12], as well as using verbal prompts to encourage food selection have also been deemed effective to facilitate healthy food choices in ad libitum dining environments.

Approximately 74% of non-deployed military personnel consume at least one meal per day in military dining facilities (DFAC)[13], and as such, interventions that encourage performance-based food choices and eating behaviors in this dining environment could influence eating patterns of large numbers of military personnel. A study of the use of labels in an Army cafeteria did not effectively increase sales of target "healthy" entrees, whereas taste and food quality had the greatest impact on menu choice.[14] Fiedler and colleagues[15] incorporated "heart-healthy" menus at one of two DFACs that served US Air Force Basic Military Trainees, and demonstrated that the "heart-healthy" facility patrons reduced daily dietary fat intake from

35% to 19% by the end of basic training, while patrons of the standard garrison DFAC increased fat intake. Additionally, the Diet Quality Index improved only in trainees frequenting the "heart-healthy" facility, and customer satisfaction indicated customer acceptability of the "heart-healthy" menus. However, diners at DFACs in basic training installations are often "coached" on food selections by cadre,[16] thus the impact of this type of food service intervention on truly ad libitum eating remains to be seen. In an earlier USARIEM DFAC intervention with five of ten DFACs on Fort Bragg, NC, modest menu enhancements resulted in reductions in energy intake, total fat, and percent energy from fat and saturated fat, with no impact on fruit or vegetable intakes between the intervention and control groups despite positive customer satisfaction ratings.[17]

DFACs that serve the Army Special Operations Forces (ARSOF) community, specifically those engaged in special operations selection, assessment, and training operations, are faced with providing nutritional support for a population that often expends 140% of the typical garrison Soldiers daily expenditure.[18] Hence, the SOF leaders have specific interest in enabling performance-based nutrition to sustain high intensity operations, and to facilitate recovery between missions, training, and ultimately during dwell time between deployments. Across the DoD, data depicts Soldiers' intakes are less than ideal, to include low intakes of fruits, vegetables, and other nutrient rich foods.[13,19] Nutrient density is the ratio of nutrients to the amount of kilocalories in a food or beverage item and contains a substantial amount of vitamin, minerals, antioxidants, and/or fiber per serving.

The US Army Special Operations Command (USASOC) Human Performance Program (HPP) includes the Tactical Human Optimization Rapid Rehabilitation & Reconditioning ($THOR^3$) program. The objective of the USASOC HPP program is to provide a comprehensive, multidisciplinary training and treatment program designed to enable the sustained operations

required of ARSOF and to ensure peak readiness, preserve unit integrity, and prolong the careers of the ARSOF operators. The USASOC HPP dietitians designed a performance nutrition intervention incorporating classroom nutrition education and dining facility changes that included performance- based recipes, revised menus, and population-specific point-of-service labeling (Figure 1) for initial execution at the US Army J.F. Kennedy Special Warfare Center & School (SWCS). After receiving approval from the Army G-4 (finance), the Defense Logistics Agency (DLA), and the Joint Culinary Center of Excellence (JCCoE) to implement the USASOC HPP DFAC intervention, USARIEM was tasked with determining whether the program was acceptable from the perspective of patrons and dining hall staff, whether the intervention improved the self-selected diet quality of the patrons, was sustainable over time, and its feasibility for expansion.

Military Relevance

The Chairman of the Joint Chiefs of Staff and The Army Surgeon General promote nutrition and healthy eating as one of the pillars/domains of health and total fitness.[20,21] Establishment of a high food quality and nutrient-rich menu in combination with a point-of-service labeling and nutrition education program has the potential to improve the eating behaviors and diet quality of Soldiers with related downstream improvements to their health, wellness, and resiliency. This research supports the Military Operational Medicine Research Program's Task Area B: Recovery Nutrition.

Study Objectives & Hypotheses

Primary Objectives

1. Assess the effectiveness of the USASOC HPP DFAC nutrition intervention (new recipes, revised menus, and an unique food labeling system) and population-specific

education on patron diet quality, quantity, and dining satisfaction compared to current practice of a garrison DFAC not exposed to the experimental interventions while controlling for confounding effects from season (e.g., weather dependent food choices, seasonal foods).

2. Determine the average food plate cost during baseline feeding, after making substitutions to DFAC foods, and after addition of labeling, to compare against diet quality. Data will be used to make informed decisions regarding benefit of the intervention relative to cost.

Secondary Objective:

1. Assess if the DFAC intervention is accompanied by changes in Soldiers' self-reported lifestyle behaviors.
2. Determine if the DFAC intervention is associated with changes in subjective rating of appetite/satiety before and after eating in the DFAC.
3. Capture DFAC staff perspectives on barriers, challenges and experiences related to their respective DFAC operations.

Hypotheses

1. Healthy Eating Index 2010 (HEI-2010) scores and nutrient quality will be higher post-HPP DFAC enhancement compared to baseline assessment and the standard garrison DFAC.
2. The DFAC customer satisfaction ratings will be higher post-HPP DFAC enhancement compared to baseline and the garrison DFAC.
3. The HPP DFAC enhancement will result in changes in self-reported non-nutritional program lifestyle choices.

4. The HPP DFAC enhancement will be associated with a greater level of satiety between meals.
5. Average plate cost at the HPP DFAC will be comparable to that of the standard garrison DFAC.

RESEARCH DESIGN AND METHODS

Study Design

The evaluation was conducted using a non-randomized control trial time-series assessment with four data collection time points (Jan 2015, March 2015, September 2015, and January 2016). It was executed at two separate dining facilities (DFAC); an experimental and a control DFAC. The Control DFAC was included to account for seasonal changes in food preferences/availability or other situations acting independently from the HPP intervention to change food selection choices. Thus, significant change was not expected for the Control DFAC. The evaluation was conducted before the new HPP DFAC intervention had taken place (0 Month), at 4-months when the majority of new foods and menus were in place, at 8-months when the point-of-service (P-O-S) labels were introduced, and at 12-months to examine if any changes persisted over time (Figure 2).

Participant Population

The experimental DFAC served US Army Special Forces Soldiers and Special Forces Qualification Course (SFQC) students within the US Army John F. Kennedy Special Warfare Center and School (SWCS), Fort Bragg, NC. The Control "Falcon Inn" DFAC served Soldiers from the 2nd Brigade Combat Team (2BCT), 82nd Airborne Division. Each DFAC served between 500-800 Soldiers per meal at the time of the study.

Inclusion and Exclusion Criteria

Participants were included if on Active Duty status, adults (18 years and older), willing to consume three meals each day at the DFAC for two consecutive testing days. A participant could volunteer for subsequent data collection, if willing and available to participate. No exclusion criteria were set. Participants were not monetarily compensated for study participation; however, they were offered the privilege of moving to the front of the line at the DFAC on study days to allow maximum time for meal consumption and tray photography.

Number of Participants/Sample Size Estimations

It was hypothesized that the Control group would not yield a significant change pre to post-test, whereas the USASOC HPP DFAC menu and point-of-service labeling enhancements would result in improved dietary intakes and Heathy Eating Index 2010 (HEI) scores. Thus a significant improvement in eating behavior between the USASOC HPP DFAC intervention and Control groups was expected. SPSS SamplePower 3.0.1 (IBM, Chicago, IL) was used to estimate sample size; power was set at 80% with an alpha at 0.05 (two-tailed analysis). HEI scores between groups at post-test was anticipated to be a 15-point mean score difference with a 30-point standard deviation (effect size of 0.5) requiring 64 participants in each DFAC group.[22]

USASOC HPP DFAC Intervention

The USASOC HPP DFAC intervention (internally known as THOR^3; Tactical Human Optimization, Rapid Rehabilitation and Reconditioning) included modifications to the recipes and menu, food choice architecture to increase accessibility of higher quality foods, and a point-of-service labeling system (Appendix A). In addition, a nutrition education component, consisting of 1-6 hours of classroom instruction external to the SWCS DFAC facility, was

introduced to the SWCS training programs. This population-specific nutrition component emphasized the importance of snacking and food choices made within the DFAC to enhance task-specific cognitive and physical performance and promote recovery. Prior to the HPP DFAC intervention, several improvements were implemented within the DFAC, such as food choice architecture (strategic food placement) of fruits and vegetables within the serving area; however all revisions were consistent with existing Army Food Service Regulations. A two-phased implementation approach was used to assess recipe and menu revisions separately from the new point-of-service labeling.

1. During Phase I (months 0-4), menu enhancements were introduced at SWCS one 21-day cycle prior to the four month assessment. These modifications included:
    a. Addition of vegetable and vegetable juice blends (enhanced potassium, antioxidants, fruit and vegetable availability/convenience).
    b. Addition of Greek yogurts (high quality lean protein) to dessert areas and minimized choices offered that were high in saturated fat and sugar (e.g., bakery).
    c. Addition of plain tuna to the salad bar (high quality lean protein and omega-3 fatty acids).
    d. Addition of walnuts and/or mixed nuts to the salad bar (monounsaturated fats and omega-3 fatty acids).
    e. All bread options included 100% whole wheat option (complex whole grains) alongside of the refined white flour bread option.
    f. Pre-cut fruits were available on the salad bar and in the dessert areas (enhanced fruit availability and convenience).
    g. Placement of vegetables at the start of the serving line (enhanced vegetable availability).
    h. Reduction or elimination of saturated fats (e.g., use low fat mayonnaise or yogurt for prepared salads; replace butter with olive oil in recipes).

i. Addition of fresh fruit-infused water as a beverage option and elimination of sugary beverages (e.g., soda) from beverage options.

Note that although more whole grains and leaner protein sources were offered, high fat protein sources, refined grains, and some other lower quality foods were still offered after the HPP DFAC enhancement intervention.

2. During Phase II (5-8 months), introduction of the point-of-service labeling system occurred. The labeling system possessed similarities to the Army's color-coded Go For Green® program, which uses the stoplight approach to describe menu items (green = eat often, yellow = eat in moderation, red = eat rarely); however the HPP nutrition color coding system (blue = lean protein, purple = combination foods, orange = performance fats, green = quality carbohydrates) promoted fresh, lean, clean and performance-based, population-specific, macronutrient descriptors. The label incorporated the THOR^3 icon and an ammunition-style graphics (bullet casing icons) to describe the acceptability of the menu items (3-bullet icons = Best choice, 2-bullet icons = Good Choice, 1-bullet icon = Limit these choices) for constructing a nutritious meal. This labeling system is user-friendly and resonated with the SOF community. Refer to Appendix B for an example.

The SOF performance-based menu standards and guidelines[23] were developed jointly by the U.S. Special Operations Command Performance Dietitian Working Group consisting of Army, Navy and Air Force personnel within SOF. These standards were developed from the U.S. Olympic Training Center menu standards[24] as the foundation of the menu, which incorporated Dietary Guidelines for Americans 2010,[25] and then scrutinized to ensure the revised menu would meet the Joint Subsistence Policy Board DoD Menu Standards 2010.[26]

USASOC HPP DFAC Evaluation Timeline

The evaluation by USARIEM was conducted over four time periods to adequately assess baseline, HPP DFAC enhancement implementation, point of service labeling and program maintenance. Recruitment, study enrollment, and data collection occurred at both DFACs (SWCS and 2BCT) at each time point (Figure 2) as highlighted below. Note that 2BCT control DFAC was encouraged to continue food service operations as usual and included the use of the Go For Green® point-of-service labeling system.[27]

1. Baseline: Intent was to provide an understanding of patron diet quality and customer satisfaction prior to implementation of the USASOC HPP DFAC program.
2. Four Months: Intent was to examine change in nutrient intake and customer satisfaction following the initial implementation of new recipes and menu modification.
3. Eight Months: Intent was to examine the impact of the new point-of-service labeling.
4. Twelve Months: Intent was to examine the maintainability and cost effectiveness of the HPP DFAC performance nutrition intervention.

Research Procedures

Digital Photography Method.

The digital photography method was used to quantify food selection and intake of enrolled study participants. At each meal, foods and beverages selected by the DFAC participants prior to and after eating were photographed using digital video cameras. Photographs of participant meal trays did not include personally identifiable information (e.g., a person's face or uniform name tag). Camera angle and distance was standardized to allow the apparent size of all foods to remain consistent across photographs (Figure 3). Up to four camera stations were set up to photograph incoming trays (food selection), and outgoing post-meal

trays (plate waste) in an expedient manner. Participants could bring and return trays to any of the photography stations. Food trays were numbered to match individual trays before and after eating according to each participant's specific de-identified code number used on the paper survey in order to link participant survey responses with nutritional intakes. Pennington Biomedical Research Center (PBRC) staff was trained to man all camera stations, assisted by USARIEM research staff. A second camera at baseline was used at two of the four camera stations in order to evaluate whether an additional camera simplified and enhanced food estimation accuracy. Tray stations using two cameras during the baseline assessment eased adjudication time and improved nutritional intake assessment; thus subsequent data collections included two cameras at each camera tray station to improve efficiency.

Prior to participant meal photography, standardized reference portions of all possible food choices were weighed and recorded for comparison to the digital photographs of the participants' food trays. Digital photographs of all reference portion, patron food selection, and patron plate waste were incorporated into a computer application designed for estimation of food portion sizes (Food Photo 2.0, PBRC). Two research associates from PBRC used the software to simultaneously view all photos and estimate each food portion in the photographs to a tenth of a gram. Patron food intake was defined as the difference between food selection and plate waste. The food intake estimates were entered into a data entry grid in the computer software application for statistical analysis and food composition analysis using the PBRC nutrient database. PBRC uses the Moore's Extended Nutrient Database (MENu), which contains USDA food composition data from both the Food and Nutrient Database for Dietary Studies (NHANES database) and the Standard Release (SR) database. Recipes specific to those used in SWCS and 2BCT DFACs were entered into this system. Information on nutrient intake specific to each participant was generated. Previous studies with adults found this procedure was highly reliable and valid.[28,29]

The dietary measures included (but were not limited to): total calories, servings of food groups (fruit, vegetables, dairy, protein, grains, discretionary calories, and food group sub-categories), macronutrient (fat, protein, carbohydrate), fiber, added sugar, fatty acids, vitamins (including antioxidant and phytonutrients), and minerals.

Healthy Eating Index 2010 (HEI-2010).

The HEI-2010 is a tool to measure diet quality as it relates to the 2010 Dietary Guidelines for Americans and is based upon nutrients per 1,000 calories (kcal). Dietary intake data, captured from the photographed meal analysis, was used to calculate the HEI-2010 diet quality scores. The HEI-2010 evaluates 12-domains with a maximum number of points per domain ranging from 5-20 points and a maximum HEI-2010 total score of 100 points (Table 1). Nine categories assess nutrient and food group adequacy, while the remaining three categories assess foods that should be consumed in moderation. The domains with their respective maximum points in parentheses are total fruit (5 pts), whole fruit (5 pts), total vegetable (5 pts), greens and beans (5 pts), whole grains (10 pts), dairy (10 pts), total protein (5 pts), seafood and plant protein (5 pts), fatty acids (10 pts), refined grains (10 pts), sodium (10 pts), and empty calories (20 pts). Complete detail on the domains of the HEI-2010 can be accessed at http://riskfactor.cancer.gov/tools/hei/. Diet quality is classified as poor quality (≤50 points), needs improvement (51-80 points) and good quality (81-100 points). The US National HEI score average over the past ten years has ranged from 48-57 points.[30-32]

Paper Surveys. Two surveys were completed immediately after participants were enrolled in each study group at the 0, 4, 8 and 12-month iterations.

1) Demographics & Lifestyle survey (Appendix C) captured self-reported participant demographic data and lifestyle information to describe the sample populations. The survey

included: age, self-reported height and weight, ethnic and racial background, highest education level, rank, two questions related to meal timing and location, as well as habits and perceptions regarding physical activity, screen time (TV, video games, computer), hours of sleep, level of sleepiness/alertness, and the impact of DFAC food choices on several performance and wellbeing factors. The short international physical activity questionnaire (IPAQ) and Stanford Sleepiness Scale (SSS) were incorporated into the survey. The IPAQ is used to quantify daily vigorous intensity activity, moderate intensity activity, walking and sitting (open access at http://www.ipaq.ki.se/scoring.htm). The SSS is a 7-point scale assessing the level of sleepiness ranging from fully alert to extremely sleepy (open access at http://web.stanford.edu/~dement/sss.html).

2) Dining Facility Satisfaction (Appendix D) questionnaire assessed the sensory qualities (e.g., taste, texture, temperature, and appearance) of foods provided and consumed in the DFAC, food availability, thoughts on quality and health impact, and labeling of performance enhancing foods. The DFAC satisfaction survey was comprised of 17-items rated on a 7-point Likert Scale ranging from Strongly Agree to Strongly Disagree.

Satiety Labeled Intensity Magnitude (SLIM) Scale.

The SLIM scale is a tool assessing the degree of hunger or fullness at the time of pre-meal and post-meal tray photography. The SLIM scale is a continuous visual analog scale on a 100-mm line with descriptive labels for self-perceived hunger and fullness. Scoring ranged from -100 points (greatest level of hunger) to +100 points (greatest level of fullness). Participants were asked to view the SLIM scale and identify the level of hunger or fullness that represents their current state by drawing a horizontal line crossing the scale (Appendices E & F). The change in SLIM score was calculated as the difference from the Pre and Post eating scores to identify the degree of fullness (satiation). Research has

identified that the level of hunger is impacted by meal and snack timing, and often influences the types of food selected and degree of post-meal fullness (satiety).[33-35] The SLIM scale has been shown to be a sensitive, reliable, and an easy-to-use scale for measuring perceived satiety.[36]

The PreSLIM scale was also used to document the tray photography time, the last time food was consumed, and the type of snack (if applicable) (Appendix E). The Post SLIM scale also documented if the participant had enough time to eat (yes or no), meal length (shorter, typical or longer than usual), and an estimation of eating rate (ranging from relatively fast to relatively slow) (Appendix F). Three additional questions (dependent on time availability) were asked at post-meal photography to provide additional insight into customer satisfaction immediately following meal consumption: How satisfied are you with your meal selection today? If you could recommend one change in the DFAC, what would it be? If a significant amount of plate waste was noted, the investigator asked for a reason for leftover food on the plate.

Foodservice Staff Focus Group Sessions.

Focus group sessions were conducted at each of the DFACs at 4, 8, and 12-month study iterations. The purpose of focus groups was to capture foodservice staff experiences, challenges, and suggestions for DFAC improvement. Published studies using focus group sessions for health-related outcomes ranged in sample size of 10-60 participants[37-40]; thus 30 DFAC staff members from each DFAC at each iteration was considered sufficient to reach thematic saturation. Focus groups were formed based upon supervisory and non-supervisory positions. Questions for the focus group were developed collaboratively with the USARIEM dietitians, the HPP dietitians, and the DFAC food service advisors. Each focus group session was designed to host up to10 volunteers and lasted approximately 60

minutes. Sessions were audio recorded, and transcribed to draw unique and common themes. Consolidated data from the sessions were provided to leadership.

Data analysis

Descriptive demographic analysis is reported as mean ± standard deviation (SD) or frequency / percent depending on the scale of measurement. Data transformations included:

Dietary Intake: Foods and beverages were consolidated into top food and beverage choices for each DFAC over the four time periods based upon frequency of foods consumed. Patron nutrient intake was assessed as a daily average of three meals; patrons who did not consume at least one of each meal were excluded. Nutrient intake was compared between DFACs (SWCS and 2BCT), meals (breakfast, lunch, and dinner), and within each DFAC over the four time periods. Macronutrients (protein, fat and carbohydrate) were converted to kilograms (kg) per body weight (BW) and percent of total calories (kcal) to account for participant differences between height and weight. SWCS food group serving data were compared to the USDA 2015 dietary recommendations, based upon energy needs set at 2800 calories per day.[41]

HEI Score: HEI-2010 total and domain scores were assessed as a daily average of 3 meals for each DFAC patron and stratified by meal. Total HEI score was adjusted to account for the liberal military sodium recommendations; military dietary reference intake (MDRI) for this population recommended at no more than 5500 mg as opposed to the USDA recommendation of no more than 2300 mg for the US adult. Total HEI and Total HEI sodium-adjusted scores were categorized into Poor Quality diet (0-50 points or less), Needs Improvement (51-80 points), and High Quality (81-100 points). HEI-2010 scores and percent HEI classification were compared between DFACs (SWCS and 2BCT), meals (breakfast, lunch, and dinner) and for each DFAC over the four time periods.

Self-reported Demographic & Behavioral Data: Data for the overall sample were stratified by Army Physical Fitness Score (APFT; < or ≥ 280 points) and compared with nutrient intake and HEI diet quality categories.

SLIM Scale: Time to eat was defined as the time participants had access to their meal tray and was calculated as the difference in time documented at pre-meal versus post-meal. Snacks were classified into six categories: no snack, prepackaged protein, carbohydrate only, healthy food mix (protein + carbohydrate), meal size mix of foods, empty calories (high fat or sugar), and caffeinated snack. The hours since last meal was calculated based upon the previous post meal time. If the post meal time was missing, the following median between meal times were used: 3.5 hours between breakfast and lunch, 5.5 hours between lunch and dinner, 2 hours for time between a snack and meal, 13 hours from evening snack to early morning snack, and 15 hours from dinner to breakfast. Perceived hunger (PreSLIM score), time to eat, time since last meal, and snack type were compared to nutrient intake to identify an association between hunger level, self-reported eating rate, and food choice. Satiation (change in SLIM scale score) was compared to eating rate, nutrient intake, food groups, and HEI-2010 scores. The data were assessed as a collective data set and also compared between DFACs and by meal.

Plate Cost: Average plate cost was determined by the actual total cost of the food prepared and divided by actual headcount (number of customers fed). The USARIEM investigators coordinated with the onsite DFAC Senior Food Advisor for this information. Data were collected monthly between Jan 2015 and May 2016; an additional six months of assessment provided a full year of plate cost data after USASOC HPP DFAC intervention implementation.

DFAC Staff Focus Group Analysis:

Focus group sessions were transcribed from audio recordings prior to analysis. Participant responses were linked to an ID code to assess between study iteration, DFAC and

supervisory or non-supervisory role. Responses were also coded for discussion categories, patterns, and themes. The major categories in each discussion were further defined by coded sub-categories. Direct quotes were utilized to illustrate major and sub-categories with specific examples. Focus groups verbatim transcripts were managed and analyzed using Microsoft Word 2007 (Microsoft, Redmond, Washington) and SPSS Version 21 (IBM, Chicago, IL).

Statistical Analysis: Independent sample t-tests were used to assess if baseline differences existed between the control and intervention groups as well as changes from Pre- to Post-intervention. One-way analysis of variance (ANOVA) were completed to assess changes in eating behaviors and related outcomes over the four time points (0, 4, 8, 12-months) for each DFAC. Tukey's post-hoc analysis was performed to identify where statistical differences resided within a DFAC and between the DFACs at discrete time points. However, data was often documented from baseline (Pre-Test) for each DFAC and compared against the consolidated mean values for 4, 8, and 12-month data (Post-Test) for viewing ease. Data for 2BCT were consolidated for 0, 4, 8, and 12 month data for tabular data representation. Pearson's r or Spearman rho correlation assessment was performed to assess associations between nutrient intakes, HEI-2010 scores, appetite / satiety data, and lifestyle habits. Multiple regression analysis was performed to identify predictors of satiety based upon significant correlations. Food photography data between the first and second cameras were compared with the established technique to assess changes in efficiency of food intake estimation and adjudication.

RESULTS

Subject Demographics

A total of 688 Soldiers were enrolled between SWCS (n=428) and 2BCT (n=260) DFACs over

four iterations (Jan 2015, May 2015, Sep 2015, Jan 2016). Demographic descriptive data for the overall sample and stratified by DFAC are depicted in Tables 2 & 3. Overall 85% of the participants were Caucasian (13% Hispanic), 69% were junior enlisted (E1-E4), and 43% had some college education. The mean age of the overall sample was 25.6 ± 5.5 yrs with a BMI of 26.0 ± 2.9 kg/m^2, 4.1 ± 4.9 yrs of active duty service and, 6.5 ± 1.1 hrs of sleep, 5-7 hrs/d of daily screen time (including computer, TV, and video games), and scored 275.1 ± 22.2 points on the Army Physical Fitness Test (APFT). There were significant, yet expected, differences between SWCS and 2BCT patrons in age, BMI, years of active duty service, race, ethnicity, education, rank, physical activity levels, APFT scores, screen time, and hours of sleep. Overall 83% of the Soldiers reported they felt like they were in 'good' to the 'best' shape of their life, however self-reports were higher for SWCS than 2BCT patrons (88% vs. 76%; p<0.005). More SWCS patrons scored 280 points or higher on the APFT than 2BCT patrons (62% vs. 34%; p<0.001). Significantly more SWCS patrons reported feeling alert during the day compared to 2BCT patrons (63% vs. 44%; p<0.001) and also had more hours of nightly sleep (6.7 hrs vs. 6.2 hrs). Sixty-seven percent of the SWCS patrons were current students at the U.S. Army John F. Kennedy SWCS and had 5 hours of nutrition education included within their program of instruction during the 12-month study.

DFAC patrons' self-reported meal pattern and common eating location are highlighted in Table 4. Of the overall sample, 80% consumed breakfast, 92% consumed lunch, and 96% consumed dinner at least 5 times per week. Significantly more SWCS DFAC patrons reported consuming early morning snack (29% vs. 13% 2BCT; p<0.001), morning snack (28% vs. 16% 2BCT; p<0.001), and lunch (96% vs. 86% 2BCT; p<0.001). Meals were most commonly consumed at a military DFAC, whereas snacks were commonly obtained from the patron's home or barracks regardless of number of days (regularity) consumed.

Changes to the HPP DFAC Enhancement Intervention

The HPP DFAC intervention implementation rolled out as scheduled except for a delay in the point-of-service labeling. The original intent was to implement the new bullet icon labels at 7 months in order to assess if labeling facilitated a change in patron dietary intake at 8-months; however, the new point-of-service labeling was not implemented until a few days prior to the 8-month assessment for a variety of reasons: 1) The Army Food & Menu Information System (AFMIS) was unavailable for new recipe addition, which impacted forecast and purchase of new food ingredient; 2) Food ingredients and recipes choices on specific menu days exceeded plate cost and required food ingredient and menu-cycle adjustment; 3) DFAC staff required additional training on new recipes and menu enhancements (e.g., quinoa should be a cooked and not served raw); and 4) Food service equipment limitations resulted in manpower inefficiencies and schedule revisions. Menu and recipe modifications, although implemented at 4-months, continued to be revised and improved upon between the 4 and 8-month data points. Dietary intake at the 12-month data collection might be a better indicator of point-of-service (P-O-S) labeling impact on food choice. Also notable was that 2BCT implemented a "Healthy Bar" consisting of high quality food items (e.g., fruit, vegetables, nuts, whole grains) just prior to the 12-month data collections, that may have influenced patron choice beyond that expected if foodservice operations continued unchanged.

Effectiveness of the HPP DFAC Intervention

1. Healthy Eating Index-2010 (HEI-2010) Scores

HEI-2010 score was the primary measure to assess effectiveness of the USASOC HPP DFAC intervention. Total HEI-2010 score (Figure 4) for SWCS DFAC patrons significantly increased +3.35 points (baseline: 56.7 pts; 4-12 month mean: 61.0 pts; p=0.002), whereas 2BCT DFAC patron Total HEI-2010 scores remained consistent over the four time periods (mean of 49 pts

with a range of 48.0 - 50.7 pts). The Total HEI score was adjusted to account for the liberal military dietary reference intake maximum sodium value of 5500 mg/day for this sample population compared to the USDA recommendation of no more than 2300 mg/day. The sodium-adjusted HEI score for SWCS patrons shifted to an intervention mean of 70.3 points with a +4.7 point significant improvement (p=0.003).

Sodium adjusted HEI-Scores for each DFAC stratified by HEI-Score quality categories (poor, needs improvement, and good quality diet) are presented in Table 5. At baseline, 18% of SWCS patron meals were categorized as poor quality (HEI score ≤ 50 pts), 81% as needing improvement (51-80 pts), and 1% as good quality (≥81 pts); whereas 46% of 2BCT patron meals at baseline were categorized as poor quality, 54% as needing improvement, and 0.5% as good quality. After the HPP DFAC Intervention, SWCS patron diet quality significantly improved as only 11% of diets were rated poor quality, while 84% rated as needing improvement and 5% as good quality (p<0.001). The distribution of HEI score categories remained consistent for 2BCT over the 12-month study. When assessed by meal (breakfast, lunch and dinner), SWCS patrons' diet quality significantly shifted to higher quality at all three meals (Table 5).

HEI-2010 total and domain scores for SWCS and 2BCT patrons are reported in Table 6. SWCS patrons had significant increases in 5 of the 12 HEI-2010 domains. The HEI domains with the greatest magnitude of change for SWCS were a 26% increase in whole fruit, a 35% increase in total protein and a 183% increase in whole grains (all p<0.02). When changes in HEI-2010 domain scores were assessed by meal (breakfast, lunch and dinner), total fruit, whole fruit, whole grains, and empty calories exhibited point increases for all three meals, while dairy decreased at all three meals (not shown in table). SWCS HEI domain points were significantly higher than 2BCT (p<0.010) except for dairy and sodium.

2. Top 10 Food Choices

Figures 5 & 6 illustrate the changes in food categories for SWCS and 2BCT DFACs from baseline (Jan 2015) to 12-Month (Jan 2016) data collection time points.

SWCS Patrons (Figure 5): While several high quality, performance-based foods (fruits, vegetables, eggs, cheese and complex grains) remained in the Top 10 list at 12-months for SWCS, several new food additions to the HPP DFAC menu enhancements (e.g., legumes, beans and Greek Yogurt) transitioned to the Top 10 food choice list. Higher-fat pork breakfast meats were predominantly replaced by turkey bacon and sausage, and no longer a Top 10 food choice. Higher quality complex grains and starches (variety of beans/legumes) replaced refined grains and simple starch foods (e.g., potatoes, rice, pasta); neither were Top 10 food choices at 12-months although both were still available. High fat or sugar desserts were removed from the menu and replaced by a yogurt parfait bar (diced fruit, granola, and nuts). Poultry, a leaner protein source, always ranked higher than beef. Nuts and seeds, also high quality protein and high fiber starch foods, ranked #11-13 through all iterations. Complex grains moved from 9th place at baseline to 7th place at 4-months and finally 3rd place for the 8 & 12-month time periods. Thus the higher quality food options were not only available but consumed in higher quantities than the lower quality foods that were still available.

2BCT Patrons (Figure 6): Despite, healthier food options being available, several poor quality foods (i.e., high in fats and sugars; low in vitamins, minerals, fiber and phytonutrients) were on the Top 10 Food Choice lists (e.g., desserts, refined grains, pork breakfast meats) along with several higher quality, performance-based foods (e.g., fruit, vegetables, and cheese). Refined grains ranked in the Top #2-4 food choices across all four study iterations. Beef, typically higher in saturated fats, consistently ranked higher than poultry.

3. Beverage Choices

Beverage choices for SWCS and 2BCT patrons over the four time periods were assessed by category. The water category captures non-caloric (or very low calorie) enhanced, infused and club soda options. Sweetened beverages contain empty calories (typically 250 kcals per 12 ounce serving) due to added sugar, and include soda, lemonade, and sweetened iced tea. Although juice is a caloric beverage it was captured in a separate category due to the natural property of fruit juice without added sugars. The sports beverage category was defined as an electrolyte beverage containing fewer calories (typically ≤80 kcal per 12 ounce serving) than the sweetened beverage category. Beverage choices over the four time periods are reported in Figures 7 & 8 for SWCS and 2BCT DFACs.

SWCS Patrons: The top two beverage choices for SWCS patrons were milk (29-46% of beverages consumed) and juice (19-23%). Sugar-sweetened beverage ranked third at baseline (11% of beverages consumed) but dropped to the least consumed beverage (3%) for months 4-12, being replaced by water products (5-17% combined) and vegetable juice (7-10%). Coffee consumption (7-12% of beverages consumed) was higher than that for 2BCT patrons (1-6%).

2BCT Patrons: The top beverage choices for 2BCT patrons were milk (18-43% of beverages consumed) and sugar-sweetened beverages (28-48%). Sports beverages increased from 1% of beverage intake to 4-10% during months 4-12.

4. Dietary Intake Food Group Distribution

Dietary intake classified by food group servings provides the detail to understand why the HEI scores changed following the HPP DFAC intervention. Figure 9 displays the fruit, vegetables and dairy intakes for SWCS patrons pre- to post-DFAC implementation (reported in cups). For comparison, the 2015 USDA guideline recommendations for adults (based upon 2800

calories/day) are denoted by a red line while the average 2BCT intake over the 12-month study is denoted as light blue column bars. SWCS patrons had a significant increase in total fruit intake (25%; +0.5 cups) to 2.4 cups/day, which was close to the 2.5 cups/day USDA recommendation and attributed to a 50% increases in citrus & melon fruit (+0.34 cups) and a 54% increase in fruit juice (+0.23 cups). SWCS patrons had an 18% decrease in total vegetables (-0.70 cups) to 3.2 cups/day just below the 3.5 cups/day USDA recommendation, which was attributed to decreases in dark green vegetables (-0.25 cups) and total starchy vegetables (-0.6 cups; predominantly as potatoes). Despite the overall decrease in vegetables, SWCS patrons exhibited a significant increase in red / orange vegetables (+0.35 cups of which 0.22 cups was attributed to tomato intake) and legumes (+0.23 cups), both met the USDA recommendations. Total dairy servings did not significantly change over time with 2.9 cups/day consumption compared to the 3.0 cup USDA recommendation; however, milk decreased 39% (-0.9 cups) while yogurt intake increased 62% (+0.16 cups). Anecdotally, SWCS DFAC patrons reported that the milk dispensers often ran out during meal times, which may have contributed to decreased milk intakes along with increased intakes of water and electrolyte products during summer months.

Figure 10 depicts the intake of grains and protein sources reported in ounces (oz). Total grains did not significantly change after the HPP DFAC intervention with consumption at 5.4 oz of the 10 oz / day USDA recommendation. Whole grains significantly increased by 161% (+0.79 oz) while refined grains decreased 10% (-0.46 cups). Total protein foods (14 oz/day) did not significantly change after the HPP DFAC intervention; however, SWCS patrons met or exceeded the USDA recommendation, including soy, nuts and seeds (1.2 oz/day) and seafood (1.7 oz/day), both high in omega 3 or 6 fatty acids.

Figure 11 depicts discretionary calories from oils, fats and added sugar, reported in grams.

Discretionary calories should be consumed in moderation and thus the USDA recommendations represent a maximum recommended quantity. SWCS patron intake exhibited a 26% decrease in oils (-10.9 g/d) to 29.7g/d and dropped below the 36 g/d USDA recommended maximum. SWCS patrons had a 13% decrease in solid fat (-6.1 g/d) to 35.8g; however, intake was still above the 16 g/d USDA recommended maximum. Added sugar remained about 15 g/d, well below the 32 g/d USDA maximum recommended intake.

Table 7 displays the complete 25 food group category list with the change from baseline to 4-12 month average intake in cups, ounces or grams along with % change, and comparisons to 2BCT and USDA recommendations.

5. Dietary Intake - Macronutrient

Military dietary reference intakes (MDRI) and performance nutrition guidelines and recommendations exist for macronutrient intake.[42,43] Table 8 reports SWCS patron macronutrient intake pre- and post-DFAC implementation, compared against the overall 12-month 2BCT patron intake and macronutrient recommendations. Energy intake did not significantly change over time; averaging ~2750 kcal/d. Protein intake increased (+13.8 g/d; +0.2 g/kg body weight; +3.1% of total energy intake; p=0.011) to 2 g/kg body weight (BW) and 25% of energy, at the top of the performance recommendations (2 g/kg BW). Fat intake decreased (-15.7 g/d; -0.2 g/kg body weight; -3.9% of total energy intake p<0.001) to 1.2 g/kg and 32.8% of energy, which was improved but still above the maximum recommendation at 1 g/kg BW and 30% of energy. Of the dietary fats, decreases were observed in saturated fat (-4.5 g/d; 10.4% of total energy) to 28.6 g; monounsaturated (-4.5 g/d) to 37.8 g; and polyunsaturated fats (-6.9 g/d) to 24.6g, linoleic (omega-6; -6.2 g/d) to 21.2 g/d, and linolenic acid (omega-3; -0.7 g/d) to 2.4 g/d. The omega 6:3 ratio remained consistent at ~8.8:1, above the desired ratio of 5:1 but below the average US adult omega 6:3 ratio at 14 to 19:1. Fiber

intake increased 4.6 g/d to 29.5 g/d illustrating an improvement although still short of the daily recommendation of 35-38 g/d.

6. Dietary Intake - Vitamins

Vitamin intake of SWCS patrons pre- to post-HPP DFAC intervention, compared to 2BCT patron intake and MDRI recommendations are reported in Table 9. Several vitamin levels significantly increased from baseline and met MDRI recommendations: Beta Carotene (+2977 μg/d to a mean 11671 μg/d), Lycopene (+6164 μg/d to 11843 μg/d), Riboflavin (+0.4 mg/d to 2.4 mg/d), Vitamin B_6 (+0.6 mg/d to 4.4 mg/d), Vitamin B_{12} (+1.3 μg/d to 9.8 μg/d), and Folate (+133 μg/d to 710 μg/d). Two vitamins significantly decreased from baseline but still met MDRI recommendations: Vitamin D (-1.6 μg/d to 1.6 μg/d) and Vitamin K (-75 μg/d to 313 μg/d). All vitamin intake values after the USASOC HPP DFAC intervention were above that of 2BCT patrons except for Vitamin D.

7. Dietary Intake - Minerals

Mineral intake for SWCS patrons pre- to post HPP DFAC intervention, compared to 2BCT patron intake and MDRI recommendations are reported in Table 10. Two minerals significantly changed: calcium (-193 mg/d to 1386 mg/d) and selenium (+13 mg/d to 206 mg/d). However, all minerals met the MDRI recommendations.

8. DFAC Customer Satisfaction

DFAC customer satisfaction results for SWCS DFAC over the four data collection time points are reported in Figures 12 & 13 with overall 12-month 2BCT patron data for comparison. A total of 17 questions were asked about DFAC satisfaction related to the availability, quality, and portion size of specific foods (13 questions), as well as usefulness of the point-of-service

labeling (P-O-S) (4 questions). SWCS patron opinions on DFAC food service significantly improved on 11 of the 13 food-related customer satisfaction items post-HPP DFAC intervention (Figure 12), whereas 2BCT patron satisfaction remained consistent over the 12-month study. SWCS patrons agreed that food choices were adequate (67%) and healthy foods (79%), performance-based foods (72%), and fresh fruits (63%) were available (all $p<0.03$), all of which significantly increased from the 40-48% reported agreeance at baseline. SWCS patrons agreed that the salad bar offered a variety of fresh vegetables (76%, up from 53% at baseline); the main dishes were healthy and performance based (73%, up from 32%) and that side dishes were prepared without added fats (57%, up from 42%) (all $p<0.01$). Significant changes were observed for availability of healthy and performance-based desserts (31-47% fluctuation, up from 17%) and vegetarian options (33-46% fluctuation, up from 20%) (both $p<0.001$) although continued increases are desirable.

P-O-S labeling was implemented two days prior to the 8-month data collection. No change in any of the four satisfaction questions occurred between baseline and 4-month data collections. P-O-S labeling results (Figure 13) depict improvement in usefulness and actual use; however, only one of four questions at the 12-month data collection was significantly different from baseline. Fifty-nine percent agree that nutrition labels provide knowledge to make performance-based food choices (up from 39%; $p=0.018$).

9. Impact of DFAC Food on Self-Reported Performance & Wellbeing Factors

Along with actual food intake, opinions about the impact of DFAC food on patron self-reported performance and wellbeing factors were assessed. Table 11 depicts baseline opinions compared to 4-12 month percentages. Significant increases were observed for SWCS patrons on 10 of 12 factors ($p<0.05$), specifically: feeling energized (+12% units to 55%), improve mental performance (+12% units to 49%), improved physical performance (+17%

units to 57%), sustained physical performance for longer periods (+13% units to 56%), feeling good about self (+20% units to 58%), recovery after vigorous (+14% units to 55%) and moderate (+13% units to 56%) activity, reduce injury (+16% units to 39%), improve sleep (+12% units to 37%), and improve responses to emotions and stress (+14% units to 45%). No change was observed in 2BCT patrons' opinion regarding the impact of DFAC food on performance and wellbeing factors over the 12-month study.

There were no changes to percentages of feeling alert, ratings of physical readiness or APFT scoring category (<280 pts vs. ≥ 280 pts) from baseline to 4-12 months data collection for either DFAC (not reported in table).

10. Food Plate Cost

Average plate cost was tracked from baseline over the 12-month study and extended an additional 6 months (18 months total; Jan 2016 to May 2016) to account for a full year of the USASOC HPP DFAC intervention (Figure 14). Basic Daily Food Allowance (BDFA) for a standard garrison DFAC is $10.49, whereas SOF-specific DFACs were granted a 125% increase for a BDFA of $13.11. Plate costs for the new HPP DFAC intervention were higher the first six months of implementation (up to $14.20/day) but then stabilized in the subsequent six months to a cost below the BDFA ranging from $12.05 to $12.95/day.

Analysis of specific plate cost fluctuations over the time period yielded a variety of causes. The new HPP performance-based menu and new catalog items originally incurred costs above the BDFA (up to $14.20/day) due to: difficulty forecasting usage of new foods and recipes due to inability to add them to the Armed Forces Menu Information System (AFMIS) until 4 months after implementation; minimum bulk and special purchase requirements by Sysco Foods (prime vendor), unfamiliarity of DFAC staff with using and preparing new

ingredients and recipes; the unexpected novelty effect of new food items by patrons resulting in increased (and unplanned) consumption rates at all three meals; special Army celebrations which result in an occasional spike in plate cost. Several management and operational adjustments led to improved food service practices efficiency and plate cost stabilization: addition of 2nd SWCS Performance Dietitian to facilitate communication and training on new recipe preparation; staff schedule adjustments to account for preparation of 'from scratch' recipes; modifying daily menu to balance out daily food costs (i.e., serving high cost items such as salmon and steak on different days); addition of new food service equipment to increase foodservice operations efficiency; access to AFMIS to improve forecasting ability; reduction or elimination of individual packaged foods (e.g., yogurt, milk, cereal, granola bars) that are easily taken as To-Go items, and substitution of some ingredients not on the catalog or missing due to forecasting issues. SWCS plate costs were consistently recorded below the BDFA of $13.11 and ranged from $12.05 to $12.95/day from Jan – May 2016 (8-12 months post DFAC intervention).

Appetite & Satiety (SLIM Scale, Meal Timing)

Time since last meal, snack type prior to meal (when applicable), appetite & satiety, meal time, self-reported meal length, and self-reported eating rate for each DFAC by meal are reported in Table 12. PreSLIM score represents the degree of hunger (-100 pts greatest imaginable hunger to 0 pts not hungry); PostSLIM score represents the degree of fullness/satiety (0 pts not full to +100 pts greatest imaginable fullness); and the change in SLIM score represents the degree of satiation (magnitude of change from hunger to fullness). The time difference from pre-meal tray photograph to post-meal tray photograph is defined as 'meal time' in minutes; however this may not reflect actual eating time. Snack type is self-reported data at the time of pre-meal tray photography based upon the question "When did

you last eat?" Meal length and eating rate are self-reported data at the time of post-meal tray photography.

There were significant differences in all variables noted in Table 12 when assessed by meal for each DFAC, but not over time. Breakfast had a higher percent of patrons reporting shorter dining times and a faster rate of eating over a mean of 17.1 minutes; whereas dinner had a greater percent reporting longer than usual dining times and slower rates of eating over a mean of 22.7 minutes. More SWCS patrons consumed snacks before breakfast (51%) compared to lunch (25%) and dinner (35%). SWCS patrons most commonly reported consuming prepackaged protein (e.g., protein shake or protein bar) or a carbohydrate-based snack (e.g., refined or whole grain, fruit, or vegetables). When the consolidated DFAC and meal data were compared to diet quality and intake, several significant correlations were obtained. Degree of hunger premeal was associated with a greater magnitude change in fullness ($r = -0.724$; $p<0.001$). The greater the degree of hunger, a higher quantity of macronutrients and total energy were consumed (protein $r =-0.152$; fat $r=-0.155$; carbohydrate $r=-0.183$; kcal $r=-0.208$; all $p<0.001$). As macronutrient and energy intake increased, a greater change in the magnitude of fullness was reported (protein $r =0.183$; fat $r=0.183$; carbohydrate $r=0.186$; kcal=0.237; all $p<0.001$) and thus a greater level of satiation.

A multiple regression was calculated to predict the degree of satiation (transition from hunger to fullness) based upon the following significant correlations observed: hours since last meal, initial hunger level (PreSLIM Score), meal time, self-reported meal length, having enough time to eat, total energy intake (kcal), macronutrient intake (protein, fat and carbohydrate intake reported as g/kg body weight), and food group servings (fruit, vegetable, starchy vegetables, grains, legumes, nuts/seeds, and dairy). A significant regression equation was found ($F(1,2342)) = 461.879$, $p<0.001$, with an R^2 of 0.542; meaning 54.2% of the variance in

magnitude of satiation was explained by the initial degree of hunger, protein and carbohydrate intake, whole grain intake, the meal length and having enough time to eat. DFAC patrons' predicted satiation is equal to 46.741 - 0.978 (PreSLIM Score points) + 7.770 (Protein g/kg BW) + 2.465 (Carbohydrate g/kg BW) + 2.76 (Meal Length) – 7.032 (Enough Time) – 1.28 (Whole Grain ounces), where Meal Length is coded 1=shorter than usual, 2=typical, 3=longer than usual, and Enough Time is coded 1=Yes and 2=No.

Fitness Level & Nutrient Intake

This study did not measure indices of performance beyond self-reported physical readiness and APFT scores. Sixty-one percent of SWCS patrons compared to 33% 2BCT patrons, scored ≥ 280 points. When DFAC patrons APFT scores were stratified by HEI diet quality category (sodium adjusted), a significant difference was observed (Figure 15). APFT score increased with increasing diet quality; 270.1 ± 20.2 points for poor diet quality, 275.8 ± 22.0 points for a needs improvement diet, and 283.1 ± 16.2 points for high quality diet (p=0.034). Inadequate sample power prevented data analysis stratified by DFAC.

DFAC Staff Food Service Operations: Focus Group Qualitative Results

A total of 205 DFAC staff members participated in focus group sessions at the 4, 8, and 12-month iterations; 13 SWCS supervisors, 76 non-supervisor SWCS staff, 25 2BCT supervisors, and 91 non-supervisory 2BCT staff. Qualitative data analysis generated six common themes from DFAC staff: 1) DFAC food choices and quality is linked to Soldier morale; 2) Staff take pride in meal service; 3) Staff recognize the need for consumer education on how to make healthy / performance-based food choices; 4) Recipe cards are old, tedious to use, should be revamped, and added to the Army Food Management Information System (AFMIS); 5) Staff morale affects foodservice operations and the quality of meals served; and 6) Staff are highly

receptive to receiving training related to new recipes, healthy cooking techniques, and to increase culinary skills.

Unique SWCS DFAC themes were identified at 4, 8, and 12-months during the SWCS DFAC staff focus group session. SWCS staff encountered initial challenges associated with new recipes, unfamiliar ingredients (including spices), time to cook from scratch, and the need for upgraded equipment to support change in cooking procedures (more baking, steaming compared to frying and grilling), consistency in portion control and questions related to the new P-O-S labeling. SWCS management experienced challenges with prime vendor procurement requirements, staff training and communication, food forecasting and food waste containment. Many of the forecasting and staff issues were resolved once new recipes and ingredients were added to AFMIS, staff training was provided, and management made a concerted effort to improve production schedule and redistribute the workload. By the 12-month end of study, SWCS DFAC staff confirmed high acceptability of the USASOC HPP DFAC intervention, took ownership of the program, and acknowledged the desire to maintain the program.

Results of multiple cameras to Improve Food Photography Estimation Accuracy

Estimation of 5667 foods was completed by 14 PBRC research analysts. The proportion of errors tended to be less for 2 cameras (33.3%) than one (35.4%), but the difference was not statistically significant (p=0.111). In terms of nutrient evaluation of all meals, energy and macronutrient content differences between the two methodologies were not different. While it was hypothesized that 2 cameras would be superior to single cameras, this ancillary study did not reveal significant differences; although a 2nd camera may resolve some questions related to viewing angle.

DISCUSSION

The Joint Chiefs of Staff, The Army Surgeon General, and top military leaders have made readiness a #1 priority and acknowledge that nutrition is one of the critical pillars / domains for health and readiness.[20,21,44] The primary finding of this evaluation was that the USASOC HPP performance-based DFAC intervention produced modest but persistent increases in diet quality. Several nutrition metrics, including food choice, diet quality, opinions about the value of food, and DFAC customer satisfaction were significantly improved. SWCS Soldiers' intake demonstrated a significant HEI-2010 score transition from lower to higher quality food choices across all three meals. The SWCS Soldiers attained an average HEI score of 70 points (adjusted for liberal military sodium recommendations), 15 points higher than Soldiers dining at the award-winning Control DFAC operating within the current DoD foodservice guidelines. The improved SWCS Soldiers' HEI scores were attributed to choosing the new USASOC HPP DFAC intervention foods rich in protein, fiber, vitamins, minerals, and phytonutrients as demonstrated by significant increases in daily intake of fruit (0.4 cups/d), red/orange vegetables (0.4 cups/d), whole grains (0.8 cups/d), protein-based legumes (0.9 cups/d), yogurt (0.2 cups/d), and lean protein (0.6 oz/d) ($p<0.05$). USASOC Soldiers also exhibited significant reductions of less desirable food choices: refined grains (0.5 cups/d), starchy vegetables (0.6 cups/d), oils (11 g/d) and solid fat (6 g/d) ($p<0.05$).

SWCS Soldier satisfaction for 11 of 13 ratings related to DFAC food appeal, options, quality, and availability increased significantly over the course of the 12-month study. In addition, after the USASOC HPP DFAC intervention, significantly more SWCS Soldiers reported that DFAC foods impacted 10 of 12 performance and wellbeing factors related to improved mental and physical performance, physical activity recovery, sleep quality, injury mitigation, and response to stress and emotions. Thus, the value of nutrition in promoting military performance and

readiness was acknowledged and validated by SWCS Soldier food choice when performance-based foods were available. These USASOC HPP intervention findings further support past study results in which diet quality was improved following a DFAC intervention, and improved diet quality was associated with beneficial changes to health and resiliency metrics.[15,17,22,45,46]

Food choice architecture and P-O-S labeling may positively influence food choice at the point of purchase; however, research on P-O-S effectiveness to promote healthy food choices has yielded mixed results.[2,14,47-49] In this study, the P-O-S labeling was introduced a few days prior to the 8-month data collection time point and continued through to the 12-month evaluation. At end-point there was a near doubling of SWCS patrons agreeing that the P-O-S labeling provided knowledge to make performance-based food choices (39% increased to 59%), yet over the same time period the HEI remained essentially unchanged (+1 point). Co-incident P-O-S marketing might in part have contributed to the lack of effectiveness at producing positive effect on diet quality. In addition, research has identified that age, formal education, income, and food costs influence diet quality.[31,50,51]

SWCS Soldiers were expected to have higher HEI scores when compared to 2BCT Soldiers, partially due to covariates of age (higher education, more years of military service, and higher military ranks), but also due to the nature of being in the Special Forces. Nutrition education on the value of food in health and resilience contribute to healthy food choice decisions.[51,52] The USASOC HPP performance nutrition program incorporates 5 hours of performance nutrition education by registered dietitian nutritionists (RDN) and this occurred prior to the DFAC intervention. As such, this may have contributed to higher than expected baseline SWCS HEI scores and the 8-point total score difference (out of 100 points) between SWCS and 2BCT Soldiers. We also cannot discount the contribution of this education to the magnitude of change that occurred after the new foods were introduced into the SWCS

DFAC.

Research supports that diet quality increases as the food costs and the monetary value of the diet ($/day) increases, due to the cost associated with fresh, lean and performance-based foods such as nuts, beans, fruits, vegetables, whole grains, and lean proteins.[52-55] USASOC DFACs are currently granted additional basic daily food allowances (BDFA) compared to the typical Army DFACs ($13.11 vs. $10.49/day) due to the additional energy and nutrient requirements associated with their physical training program. The HPP DFAC intervention offers foods that are colorful, flavorful, incorporate fresh and seasonal foods as well as new seasonings; and utilize cost-saving strategies such as bulk purchase and reduction in pre-packaged foods. Changes were implemented across all meals and sections of the DFAC including short order grill, hot entrees, side dishes, salads, fruits, desserts, condiments, and beverages. The USASOC HPP DFAC intervention was sustainable within the authorized 125% SOF BDFA, but exceeded the standard garrison BDFA. The USASOC HPP DFAC intervention (education, menu, and labeling) was developed to be population- and task-specific for the SOF, not for a broad-reaching population. Therefore, the USASOC HPP DFAC program will require modifications to successfully transition to larger Army and DoD.

Several study strengths exist. Deviation from the foodservice policy was granted for the implementation of the USASOC HPP DFAC intervention and provided the opportunity for an evidence-based approach to verify whether the intervention truly promoted optimal Soldier fueling. The use of food photography to quantify daily nutrient intakes (over 3-6 meals) strengthened the evidence through enhanced accuracy of actual dietary consumption compared to studies relying on self-reported food intake data through dietary recall or food frequency questionnaires. The mixed model design of quantitative nutrient intake analysis in conjunction with focus group sessions with DFAC staff provided the opportunity to accurately

account for program implementation challenges and successes to inform future program expansion.

Several study limitations exist. The USASOC HPP DFAC program implementation did not follow the prescribed plan. New food and recipe introductions continued over a four month period after program initiation, and P-O-S labeling occurred during the planned sustainment phase, preventing a clear-cut assessment of each program subcomponent. The study also followed a time-series research design with new Soldiers participating at each data collection time point thereby introducing potential biases in the group differences observed. Future assessment of the USASOC HPP DFAC intervention would benefit from a repeated measures longitudinal design assessing nutrient intake of the same Soldiers at subsequent time points. Another potential confounder was that 5-hours of nutrition education were introduced prior to the HPP DFAC intervention. Future research should examine the independent effectiveness of educational strategies to promote performance-based eating behaviors. The study was not designed to measure the impact of food choice on military performance over time. An association exists between Soldiers' dietary intake and fitness scores but conclusions cannot be drawn from this study. Future research should examine the impact of optimal fueling on military readiness and important health metrics over a longer period than a few months.

CONCLUSIONS

This study demonstrated that USASOC HPP performance dietitians were successful in designing and implementing a performance-based program to address nutrition in support of the US Special Operations Command (USSOCOM) HPP goals and also the US Army's Performance Triad and Chairman of the Joint Chiefs of Staff Total Force Fitness framework.[20,44] The USASOC HPP DFAC nutrition intervention was determined to be feasible, effective and sustainable in a high-paced SOF DFAC. Classroom nutrition education in

conjunction with the USASOC HPP DFAC intervention provided the knowledge and dining environment conducive to promoting the most appropriate food choices and support optimal Soldier readiness. The SOF community places value on the engagement of RDNs as the subject matter experts by embedding them into unit infrastructure. Military leaders can capitalize on the RDN expertise as well as understanding of evidence-based performance nutrition research to reassess current regulations and policies related to warfighter fueling.

RECOMMENDATIONS

To achieve success with future USASOC HPP DFAC intervention expansion, multiple considerations must be addressed: ensuring patron acceptability; foodservice staff self-efficacy with meal preparation; meal cost containment through efficient food production practices; prime vendor limitations; and most importantly leadership support and shared vision that nutrition is a tool to promote short and long-term health and readiness benefits.

Suggested strategies to sustain and expand this program are: 1) Empower DFAC staff by investing in training, education and appreciation. 2) Provide a voice to DFAC staff during the planning, implementation and evaluation phases to ensure buy-in and ownership of the program. Staff focus group sessions during this study identified inconsistency in food portion control, food waste management, and unexpected patron demands impacting accurate food forecasting. In addition, they provided shift schedule suggestions to improve efficiency in operations, identified that food quality changes positively impacted dish room activities (e.g., elimination of fats and gravies eases cleaning), admitted to making DFAC recipes at home with a positive family reaction, acknowledged a positive response from Soldier patrons, and discussed uncertainty about how to use the new P-O-S labeling. 3) Incorporate on-going assessment and provision of necessary resources (such as ingredients, recipes, and equipment) and monitoring of food service operations to reduce food waste and control food

costs. 4) Promote on-going communication between key stakeholders both internal and external to the DFAC. 5) Promote consumer / Soldier performance-based nutrition education internal and external to the DFAC. This may require developing a HPP DFAC program marketing plan with specific educational materials and consultation from local and SOF RDNs. A transferable USASOC HPP DFAC intervention package should include: new AFMIS recipes, menu cycle and equipment recommendations, a work shift plan, a training plan for new recipe preparation and P-O-S label use, templates for P-O-S materials, specific food service operations lessons learned with recommended corrective actions, and guidance on conducting staff feedback sessions to mitigate potential threats to implementation success.

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Table 1. Healthy Eating Index-2010 (HEI) Domains & Scoring Standards.

Domains	Maximum Points	Standard for Maximum Score	Standard for Minimum Score of Zero
Adequacy:			
Total Fruit	5	≥0.8 cup equiv/ 1,000 kcal	No Fruit
Whole Fruit	5	≥0.4 cup equiv/1,000 kcal	No Whole Fruit
Total Vegetables	5	≥1.1 cup equiv/ 1,000 kcal	No Vegetables
Greens and Beans	5	≥0.2 cup equiv/1,000 kcal	No Dark Green Vegetables or Beans and Peas
Whole Grains	10	≥1.5 oz equiv/1,000 kcal	No Whole Grains
Dairy	10	≥1.3 cup equiv/1,000 kcal	No Dairy
Total Protein Foods	5	≥2.5 oz equiv/1,000 kcal	No Protein Foods
Seafood and Plant Proteins	5	≥0.8 oz equiv/ 1,000 kcal	No Seafood or Plant Proteins
Fatty Acids	10	(PUFAs + MUFAs)/SFAs ≥2.5	(PUFAs + MUFAs)/SFAs ≤1.2
Moderation:			
Refined Grains	10	≤1.8 oz equiv/1,000 kcal	≥4.3 oz equiv. per 1,000 kcal
Sodium	10	≤1.1 gram/1,000 kcal	≥2.0 grams per 1,000 kcal
Empty Calories	20	≤19% of energy	≥50% of energy

Note: equiv=equivalent; oz=ounces; PUFA=Polyunsaturated Fats; MUFA=Monounsaturated Fats; SFA=Saturated Fats; kcal=kilocalorie

Table 2. DFAC Patron Demographic Descriptive Data of Continuous Variables.

Overall Demographics	Overall (n=688)	SWCS (n=428)	2BCT (n=260)	*p*-value between DFACs
	Mean (SD)	Mean (SD)	Mean (SD)	
Current age (years)	25.6 (5.5)	27.5 (5.7)	22.4 (3.1)	<0.001
BMI (kg/m^2)	26.0 (2.9)	26.2 (2.7)	25.6 (3.3)	0.007
Years of Active Duty	4.1 (4.9)	5.1 (5.7)	2.4 (2.6)	<0.001
Vigorous PA (hours/day)	1.2 (0.8)	1.2 (0.9)	1.1 (0.8)	ns
Moderate PA (hours/day)	0.7 (0.9)	0.6 (0.7)	0.9 (1.2)	<0.001
Walking (hours/day)	1.3 (2.1)	0.9 (1.3)	1.9 (2.8)	<0.001
Sitting (hours/day)	5.3 (3.4)	5.4 (3.3)	5.0 (3.6)	ns
APFT score	275.1 (22.2)	280.2 (20.5)	266.8 (22.3)	<0.001
Number of pushups	70.6 (12.1)	71.9 (10.9)	68.3 (13.7)	0.001
Number of sit ups	75.5(9.9)	76.9 (9.7)	73.1 (9.9)	<0.001
Total Run Time (minutes)	13.6 (1.3)	13.4 (1.2)	14.0 (1.3)	<0.001
Weekday Screen Time (hours/day)	5.2 (3.5)	4.9 (3.5)	5.7 (3.6)	0.006
Weekend Screen Time (hours/day)	7.6 (4.1)	6.9 (3.9)	8.7 (4.3)	<0.001
Hours of sleep	6.5 (1.1)	6.7 (1.0)	6.2 (1.3)	<0.001

Note: SWCS=Special Warfare Center & School; 2BCT=2nd Brigade Combat Team; DFAC=Dining Facility; ns=not significant

Table 3. DFAC Patron Demographic Descriptive Data of Categorical Variables.

	Overall (n=688) n (%)	SWCS (n=428) n (%)	2BCT (n=260) n (%)	*p*-value between DFACs
SWCS DFAC	428 (62)			
2BCT DFAC	261 (38)			
Males	666 (98)	426 (100)	240 (95)	<0.001
Race				
Caucasian	579 (85)	384 (90)	195 (77)	<0.001
African American/Black	45 (7)	14 (3)	31 (12)	
Asian	12 (2)	7 (2)	5 (2)	
Other	45 (6)	21 (5)	21 (9)	
Hispanic	86 (13)	33 (8)	53 (21)	<0.001
Education				
Up to High School	193 (28)	55 (13)	138 (54)	<0.01
Some up to AAS	289 (43)	192 (45)	97 (38)	
Bachelor's Degree	179 (26)	162 (38)	17 (7)	
Graduate Degree	19 (3)	17 (4)	2 (1)	
Rank				
E1-E4	486 (69)	237 (56)	229 (91)	<0.001
E5-E7	95 (14)	71 (17)	24 (10)	
WOC	60 (9)	60 (14)	0 (0)	
WO1-CW4	35 (5)	35 (8)	0 (0)	
O1-O3	21 (3)	21 (5)	0 (0)	
SWCS Student	286 (42)	285 (67)	0 (0)	<0.001
APFT Score ≥ 280 points	337 (51)	254 (62)	83 (34)	<0.001
Alert / Sleepiness				
Alert	378 (56)	265 (63)	112 (44)	<0.001
Not Fully Alert / Sleepy	293 (44)	153 (37)	140 (56)	
Physical Readiness				
Good to Best Shape	560 (83)	370 (88)	190 (76)	<0.001
Neither Good nor Bad, Bad or Worst Shape	112 (17)	52 (12)	60 (24)	

Note: SWCS=Special Warfare Center & School; 2BCT=2nd Brigade Combat Team; DFAC=Dining facility; AAS=Associates Degree; E1-E4=Enlisted ranks (Private through Specialist); E5-E7=noncommissioned officer ranks (Sergeant through Sergeant First Class); WOC=Warrant Officer Candidate; WO1-CW4=Warrant Officer ranks (Warrant Officer through Chief Warrant Officer); O1-O3=Officer ranks (2nd Lieutenant through Captain).

Table 4. DFAC Patron Meal and Snack Pattern & Most Common Dining Location.

Meals / Snacks Consumed (5 times/week) & Top Locations (regardless of times/week)	Overall %	SWCS %	2BCT %	*p*-value between DFACs
Early Morning Snack	23	29	13	<0.001
Home / Barrack	24	31	12	<0.001
Convenience source	3	2	4	
Breakfast	82	80	84	ns
DFAC	58	56	62	0.021
Home / Barracks	37	40	31	
Convenience source	5	4	7	
Morning Snack	23	28	16	<0.001
Home / Barracks	20	25	12	ns
Convenience source	11	8	16	
Lunch	92	96	86	<0.001
DFAC	69	75	59	ns
Home / Barracks	18	17	19	
Convenience source	13	8	20	
Afternoon Snack	38	41	34	ns
Home / Barracks	29	33	24	ns
Convenience source	18	15	25	
Dinner	96	97	94	ns
DFAC	59	64	50	ns
Home / Barracks	20	18	24	
Convenience source	21	18	26	
Evening Snack	55	56	53	ns
Home / Barracks	46	49	41	ns
Convenience source.	16	13	22	

Note: SWCS=Special Warfare Center & School; 2BCT=2nd Brigade Combat Team; DFAC=Dining Facility; ns=not significant. Location of meals was assessed by the question “During the past 7 days, where did most of your (specific meal/snack inserted) come from?” The meal options were consolidated to: did not eat, home/barracks, military dining facility, or convenience source (store, fast food, buffet, restaurant, vending, etc.) and may reflect meals / snacks eaten less than 5 times per week.

Table 5. Healthy Eating Index-2010 (HEI-2010) Diet Quality Category (Adjusted for Sodium) by DFAC Pre to Post-DFAC Intervention.

HEI Score Categories (adjusted for sodium)	SWCS			2BCT		
	0-Month %	4-12 Month %	Pre-Post *p*-value	0-Month %	4-12 Month %	Pre-Post *p*-value
All Meals						
Poor Quality	17.8	11.1	<0.001	45.7	39.6	ns
Needs Improvement	80.8	83.6		53.8	60.4	
Good Quality	1.4	5.3		0.5	0.0	
Breakfast						
Poor Quality	12.5	7.4	0.042	29.1	23.4	ns
Needs Improvement	83.1	85.5		69.6	76.6	
Good Quality	4.4	7.1		1.3	0.0	
Lunch						
Poor Quality	21.7	12.5	<0.001	59.2	52.6	ns
Needs Improvement	78.3	83.5		40.8	47.4	
Good Quality	0.0	4.0		0.0	0.0	
Dinner						
Poor Quality	18.2	13.4	0.027	52.3	44.6	ns
Needs Improvement	81.8	81.8		47.7	55.4	
Good Quality	0.0	4.7		0.0	0.0	

Note: Poor Quality = HEI Score ≤50 pts; Needs Improvement = HEI Score 51-80 pts; Good Quality = 81-100 pts; SWCS 0-month included 443 meals and 4-12-months 1197 meals); 2BCT 0-month included 199 meals and 4-12 months 565 meals); percentage based upon the HEI score adjusted for sodium score to account for liberal military dietary reference intake of no more than 5500 mg for this sample population.

Table 6. Healthy Eating Index-2010 (HEI-2010) Scores of SWCS DFAC Patrons Pre- to Post-DFAC Intervention.

HEI-2010 Domains & Total Score (points)	Comparison		SWCS DFAC Patron Intake				Pre-Post *p*-value
	Max Points Available	2BCT (n=200) 0-12 Month Mean (SD)	0-Mo (n=97) Mean (SD)	4-12 Month (n=285) Mean (SD)	Change	Percent Change	
Total Fruit *	5	2.80 (1.9)	2.87 (1.6)	3.26 (1.7)	+0.39	**+13%**	ns
Whole Fruit *	5	1.43 (1.8)	1.83 (1.6)	2.30 (1.9)	+0.47	**+26%**	0.018
Total Vegetables *	5	3.52 (1.3)	4.33 (1.0)	4.04 (1.2)	-0.29	-7%	0.021
Greens & Beans *	5	1.68 (1.8)	3.66 (1.6)	3.26 (1.9)	-0.40	-11%	0.042
Total Protein *	5	3.38 (1.4)	2.96 (1.3)	3.99 (1.2)	+1.03	**+35%**	<0.001
Seafood & Plant Protein *	5	1.64 (1.8)	2.52 (1.9)	3.05 (1.8)	+0.53	**+21%**	0.014
Whole Grains *	10	1.45 (2.1)	1.05 (1.5)	2.98 (2.7)	+1.93	**+183%**	<0.001
Dairy	10	6.62 (3.1)	7.20 (3.0)	6.38 (3.0)	-0.82	-11%	0.022
Fatty Acids *	10	5.88 (2.4)	7.59 (2.3)	6.90 (2.5)	-0.69	-9%	0.018
Refined Grains *	10	7.46 (2.6)	8.72 (1.8)	8.75 (2.0)	-0.03	0%	ns
Sodium *	10	2.73 (2.6)	1.04 (1.6)	1.32 (1.8)	+0.28	+27%	ns
Empty Calories *	20	13.91 (4.8)	17.24 (3.3)	17.86 (2.5)	+0.62	+4.6%	ns
Total HEI Score *	100	49.01 (10.4)	56.7 (8.8)	60.1 (9.0)	+3.35	+6%	0.002
Total HEI Score * (Sodium adjustment)	100	56.28 (10.8)	65.7 (8.7)	68.7 (8.6)	+3.0	+5%	0.003

Note: SWCS=Special Warfare Center & School; Mo=Month; SD=standard deviation; ns=not significant; * $p<0.01$ between SWCS and 2BCT patrons.

Table 7. Food Group Intake of SWCS DFAC Patrons Pre- to Post-DFAC Intervention.

Food Group with Subgroups	Comparison		SWCS DFAC Patron Intake				Pre-Post *p*-value
	USDA Reco	2BCT (n=169) 0-12 Mo Mean	0-Month (n=87) Mean	4-12 Month (n=233) Mean	Change	% Change	
Total Fruit (cups)	2.5	2.01	1.92	2.40	+0.48	**+25%**	0.013
Citrus & Melon	N/A	0.63	0.68	1.02	+0.34	**+50%**	0.001
Other Fruit	N/A	0.90	0.82	0.73	-0.09	-11%	ns
Fruit Juice	N/A	0.48	0.42	0.65	+0.23	**+54%**	0.014
Total Vegetables (cups)	3.5	2.66	3.90	3.20	-0.70	-18%	<0.001
Dark Green Veg	0.36	0.33	0.82	1.62	-0.25	**-98%**	<0.001
Total Red Orange Veg	1.0	0.43	0.75	1.09	+0.34	**+45%**	<0.001
Total Starch Veg	1.0	1.1	1.30	0.72	-0.58	-29%	<0.001
Legumes (Vegetables)	0.36	0.07	0.10	0.32	+0.22	**+220%**	<0.001
Total Grains (ounces)	6.9	6.88	5.06	5.39	+0.33	+7%	ns
Whole Grains	5	0.61	0.49	1.28	+0.79	**161%**	<0.001
Refined Grains	5	6.28	4.57	4.11	-0.46	-10%	ns
Total Protein (ounces)	7	9.78	13.32	14.17	+0.85	+6%	ns
Meat, Poultry, Eggs	4.7	6.75	8.51	8.89	+0.82	+4%	ns
Cured Meats	N/A	1.62	2.28	2.37	+0.09	+4%	ns
Seafood	1.4	0.84	1.40	1.68	+0.28	+20%	ns
Soy, Nuts & Seeds	0.85	0.55	1.12	1.23	+0.11	+10%	ns
Legumes (Protein)	N/A	0.28	0.40	1.29	+0.89	**+223%**	<0.001
Total Dairy (cups)	3.0	3.25	3.46	2.30	-1.16	-34%	0.025
Milk	N/A	2.04	2.30	1.40	-0.90	-39%	<0.001
Yogurt	N/A	0.19	0.29	0.47	+0.18	**+62%**	0.001
Cheese	N/A	1.02	0.87	1.00	+0.15	+15%	ns
Oils (grams)	≤36	34.53	40.32	29.68	-10.64	**-26%**	<0.001
Solid Fats (grams)	≤16	47.56	41.30	35.78	-5.52	-13%	0.004
Added Sugar (grams)	≤32	23.40	16.06	14.99	-1.07	-7%	ns

Note: SWCS=Special Warfare Center & School; 2BCT=2nd Brigade Combat Team; DFAC=Dining Facility; USDA Reco=2015 Dietary Recommendation at 2800 kcal/day (solid fat & added sugar based upon 2010); N/A=Not Available; ns=not significant

Table 8. Macronutrient Intake of SWCS DFAC Patrons Pre- to Post-DFAC Intervention.

Macronutrients	Comparison		SWCS DFAC Patron Intake				Pre-Post *p*-value
	Recommend-ation	2BCT (n=169) 0-12 Month Mean (SD)	0-Mo (n=87) Mean (SD)	4-12 Month (n=233) Mean (SD)	Change	% Change	
Food Energy (kcal)	3000-4600	2860 (858)	2842 (613)	2750 (670)	-92.0	-3%	ns
Kcal / kg BW	N/A	36.8 (11.8)	33.9 (7.6)	32.7 (8.4)	-1.2	-4%	ns
Protein (g/kg BW)	1.2-2.0	1.6 (0.5)	1.8 (0.5)	2.0 (0.5)	+0.2	+11%	0.011
Fat (g/kg BW)	≤ 1.0	1.4 (0.5)	1.4 (0.4)	1.2 (0.4)	-0.2	-14%	<0.001
Carbohydrate (g/kg BW)	3-13	4.5 (1.6)	3.7 (1.1)	3.6 (1.3)	-0.1	-3%	ns
% PRO of Total Kcal	20-25	18.1 (4.3)	21.6 (3.7)	24.7 (4.9)	+3.1	+14%	<0.001
% FAT of Total Kcal	≤ 30	34.8 (6.6)	36.7 (6.1)	32.8 (6.5)	-3.9	-11%	<0.001
% CHO of Total Kcal	≤ 55	48.4 (8.8)	42.9 (7.2)	43.7 (8.4)	+0.8	+2%	ns
Total Dietary Fiber (g)	35-38	21.1 (8.1)	24.9 (8.2)	29.5 (10.7)	+4.6	+18%	<0.001
Cholesterol (mg)	<300	656 (236)	714 (236)	702 (280)	-12	-2%	ns
Saturated Fat (g)	≤10% of kcal	34.5 (12.9)	33.1 (9.5)	28.6 (9.3)	-4.5	-14%	<0.001
Monounsaturated Fat (g)	N/A	41.1 (14.3)	42.3 (13.3)	37.8 (13.2)	-4.5	-11%	0.008
Polyunsaturated Fat (g)	N/A	27.1 (11.8)	31.5 (10.4)	24.6 (9.9)	-6.9	-22%	<0.001
Omeag-6 (g) *(18:2 Linoleic)*	18	23.6 (10.5)	27.4 (9.2)	21.2 (8.8)	-6.2	-23%	<0.001
Omega-3 (g) *(18:3 Linolenic)*	1.7	2.6 (1.3)	3.1 (1.2)	2.4 (1.2)	-0.7	-23%	<0.001

Note: SWCS=Special Warfare Center & School; 2BCT=2nd Brigade Combat Team; DFAC=Dining Facility; SD=Standard deviation; PRO=Protein; CHO=Carbohydrate; g=grams; kcal=calories; BW=body weight; mg=milligrams; ns=not significant; N/A=Not Available; Recommendation based upon the military dietary reference intakes (MDRI) for males in the mean age group or the performance guidelines for athletes.

Table 9. Vitamin Intake of SWCS DFAC Patrons Pre- to Post-DFAC Intervention.

Vitamins	Comparison		SWCS DFAC Patron Intake				Pre-Post *p*-value
	Recommend-ation	2BCT (n=169) 0-12 Month Mean (SD)	0-Month (n=87) Mean (SD)	4-12 Month (n=233) Mean (SD)	Change	% Change	
Vitamin A (µg RAE)	1000	1167 (577)	1547 (646)	1660 (909)	+112	+7%	ns
Carotene beta (µg)	10000	4185 (3791)	8694 (6328)	11671 (10093)	+2977	+34%	0.002
Vitamin E (mg)	15	11.2 (4.7)	13.7 (4.6)	14.4 (5.9)	+0.7	+5%	ns
Vitamin D (µg)	5	12.5 (9.4)	11.3 (5.9)	9.7 (6.7)	-1.6	-14%	0.048
Lycopene (µg)	10000	4604 (4933)	5679 (7752)	11843(12928)	+6164	+108%	<0.001
Lutein zeaxanthin (µg)	6000	3615 (2884)	7367 (5621)	6863 (6237)	-504	-7%	ns
Vitamin C (mg)	90	161 (135)	241 (142)	235 (156)	-6.4	-2%	ns
Thiamin (mg)	1.2	2.1 (0.7)	2.0 (0.5)	2.4 (0.8)	+0.4	+20%	<0.001
Riboflavin (mg)	1.3	3.1 (1.3)	3.4 (1.0)	3.5 (1.2)	+0.1	+3%	ns
Niacin (mg)	16	30.8 (10.0)	36.1 (10.5)	38.3 (10.5)	+2.2	+6%	ns
Vitamin B_6 (mg)	1.3	3.2 (1.2)	3.8 (1.1)	4.4 (1.5)	+0.6	+16%	<0.001
Folate DFE (µg)	400	679 (314)	687 (250)	898 (507)	+211	+31%	<0.001
Vitamin B_{12} (µg)	2.4	1.3 (2.2)	8.5 (3.8)	9.8 (5.6)	+1.3	+15%	0.025
Vitamin K (µg)	80	187 (139)	388 (252)	313 (275)	-75	-19%	0.026

Note: SWCS=Special Warfare Center & School; 2BCT=2nd Brigade Combat Team; DFAC=Dining Facility; SD=Standard deviation; µg=micrograms; mg=milligrams; DFE=dietary folate equivalent; ns=not significant; Recommendation based upon the military dietary reference intakes (MDRI) for males in the mean age group.

Table 10. Mineral Intake of SWCS DFAC Patrons Pre- to Post-DFAC Intervention.

Minerals	Comparison		SWCS DFAC Patron Intake				Pre-Post *p*-value
	Recommend-ation	2BCT (n=169) 0-12 Month Mean (SD)	0-Month (n=87) Mean (SD)	4-12 Month (n=233) Mean (SD)	Change	% Change	
Calcium (mg)	1000	1542 (785)	1579 (762)	1386 (647)	-193	-12%	0.024
Iron (mg)	10	18.3 (6.7)	18.6 (5.0)	19.2 (6.0)	+0.6	+3%	ns
Magnesium (mg)	420	373 (127)	446 (125)	464 (131)	+18	+4%	ns
Phosphorus (mg)	700	2242 (799)	2472 (715)	2487 (713)	+15	+1%	ns
Potassium (mg)	3200	4182 (1507)	5090 (1317)	5034 (1368)	-55	-1%	ns
Sodium (mg)	<5000	5288 (1602)	5931 (1203)	5887 (1337)	-44	-1%	ns
Zinc (mg)	15	16.0 (6.0)	16.9 (5.3)	17.9 (5.5)	+1.0	+6%	ns
Selenium (µg)	55	169.4 (49.1)	193 (47)	206 (50)	+13	+7%	0.033

Note: SWCS=Special Warfare Center & School; 2BCT=2nd Brigade Combat Team; DFAC=Dining Facility; SD=Standard deviation; µg=micrograms; mg=milligrams; ns=not significant; Recommendation based upon the military dietary reference intakes (MDRI) for males in the mean age group.

Table 11. Impact of DFAC Food on Self-Reported Performance & Wellbeing Factors.

Reported Most/Always	SWCS			2BCT		
	% 0-Mo	% 4-12 Mo	Pre-Post *p*-value	% 0-Mo	% 4-12 Mo	Pre-Post *p*-value
Feel Energized	43.0	55.2	0.038	40.5	35.9	ns
Improve Mood	49.5	51.7	ns	37.3	35.5	ns
Feel Satisfied Hours after Meal	51.6	55.4	ns	47.6	45.2	ns
Improve Mental Performance	37.4	49.2	0.045	38.1	47.6	ns
Improve Physical Performance	40.9	57.4	0.005	41.7	52.4	ns
Sustain Physical Performance Longer	43.0	56.3	0.023	42.9	50.0	ns
Feel Good about Self	37.6	57.5	0.001	42.5	48.2	ns
Recovery after Vigorous Activity	40.9	54.6	0.019	48.8	52.9	ns
Recovery after Moderate Activity	42.9	56.2	0.024	50.0	49.4	ns
Reduce Injury	22.6	38.8	0.004	34.5	35.3	ns
Improve Sleep	25.0	37.4	0.027	30.1	31.8	ns
Improve response to Emotions and Stress	31.2	44.5	0.02	31.0	38.2	ns

Note: DFAC=Dining Facility; SWCS=Special Warfare Center & School; 2BCT=2nd Brigade Combat Team; Mo=month; ns=not significant

Table 12. Hunger, Satiety, Meal Timing, Meal Length and Snack Type between Meals by DFAC.

	SWSC Mean (SD)			2BCT Mean (SD)		
	Breakfast (n=528)	Lunch (n=597)	Dinner (n=510)	Breakfast (n=284)	Lunch (n=270)	Dinner (n=209)
Hours Since Last Meal	10.7 (4.6)	4.1 (3.3)	4.6 (2.4)	13.2 (3.5)	4.5 (4.3)	5.1 (2.2)
Meal Time (minutes) *	17.1 (8.4)	19.6 (8.4)	22.7 (10.5)	15.5 (6.1)	19.4 (10.2)	17.0 (6.3)
Pre SLIM Score (Hunger) *	-37.4 (17.4)	-31.4 (18.6)	-33.9 (20.8)	-38.5 (20.6)	-27.3 (20.8)	-30.0 (23.4)
Post SLIM Score (Satiety) *	53.0 (16.6)	50.9 (18.7)	52.3 (20.5)	51.8 (19.1)	51.4 (22.1)	48.8 (23.2)
Change in SLIM Score *	90.4 (25.5)	82.5 (25.7)	86.4 (28.1)	90.8 (26.9)	78.8 (29.3)	78.9 (30.0)
	Percent			Percent		
Meal Length *						
Shorter than Usual	18.9	16.3	6.1	18.1	17.2	15.7
Typical	73.5	73.4	73.9	75.5	67.4	69.6
Longer than Usual	7.6	10.3	20.0	6.4	15.4	14.7
Eating Rate *						
Slow / Very Slow	7.1	13.0	24.7	10.3	23.2	23.6
Medium	58.3	56.9	61.8	58.9	56.9	54.9
Fast / Very Fast	34.7	30.2	13.5	30.8	19.8	21.6
Snack Type Prior to Meal *						
No Snack	49.8	75.3	66.2	73.7	90.3	83.7
Prepackaged Protein	20.8	9.6	13.9	4.3	1.1	1.9
Carb-only Snack	10.9	6.4	4.3	5.0	3.0	4.8
Healthy Food Mix (protein and carb)	6.5	4.2	7.0	3.2	1.1	2.9
Meal Size Mix of Foods	6.5	3.2	4.9	6.4	1.9	3.3
Empty Calorie Snacks (high fat or sugar)	4.8	1.2	3.3	7.5	2.2	2.9
Caffeine Product	0.8	0.2	0.4	0	0.4	0.5

* $p<0.05$ between meals for both DFACs; PreSLIM score represents degree of hunger (-100 pts greatest imaginable hunger to 0 pts not hungry); PostSLIM score represents degree of fullness/satiety (0 pts not full to +100 pts greatest imaginable fullness); Change in SLIM score represents the degree of satiation (transition from hunger to fullness).

Figure 1. US Special Operations Forces Performance-Based Menu Standards.

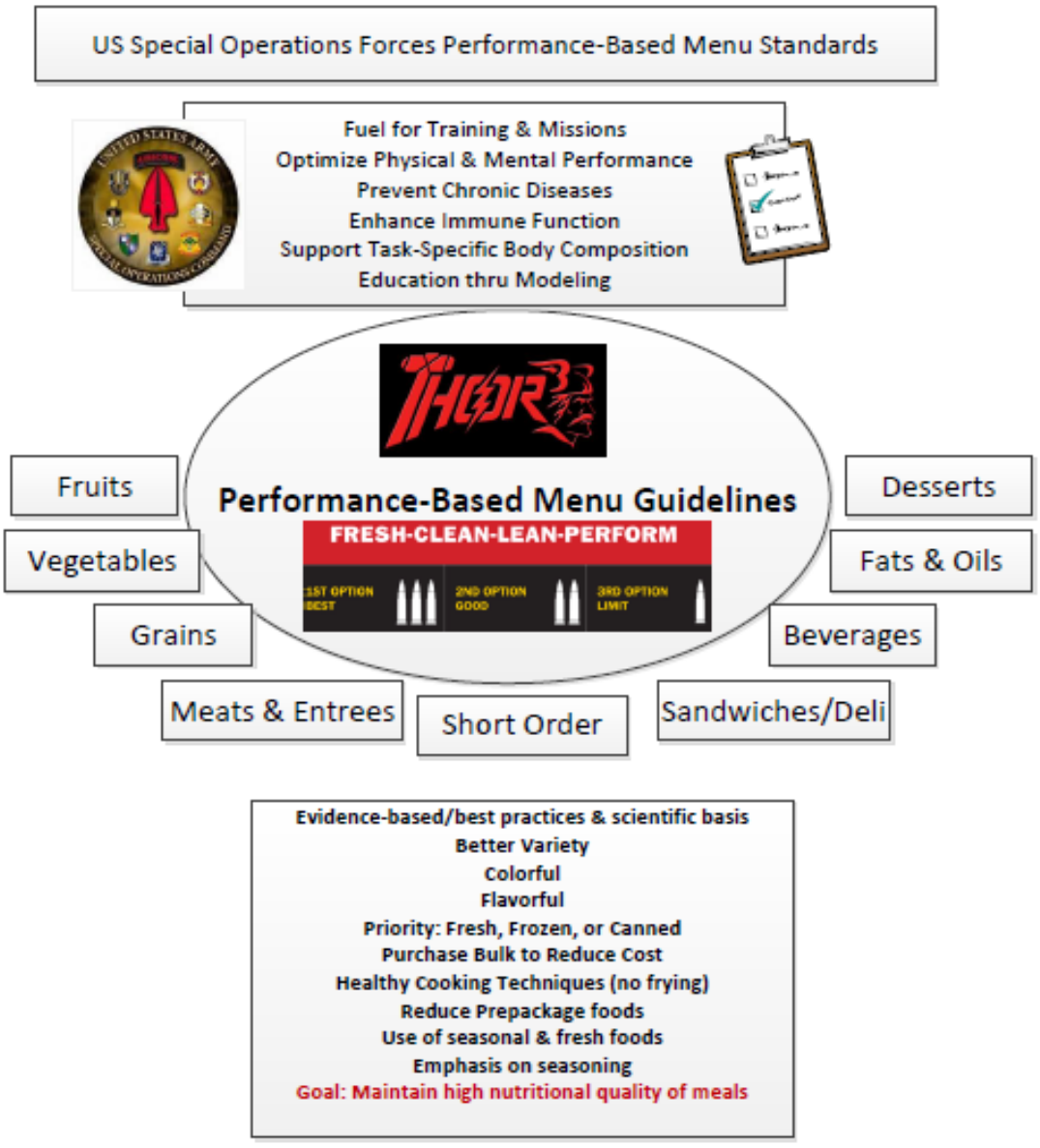


Figure 2. Human Performance Program DFAC Implementation & Evaluation Timeline.

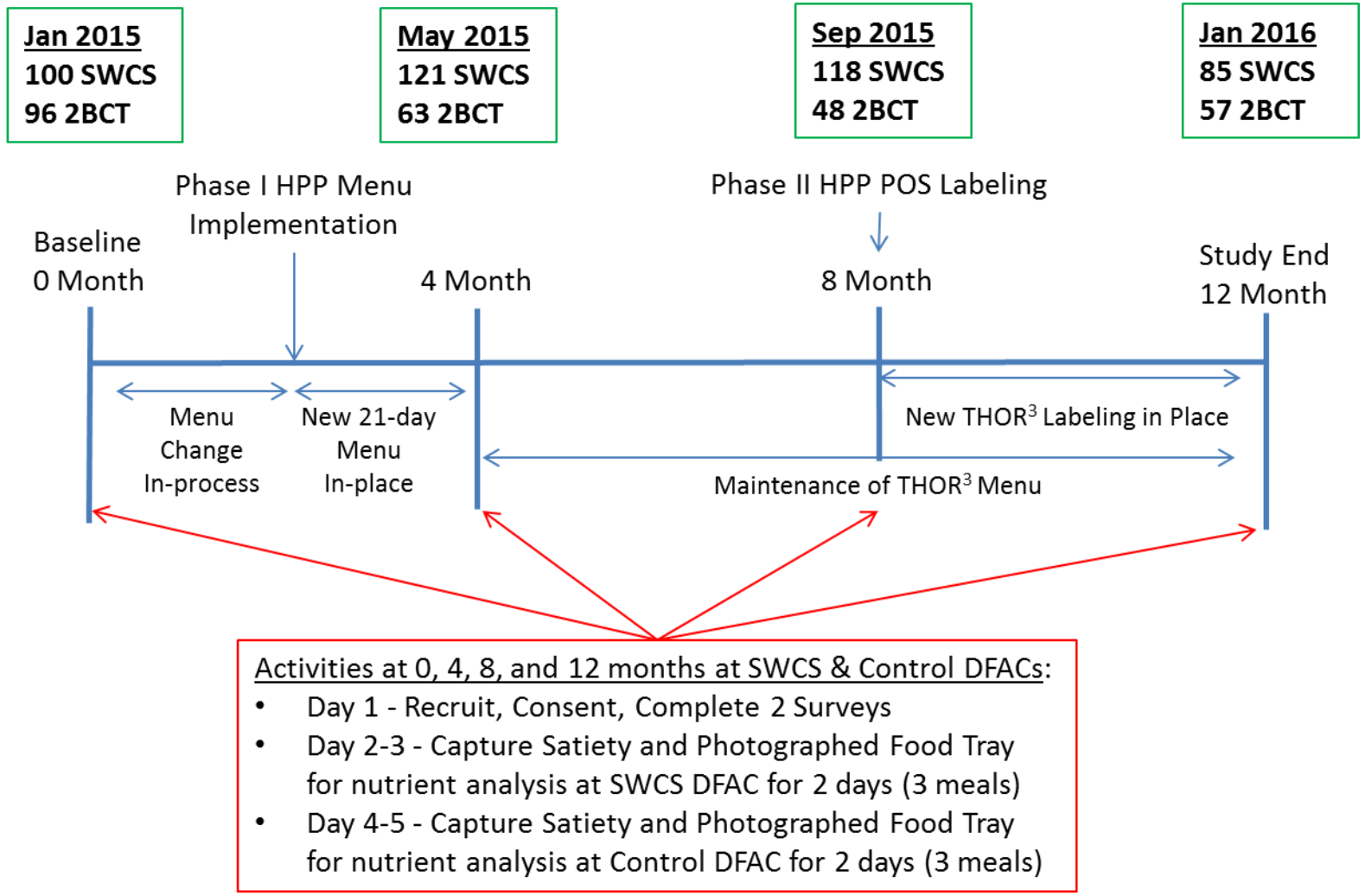
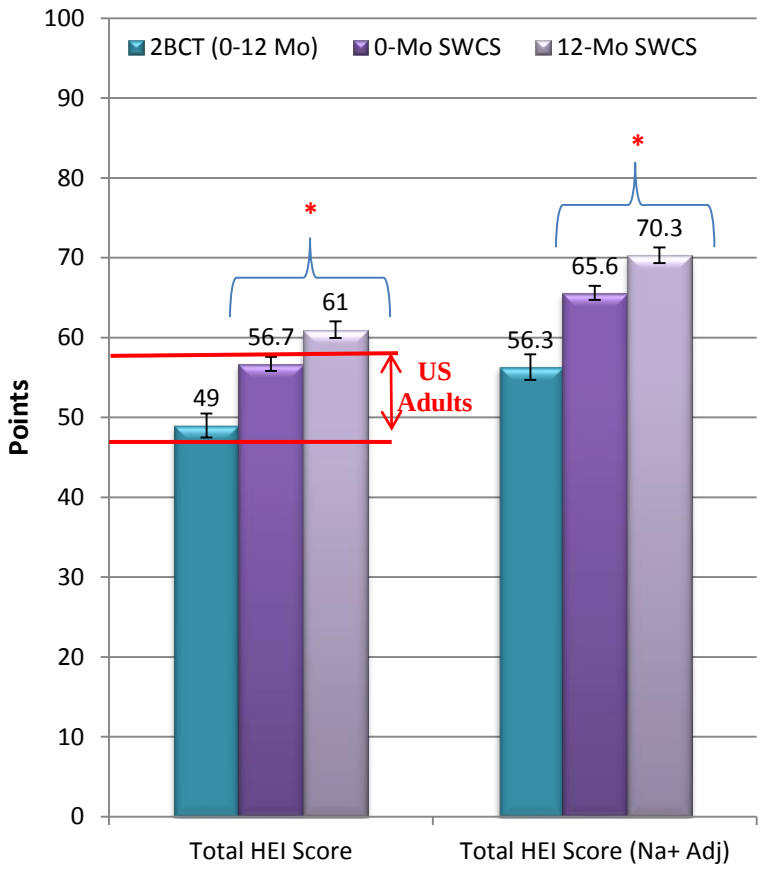


Figure 3. Digital Food Photography Station Example.

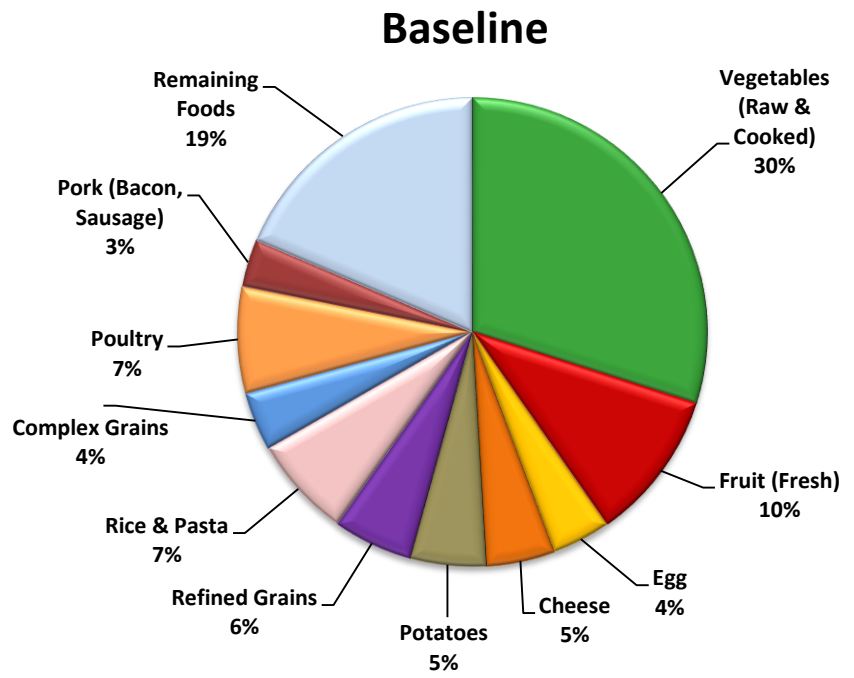
Figure 4. Total Healthy Eating Index-2010 (HEI) Score Change by DFAC (Baseline compared to 12-months).



* p <0.005

Note: SWCS=Special Warfare Center & School; 2BCT=2nd Brigade Combat Team; Mo=month; Maximum of 100 points possible; Na+Adj=HEI score adjusted for sodium score to account for liberal military dietary reference intake of no more than 5500 mg for this sample population.

Figure 5. Top 10 Food Choice Categories of SWCS DFAC Patrons Pre-Post Assessment.



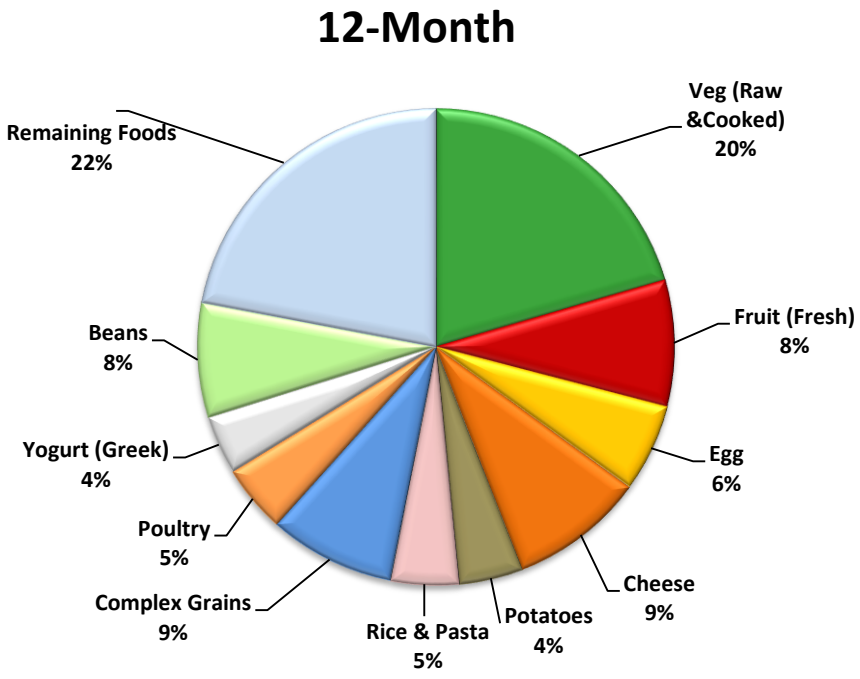


Figure 6. Top 10 Food Choice Categories of 2BCT DFAC Patrons Pre-Post Assessment.

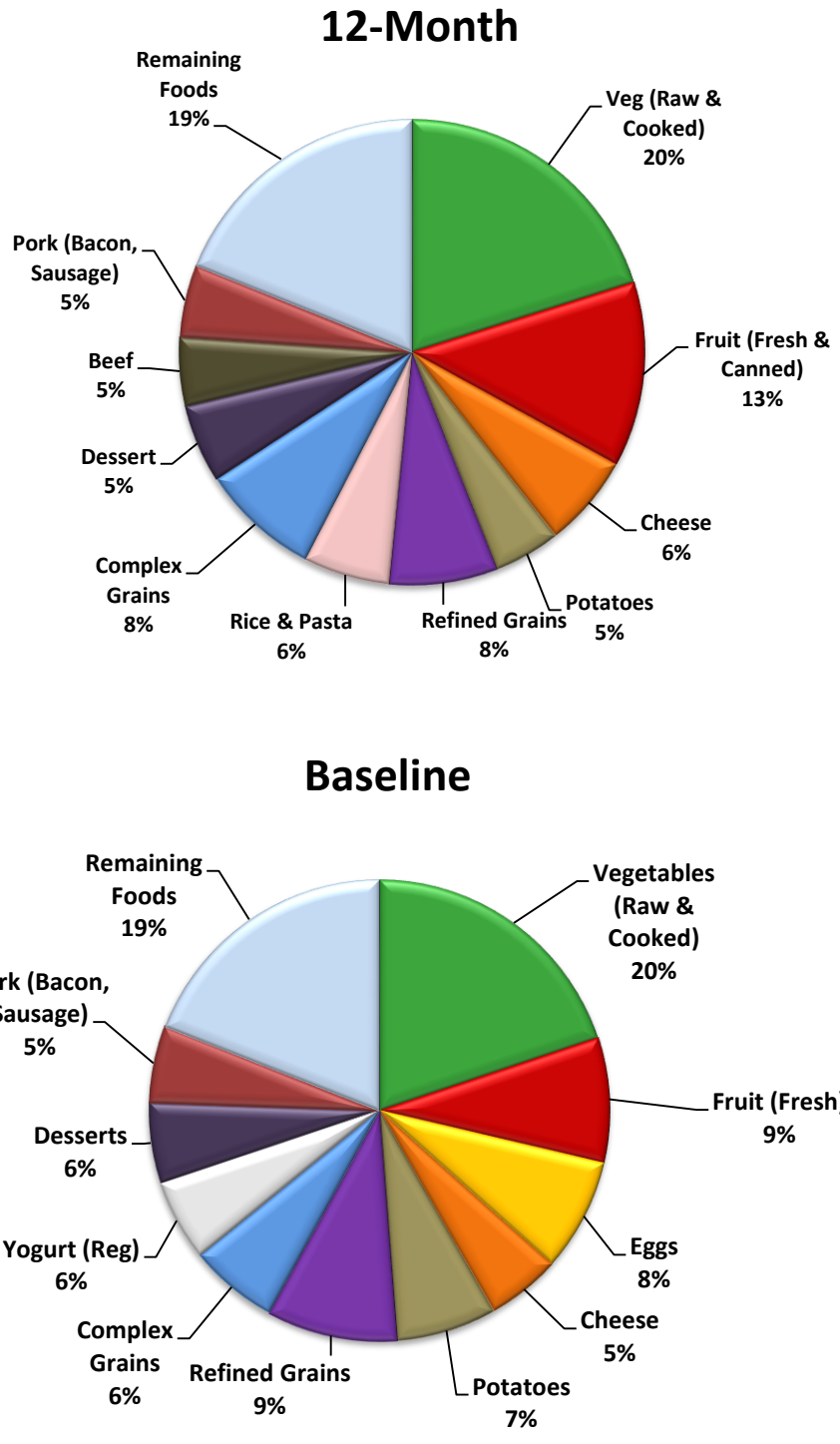


Figure 7. Beverage Choice of SWCS DFAC Patrons Pre-Post Assessment.

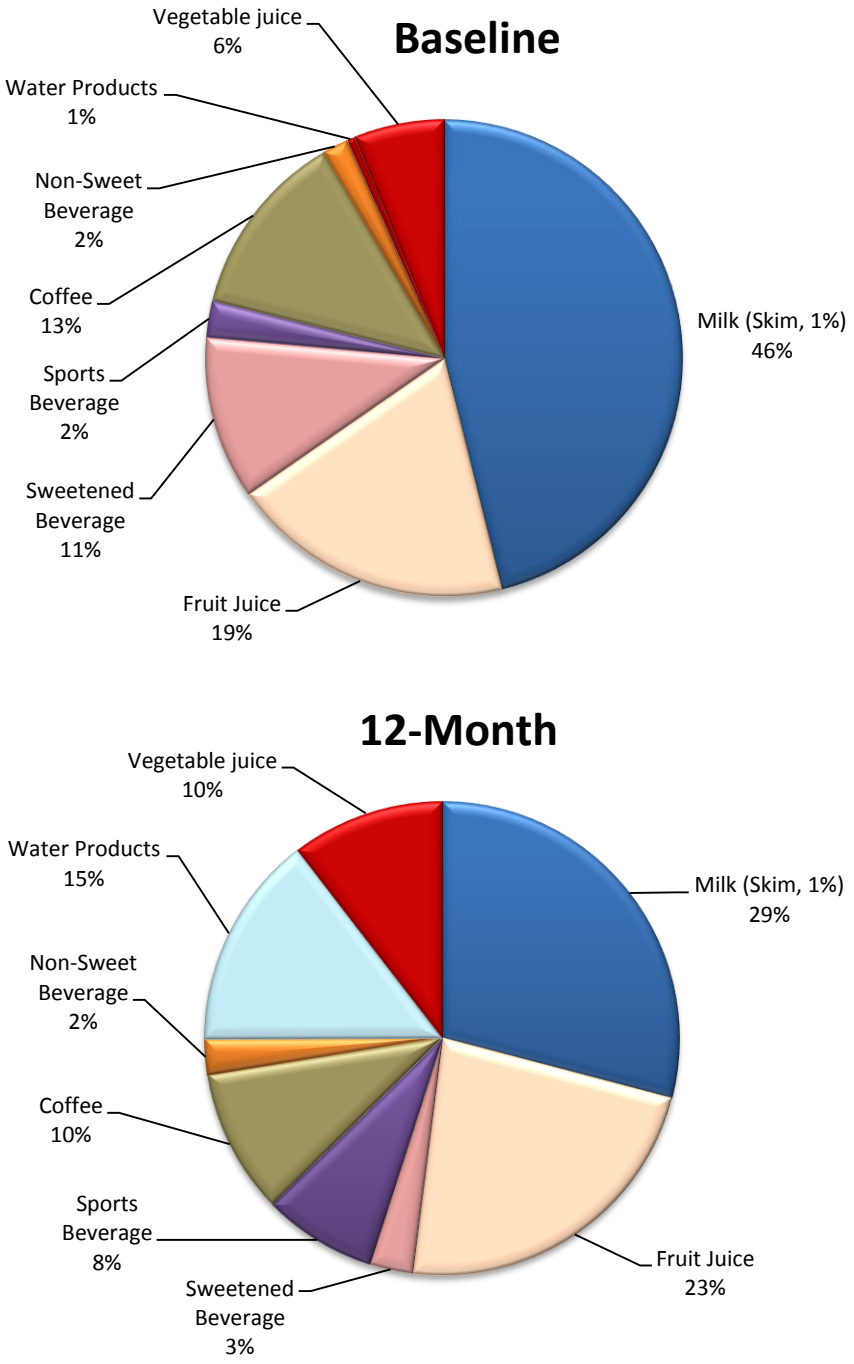


Figure 8. Beverage Choice Categories of 2BCT DFAC Patrons Pre-Post Assessment.

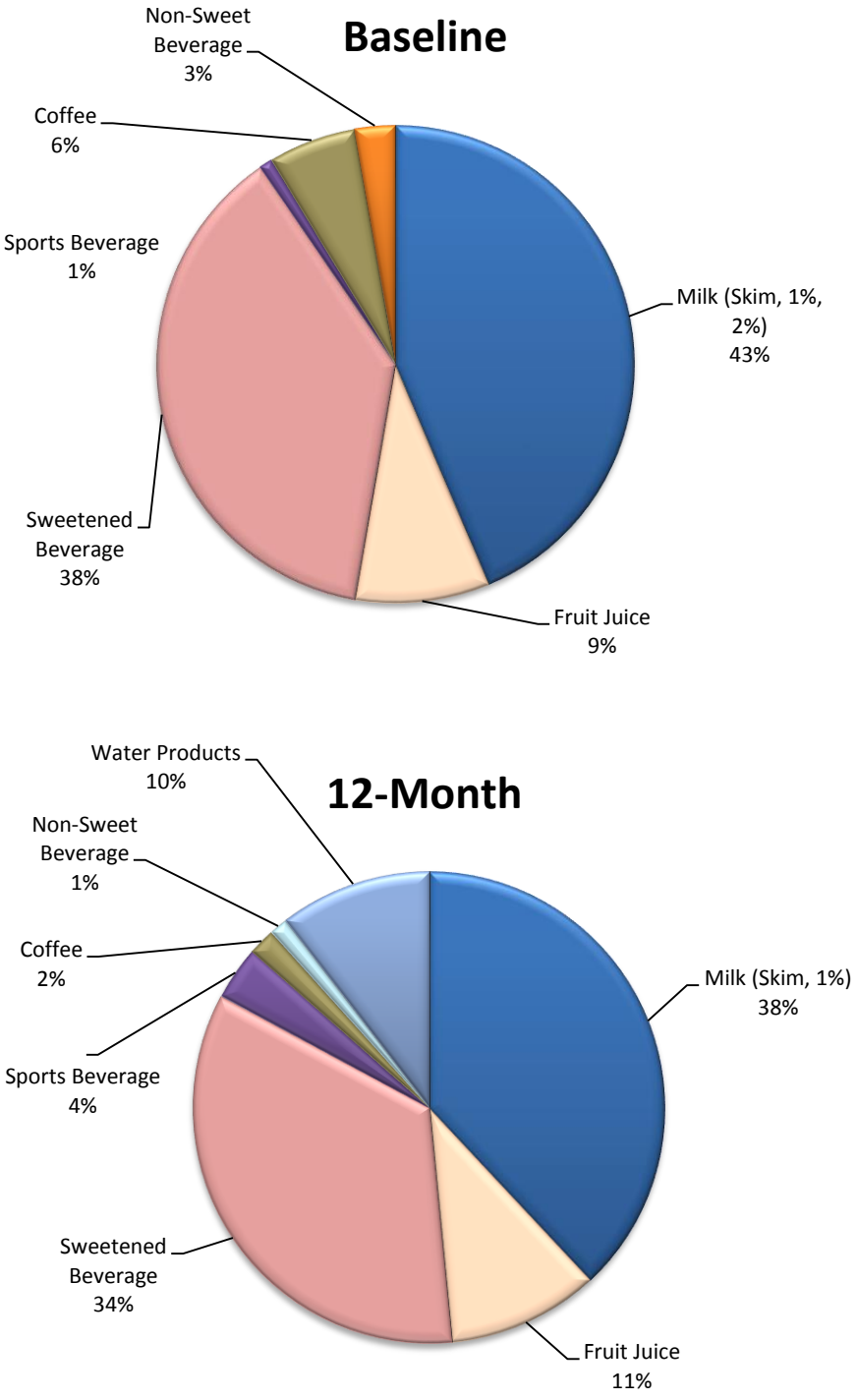


Figure 9. SWCS Patron Fruit, Vegetable, and Dairy Servings (Cups) Pre- to Post-HPP DFAC Intervention Compared to 2BCT & USDA Recommendations

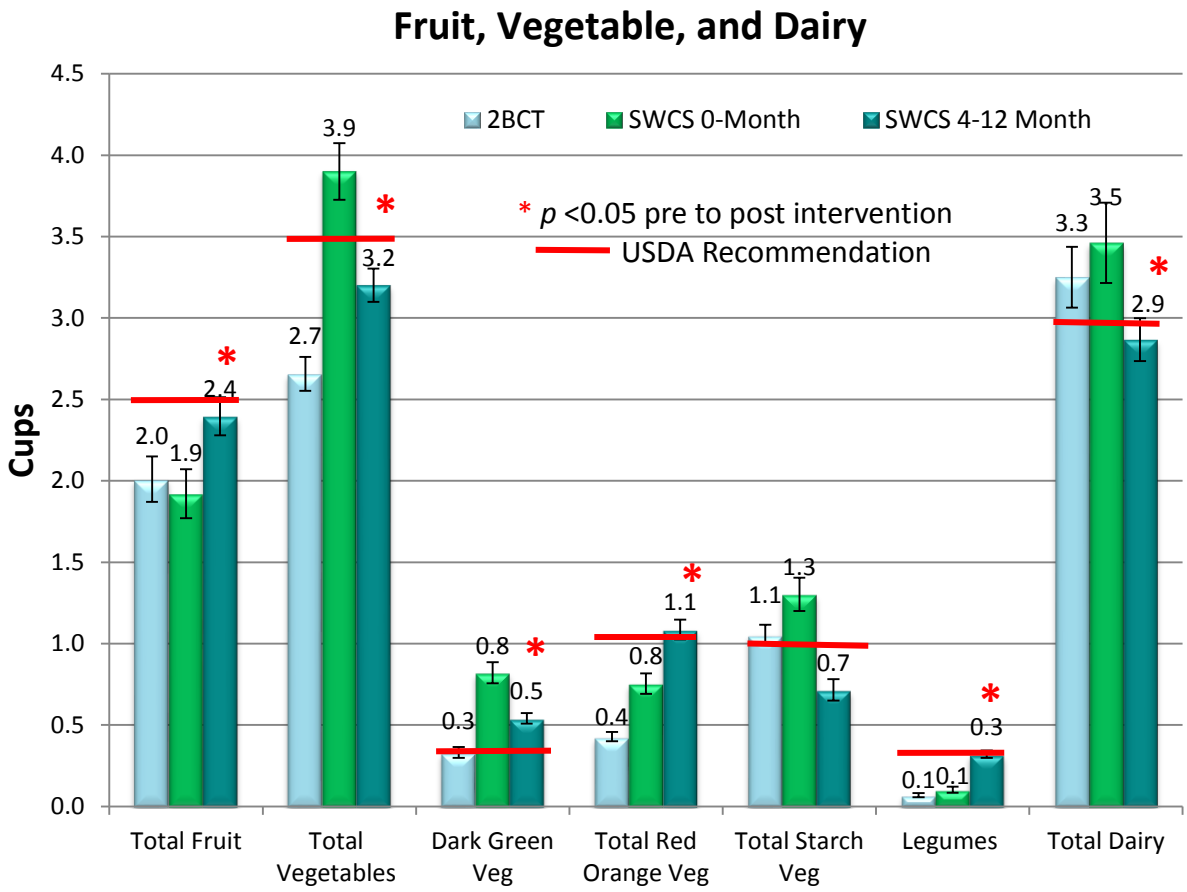


Figure 10. SWCS Patron Grains and Protein Food Servings (Ounces) Pre- to Post-HPP DFAC Intervention Compared to 2BCT & USDA Recommendations

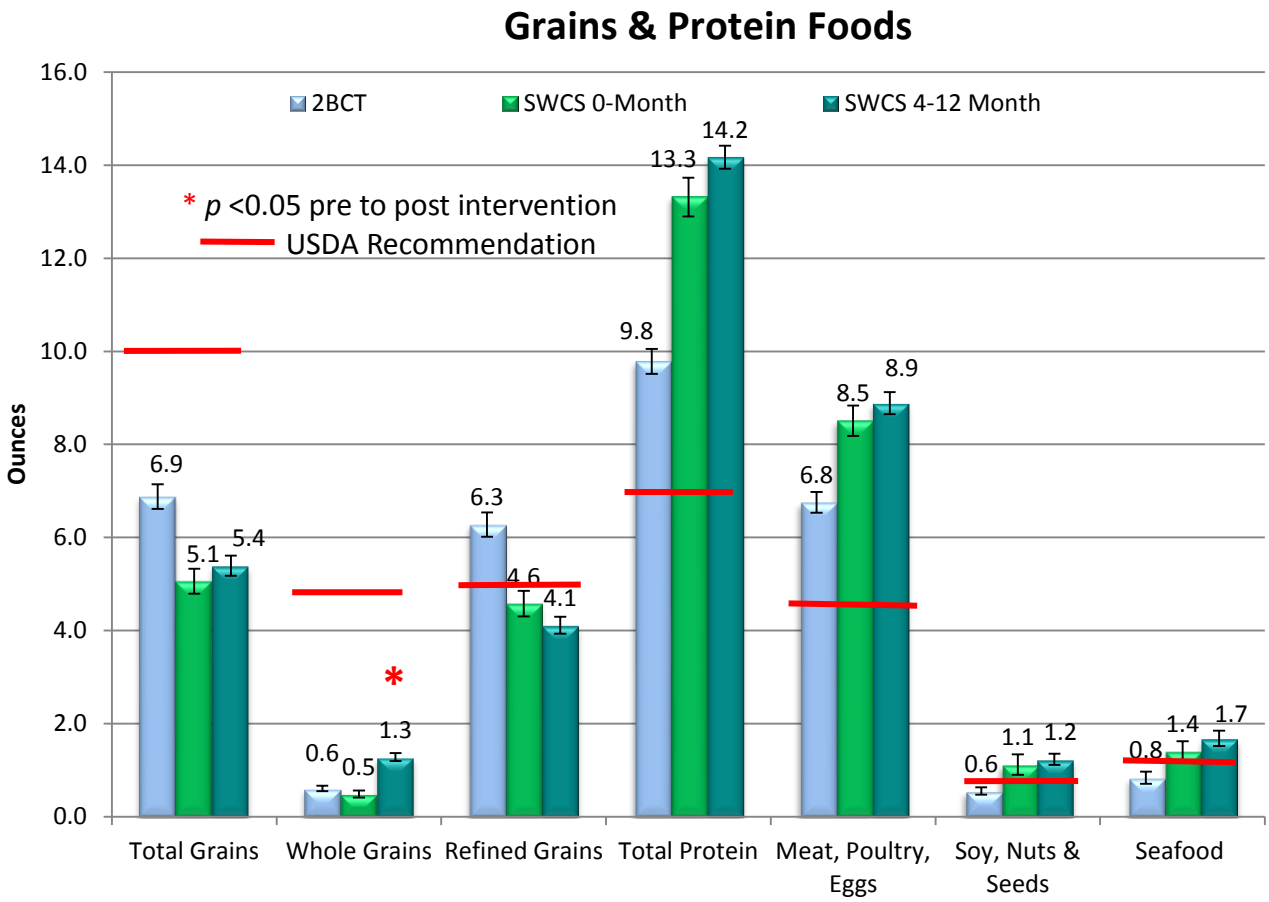


Figure 11. SWCS Patron Discretionary Calories (Grams) Pre- to Post-HPP DFAC Intervention Compared to 2BCT & USDA Recommendations

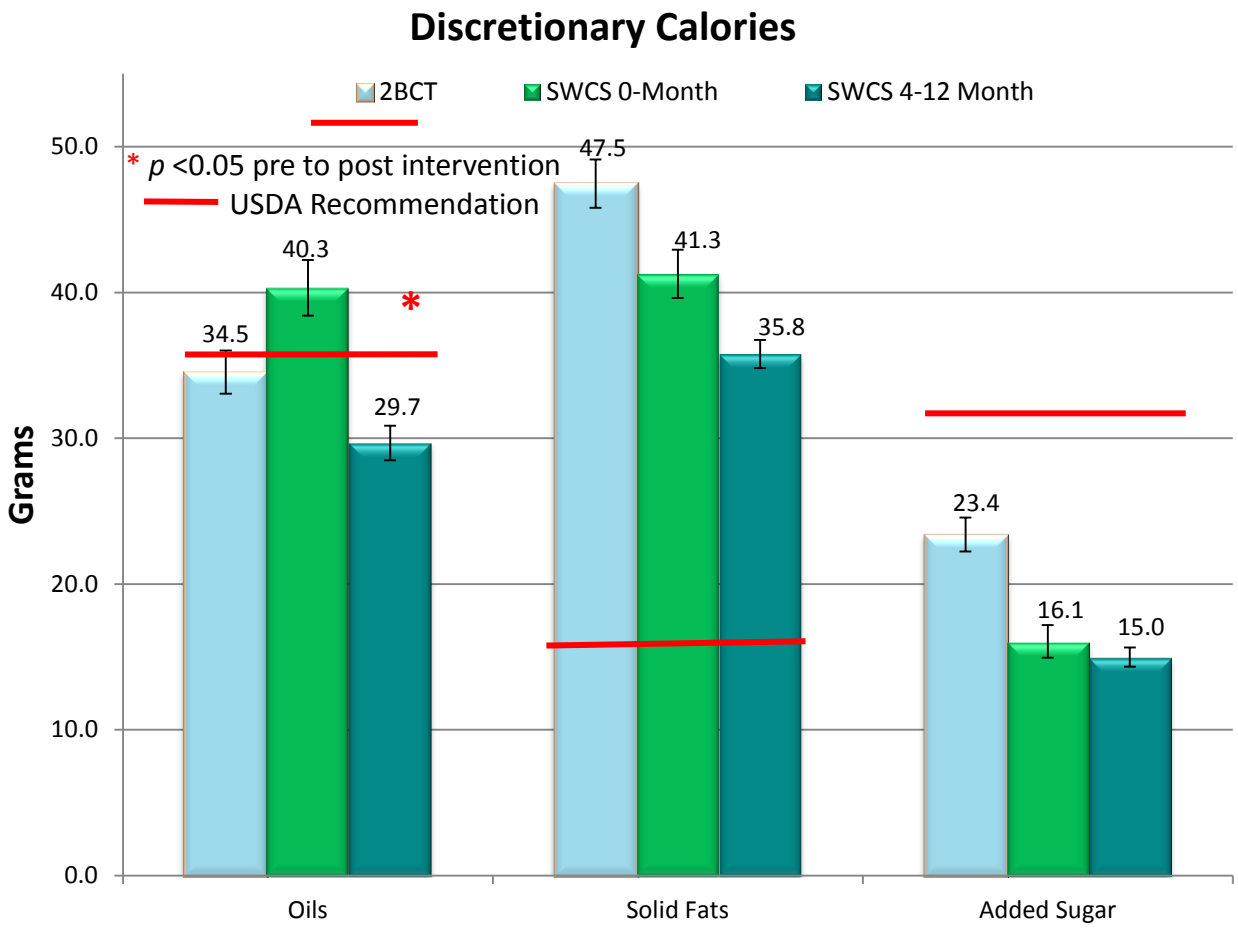


Figure 12. Customer Satisfaction Related to Food Appeal, Options and Availability of SWCS DFAC Patrons by Iteration.

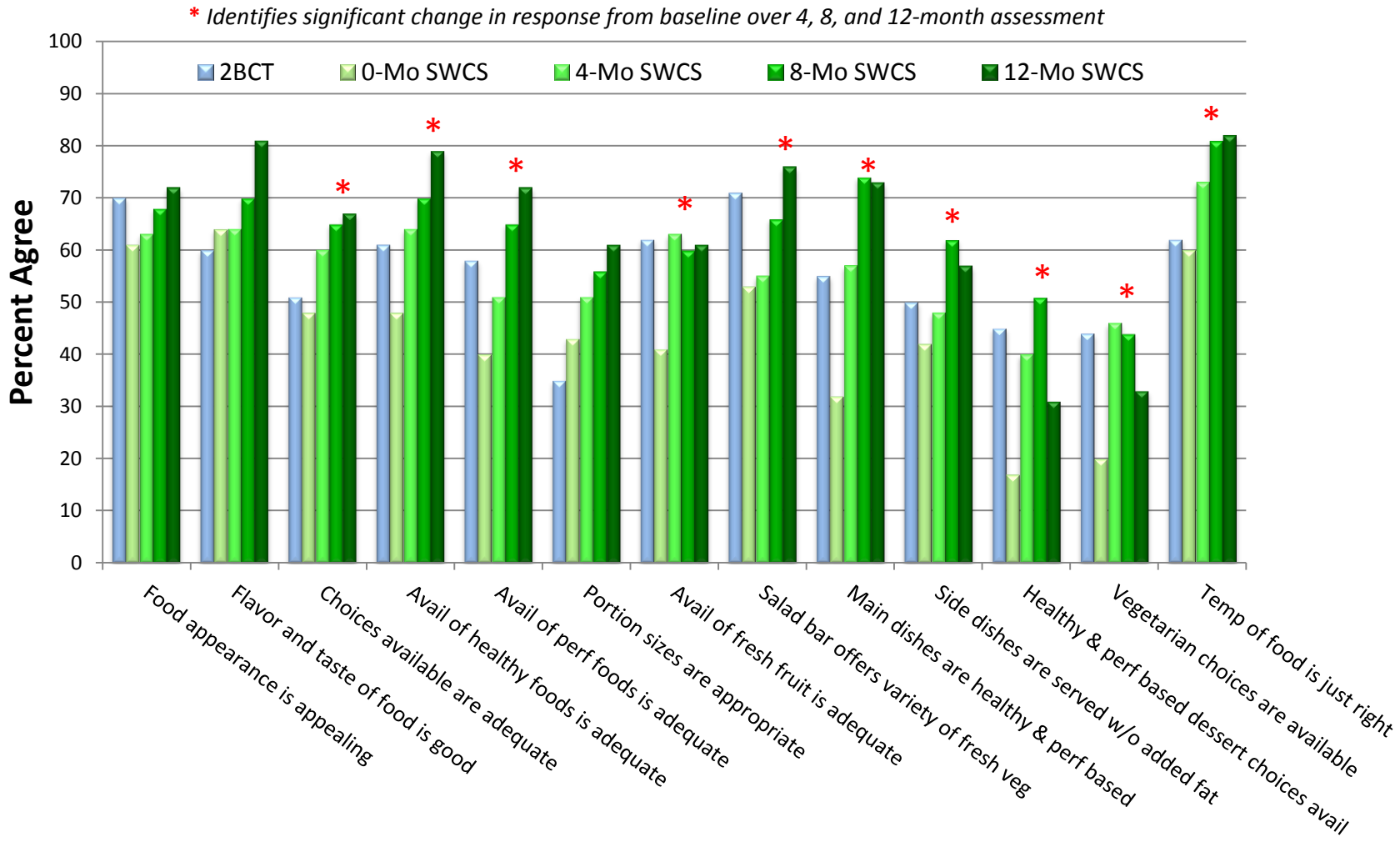


Figure 13. Customer Satisfaction Related to Point-of-Service Labeling of SWCS DFAC Patrons by Iteration.

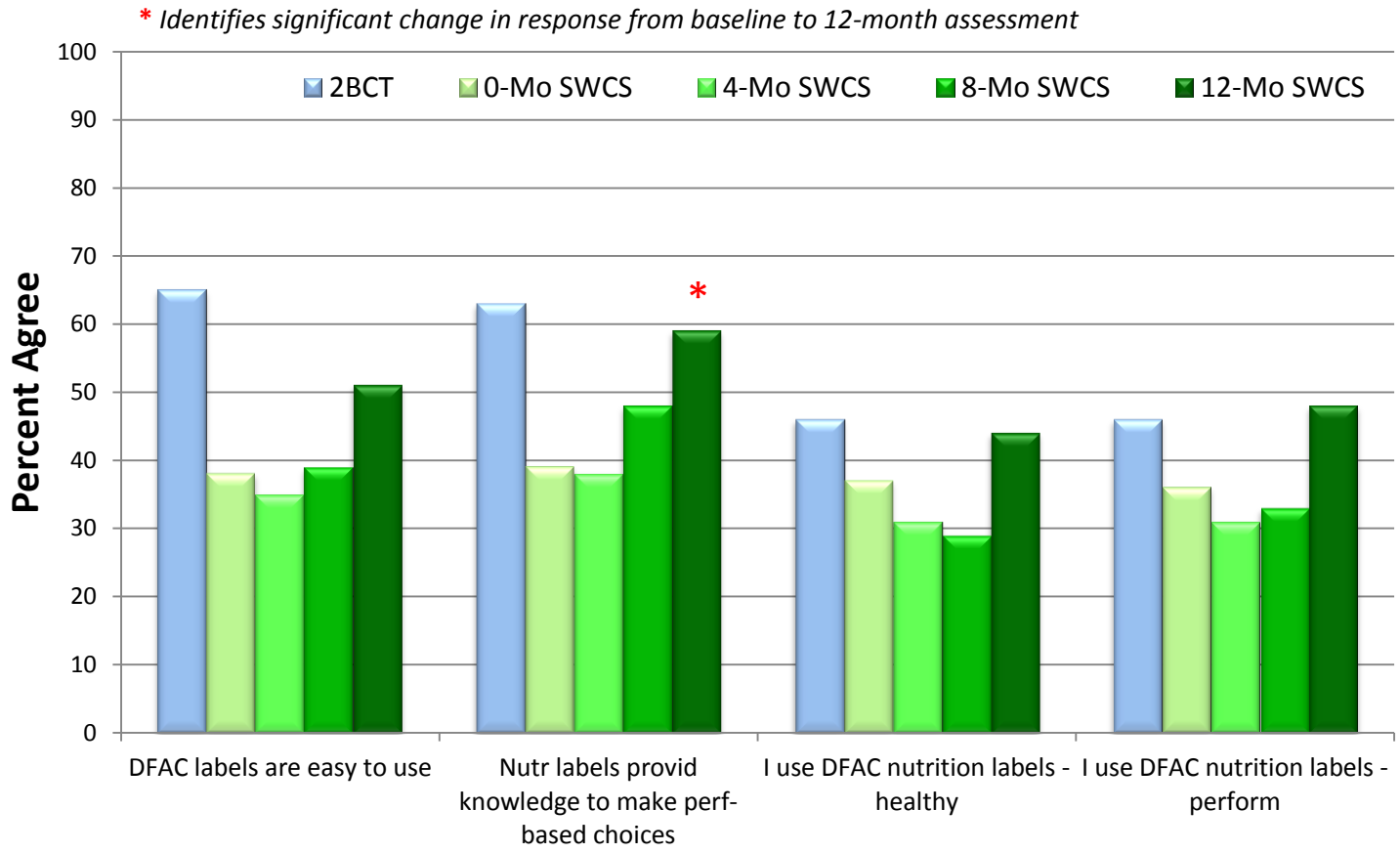
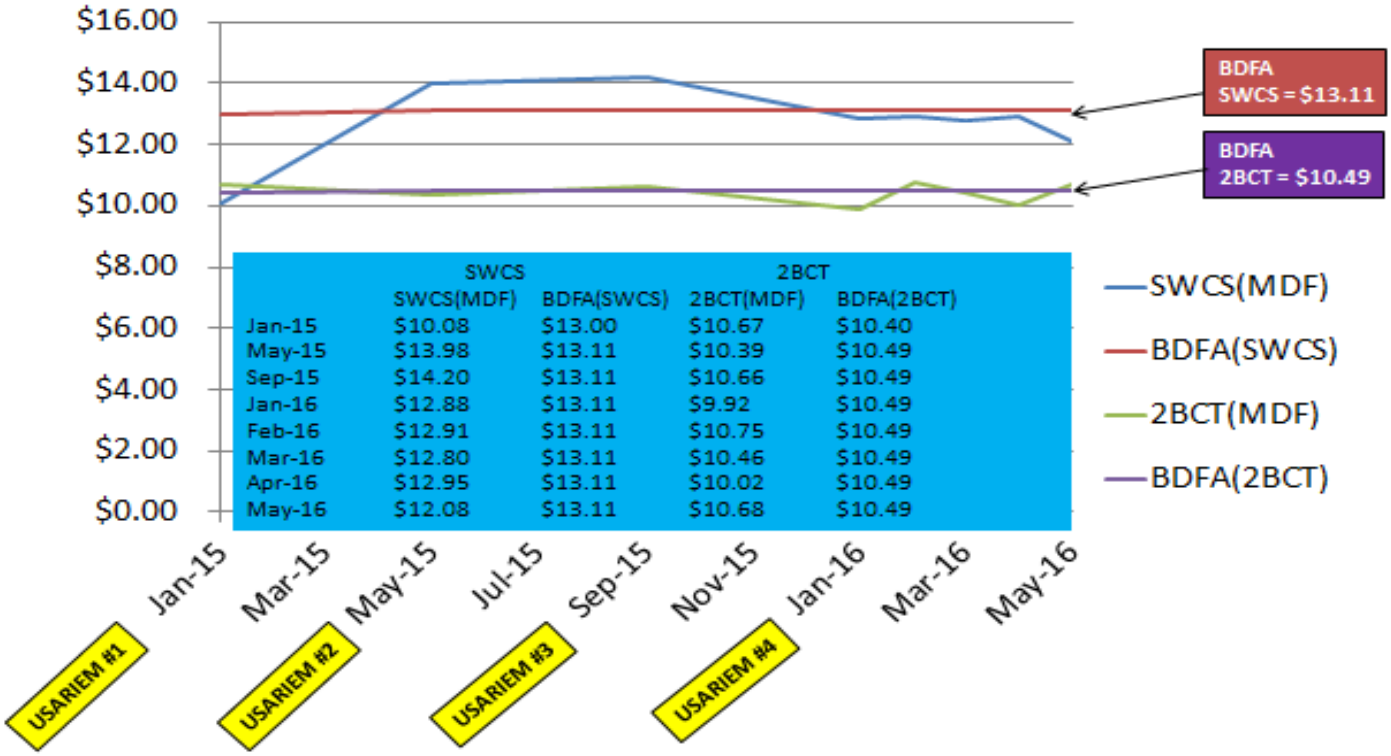
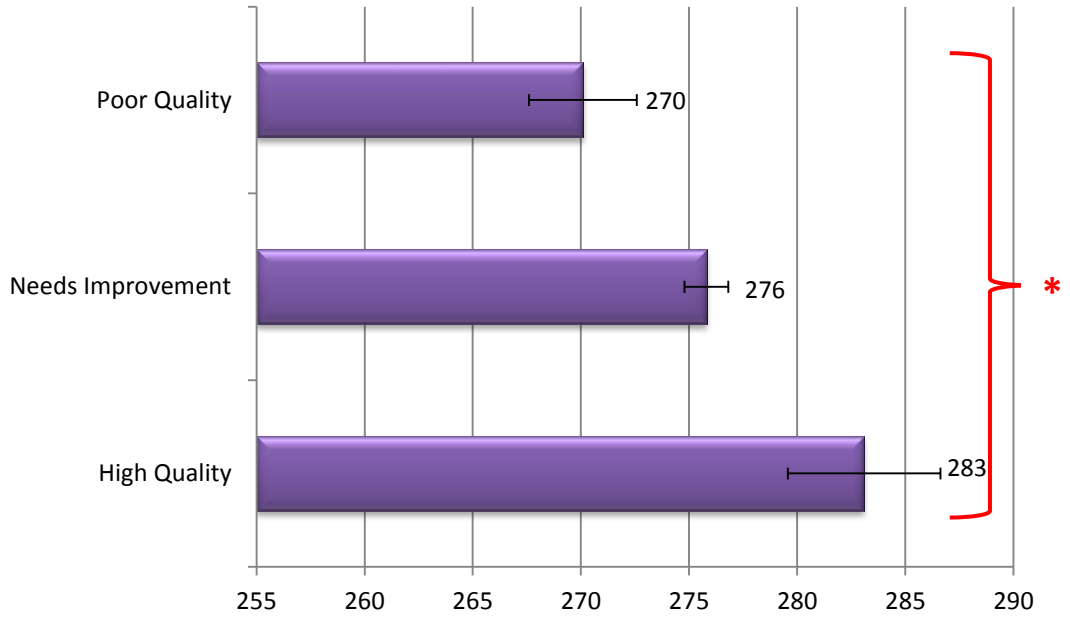


Figure 14. Average Plate Cost Analysis (Jan 2015 to May 2016) by DFAC.



	SWCS		2BCT	
	SWCS(MDF)	BDFA(SWCS)	2BCT(MDF)	BDFA(2BCT)
Jan-15	$10.08	$13.00	$10.67	$10.40
May-15	$13.98	$13.11	$10.39	$10.49
Sep-15	$14.20	$13.11	$10.66	$10.49
Jan-16	$12.88	$13.11	$9.92	$10.49
Feb-16	$12.91	$13.11	$10.75	$10.49
Mar-16	$12.80	$13.11	$10.46	$10.49
Apr-16	$12.95	$13.11	$10.02	$10.49
May-16	$12.08	$13.11	$10.68	$10.49

Figure 15. Mean Total APFT Score by Healthy Eating Index Score-2010 (Sodium Adjusted) Diet Quality Category.



* Significant difference in APFT score between HEI quality category (p<0.034)

Note: Poor Quality Diet = 0-50 points; Diet Needs Improvement = 51-80 points; High Quality Diet = 81-100 points

Appendix A: Special Operations Forces Performance-Based Menu Standards and Guidelines

1 May 2012

MEMORANDUM FOR RECORD

SUBJECT: Special Operations Forces (SOF) Performance-Based Menu Standards and Guidelines

1. Due to the long-term, high physical and cognitive performance demands on SOF human weapon systems, dining facilities identified as serving primarily SOF personnel should provide a performance-based menu that fuels training and missions, optimizes physical and mental performance, prevents chronic disease, enhances the immune system, supports task-specific body composition and provides education through modeling.

2. The SOF performance-based menu standards and guidelines were developed from the Dietary Guidelines for Americans 2010, Joint Subsistence Policy Board DoD Menu Standards 2010, U.S. Olympic Training Center (OTC) menu standards and sport and performance optimization scientific evidence in order to meet these criteria.

3. These standards were developed by the U.S. Special Operations Command (USSOCOM) Performance Dietitian (PD) Working Group. The USSOCOM PD Working Group included performance dietitians working with Army, Navy and Air Force SOF personnel.

4. SOF Performance-Based Menu Standards and Guidelines should be incorporated into all dining facilities serving predominately SOF populations, within the limits of each Service's applicable food operations regulations and policies.

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Special Operations Forces (SOF) Performance-Based Menu Standards and Guidelines

Goals of SOF Performance-Based Menu Guidelines

- Provide a performance-based menu that fuels training and missions, optimizes physical and mental performance, prevents chronic disease, enhances the immune system, supports task-specific body composition and provides education through modeling
- Provide a varied, colorful, flavorful and balanced menu based on national guidelines, sport nutrition best-practices and current scientific evidence.
- Minimize/reduce food costs and waste by serving foods in bulk and reducing use of individual serving products (PC)
- Enhance nutritional quality and safety of the menu through seasonally grown foods as budgets allow
- Maintain and enhance nutritional quality of menu by appropriate cooking techniques
- Reduce pre-packaged and convenience foods and food products served and replace with freshly prepared items or more natural alternatives

1. Fruit/Fruit Juices
   a. Standards:
      (1) Juices served will be 100% juice
      (2) Two or more fresh fruit choices per meal, cut up and ready to eat
      (3) Seasonal fruits incorporated into menu as much as possible
   b. Guidelines
      (1) Bananas available at breakfast daily
      (2) Frozen or canned (light syrup or own juice) fruits when fresh fruit not available. Priority: 1. Fresh 2. Frozen 3. Canned
      (3) Unsweetened dried fruits available at meals (e.g. purple of gold raisins, apricots, figs, apples, mangos, pineapple, cranberries, blueberries, cherries, pears, plums, etc.)
      (4) If dried fruit unavailable, offer unsweetened pureed fruit or compote (e.g., apple sauce, stewed fruit, etc.)
      (5) Fruits on dessert and salad bars to be cut up and ready to eat

2. Vegetables
   a. Standards
      (1) At least two hot vegetables per meal without added fat; one starch and one non-starch deep-colored vegetable
      (2) One legume/bean served ≥ 3 times per week
      (3) Seasonal vegetables incorporated into menu as much as possible
   b. Guidelines
      (1) No fried vegetables
      (2) Legumes/beans include, but are not limited to, peanuts, black eyed peas, lentils, lima beans, black beans, kidney beans, cannellini beans, pinto beans,

baked beans, chickpeas/garbanzo beans, navy beans, and refried beans with ≤10% saturated fat

(3) Starchy vegetables include, but are not limited to, potatoes, corn, peas, sweet potatoes, yams, acorn squash, butternut squash and pumpkin

3. Salad Bar
   a. Standards
      (1) Salad bar includes leafy green salad and one fresh topping from each of the following categories:
         (i) Red colored produce
         (ii) Orange/yellow colored produce
         (iii) Green colored produce
         (iv) Blue/purple colored produce
         (v) White/tan/brown colored produce
         (vi) Unmixed proteins
         (vii) Dried fruits
         (viii) Nuts
      (2) Two colors of fruits, cut up and ready to eat
      (3) Seven salad dressings (two regular, four containing ≤10% saturated fat)
      (4) Cold salads prepared with dressing that contain ≤10% saturated fat
      (5) Seasonal fruits and vegetables incorporated into menu as much as possible
   b. Guidelines
      (1) Leafy green salad minimum standard is 50% dark green leaves such as romaine or spinach leaves
      (2) Cold salad recipes are made with dressings that contain ≤10% saturated fat
      (3) Salad dressings must meet the following criteria:
         (i) Two regular creamy dressings
         (ii) Five dressings that contain ≤10% saturated fat
      (4) Appropriate fresh toppings include one item from each of the following categories, preferably seasonal
         (i) Red vegetables (e.g. beets, red peppers, radishes, radicchio, red onions, red potatoes, tomatoes, etc.) and fruits (e.g. red apples, cranberries, red grapes, pink/red grapefruit, raspberries, strawberries, watermelon, red pears, pomegranates, blood organs, cherries, etc.)
         (ii) Orange/yellow vegetables (e.g. butternut squash, carrots, yellow peppers, yellow potatoes, pumpkin, squash, yellow beets, rutabaga, etc.) and fruits (yellow apples, apricots, cantaloupe, grapefruit, nectarines, organs, peaches, yellow pears, pineapple, tangerines, mangoes, papaya, yellow figs, yellow watermelon, golden kiwifruit, persimmons, etc.)
         (iii) Green vegetables (e.g. asparagus, broccoli, Brussels sprouts, Chinese cabbage, green beans, green cabbage, celery, cucumbers, endive, leafy greens, leeks, lettuce, green onions, okra, peas, green peppers, snow peas, spinach, sugar snap peas, zucchini, arugula, broccoflower,

broccoli rabe, chayote squash, watercress) and fruits (e.g. green apples, green grapes, honeydew, kiwifruit, limes, avocados, etc.)

(iv) Blue/purple vegetables (e.g. black olives, purple cabbage, eggplant, purple-flesh potatoes, purple asparagus, purple carrots, purple Belgian endive, purple peppers, etc.) and fruits (e.g. blackberries, blueberries, concord grapes, dried plums, purple grapes, plums, raisins, purple figs, black currants, elderberries, etc.)

(v) White/tan/brown vegetables (e.g. cauliflower, garlic, mushrooms, onions, parsnips, white-flesh potatoes, shallots, turnips, white corn, ginger, Jerusalem artichokes, jicama, kohlrabi, etc.) and fruits (e.g. bananas, dates, white nectarines, white peaches, brown pears, etc.)

(vi) Unmixed proteins (i.e. no added mayonnaise) (e.g. canned tuna, canned and deboned salmon, hard boiled eggs, diced ham, diced chicken, diced turkey, green soybeans, hard boiled eggs, 1% or 2% fat cottage cheese, etc.)

(vii) Dried fruits (e.g. apricots, apples, purple or gold raisins, cranberries, pineapples, mangos, blueberries, cherries, figs, blueberries, pears, plums, etc.)

(viii) Nuts (e.g. almonds, Brazil nuts, cashews, chestnuts, hazelnuts/filberts, macadamia, pecans, pine nuts, pistachio, walnuts, sunflower seeds, pumpkin seeds, pecans, mixed nuts, etc.)

4. Grains/Starches
   a. Standards
      (1) Bread varieties, minimum standards: three choices of breads, and one variety bread (i.e. roll, cornbread, garlic bread, muffin, bagel, English muffin, Sandwich/bagel thins, fruit or vegetable breads, or biscuit) offered with meals
      (2) At least one bread offered that is 100% whole grain (i.e. 3 grams fiber per serving) and at least one bread offered is folate and iron-fortified
      (3) Choice of six whole grain, ready to eat cold cereals: four must be without sugar coating and at least 2 contain 3 grams fiber per serving
      (4) Bulk dispensing of two highest volume cereals is mandatory
      (5) One hot cereal without added fat or sugars at breakfast
      (6) At least one non-fried hot starch served per meal without added fat (e.g. potatoes, rice, pasta, quinoa, couscous, etc.)
   b. Guidelines
      (1) Recommend the following bread options:
         (i) One sliced, white option
         (ii) One sliced, 100% whole-grain option
         (iii) One specialty bread (e.g. cinnamon-raisin, zucchini, banana, pumpkin bran, etc.)
         (iv) Two English muffin type options (one white, one 100% whole-grain or sandwich/bagel thins)
         (v) Two bagel options, at least one with ≥3 grams of fiber
      (2) Cereal bars offered will contain at least 3 grams of fiber per serving

(3) Hot cereal options include, but are not limited to, oatmeal, grits, cream of wheat, malt-o-meal, and quinoa

5. Short Order Station
   a. Standards
      (1) Four grilled short order type items (i.e. two hot meat sandwiches, one grilled stir-fry, and one hot vegetarian sandwich)
      (2) One grilled, fresh vegetable or one hot vegetable on short order line
      (3) Choice of two additional short order entrees (e.g., but not limited to, grilled chicken breast, pizza, wrap or flatbread sandwich, lean meat or vegetarian burrito, lean meat or vegetarian fajitas, etc.)
      (4) Offer one baked side item throughout entire meal service
      (5) Offer assorted chips and pretzels. Offer at least one baked or low fat chip product.
   b. Guidelines
      (1) As a standard, offer whole grain buns for burgers and hotdogs
      (2) Use non-fat cooking sprays as needed instead of buttering breads for grilled sandwiches, stir-fry, vegetables, etc.
      (3) Only lean ground beef and turkey (≥90% lean) burgers with no fillers or extenders
      (4) Offer variety in cheeses used for grilled sandwiches (to include, but not limited to, provolone, Swiss, American, cheddar, feta, bleu, jalapeno, etc.)
      (5) Offer grilled fresh vegetable or hot non-starchy vegetable on short order line
         (i) As a cost saver, consider using previous day's pre-cut salad/fruit bar options for placement in pizzas and stir-fry or as grilled fresh vegetable options
      (6) Pizza topping options (lists are not all-inclusive):
         (i) Lean meats (e.g. marinated chicken such as pesto, jerk, bar-b-que, Buffalo, plain, herb vinaigrette; lean beef, lean meatballs, chicken or turkey sausages, pepperoni, turkey, ham, etc.)
         (ii) Cheeses (e.g. bleu, part-skim mozzarella, fresh mozzarella, cheddar, feta, parmesan, provolone, ricotta, etc.)
         (iii) Bases/sauces (e.g. red sauce, pesto, olive oil and garlic, bar-b-que, etc.)
         (iv) Vegetables/fruits (e.g. green, black or Kalamata olives, mushrooms, tomatoes, sun dried tomatoes, jarred or canned artichoke hearts, bell peppers of various colors, banana peppers, broccoli, onions [red, white, yellow or caramelized], garlic, jalapenos, pineapple, roasted red potatoes, spinach, fresh basil, etc.)
      (7) Grilled stir-fry options, one from each of the following categories:
         (i) Starch (e.g. pasta, rice, potato, etc.)
         (ii) Lean meat (e.g. chicken, lean beef, lean pork, turkey, etc.)
         (iii) Vegetable
         (iv) Sauce (e.g. broth, bar-b-que, olive/canola oil and garlic, pesto, jerk, sweet and sour, General Tso's sauce, sesame, French onion, etc.) and

seasoning (e.g. garlic, pepper, salt , seasoning salt, lemon pepper, sesame seeds, pesto, basil, oregano, onion powder, etc.)

(a) As a cost saver, use previous day's pastas, cooked meats, and precut salad/fruit bar options. Combine pasta, rice potato, etc. with fresh cut vegetables and fruits with pre-cooked cut up lean meats and seasoning/sauces

(b) The following examples are not all-inclusive, but are meant to provide concept clarification:

- Option A: pasta, spinach, tomatoes, mushrooms, chicken or lean beef, garlic, olive/canola oil
- Option B: rice, broccoli, carrots, onions, mandarin organs, chicken or lean beef, and sweet and sour or General Tso's sauce
- Option C: potato, carrots, beets, bell peppers, onions, chicken or lean beef, light oil and onion soup seasoning with broth

(8) Baked short order side item options to include, but not limited to, baked or roasted (whole or cut up) white or sweet potatoes with no added fats or sugars, baked onion rings, or baked French fries (e.g. shoe string, sweet potato, steak cut, potato gems, etc.)

6. Sandwich/Deli Station
   a. Standards
      (1) Two varieties of bread offered: one 100% whole grain (i.e. 3 grams fiber per serving) and one variety bread choice
      (2) Two sliced lean meat options (e.g. turkey, lean ham, chicken, turkey pastrami, roast beef, etc.)
      (3) Two sliced cheese options
   b. Guidelines
      (1) One 100% whole-grain (3 grams fiber per serving) and one variety bread choice
      (2) Lean, sliced deli meat options (e.g. turkey, lean ham, chicken, turkey pastrami, roast beef, chicken, etc.)

7. Meats and Entrees
   a. Standards
      (1) One red-meat option (i.e., beef or game meat) and either one white-meat (i.e., poultry or pork) or fish/seafood option that are ≤30% fat, ≤10% saturated fat
      (2) Fish served a minimum of two times per week
         (i) At least one fish served per week that is high in omega-3 fats (i.e., salmon, tuna, trout, herring, mackerel, and sardines)
   b. Guidelines
      (1) Methods of preparation to include, but not limited to, baking, braising, broiling, grilling, poaching, roasting, sautéing, steaming, stir-frying, stewing, searing, etc.

(2) Visible fat removed
(3) No fried entrees
(i) Exceptions for special occasions such as holiday meals, Service birthdays or other celebrations
(ii) No more than two special occasions per month
(iii) Special occasion menus will be approved by the command dietitian
(4) Preferred cuts may include, but are not limited to, round, loin, leg, breast, tenderloin, etc.
(5) Only lean ground beef and turkey (≥90% lean) with no fillers or extenders
(6) One or more vegetarian options at the lunch and dinner meals
(7) Serve gravy and sauces separately
(8) Choice of two breakfast meats, one of which must be ≤30% total fat, ≤10% saturated fat (e.g. turkey, lean ham, Canadian bacon, etc.)

8. Dairy and Eggs
a. Standards
(1) All milk, cheese, egg products to be ≤10% saturated fat (with the exception of whole eggs)
b. Guidelines
(1) All mile fortified with vitamin A and vitamin D
(2) Offer 1% or skim milk as the primary milk option (white and chocolate varieties offered at all meals)
(3) Use 1% or skim milk in recipes when feasible
(4) Offer dairy choices that contain ≤10% saturated fat (e.g. cottage cheese, sour cream, cream cheese, shredded cheese, sliced cheese, etc.)

9. Fats and Oils
a. Standards
(1) Use fats containing ≤10% saturated fat
b. Guidelines
(1) No oils or fats containing trans-fats will be used in oils, shortenings, or spreads for cooking, baking or dressing
(2) Salad dressings, mayonnaise and dairy products will be ≤10% saturated fat
(3) Use olive oil or olive/canola oil blends for most food preparations
(4) Use sesame, peanut or coconut oil/milk for Asian cooking
(5) Avoid partially hydrogenated oils and margarines
(6) Avoid frying of food
(7) Serve avocado in season at salad bars and with Mexican/Southwestern foods

10. Beverages
a. Standards
(1) The following options offered at every meal:
(i) Water
(ii) Skim milk

(iii) 1% white milk
(iv) 1% chocolate milk
(v) Sports (5-8% carbohydrate and electrolyte) beverage
(vi) Two 100% fruit and/or vegetable juice (may contain more than one)
(vii) Coffee (with no ingredients added)
(viii) Tea (herbal and caffeinated)
(ix) Carbonated beverages (two regular, two diet)

(2) All milk options must be labeled to reflect fat content (i.e., skim/fat free, 1% low fat)

b. Guidelines

(1) Offer lactose-free alternative (e.g. lactose free milk, soy milk, etc.) based on customer demand
(2) Sugar-free beverage flavoring powders or low-calorie flavoring packets for addition to water will be offered for all meals if used in feeding operations
(3) Water dispenser available in beverage area
(4) Coffee and hot tea available at all meals
(5) Brewed decaffeinated coffee available at all meals based on customer demand
(6) If soda is served, offer one of each of the following options at every lunch and dinner meal:
(i) One low-calorie dark soda
(ii) One low-calorie clear soda
(iii) One caffeine-free soda
(7) If ice tea is served, offer at least one sugar-free option

11. Condiments

a. Standards

(1) All sauces, condiments and spreads should be ≤15% saturated fat

b. Guidelines

(1) Coffee and tea creamers:
(i) Low-fat (i.e. 1% fat) milk and artificial sweetener
(ii) Flavored creamers if operating budget permits, at least one reduced-fat variety
(iii) No trans-fat
(2) Offer only mayonnaise, margarine, sour cream and cream cheese with ≤10% saturated fat
(3) Sodium restriction is not warranted in this population; no sodium limitations on condiments

12. Soups

a. Standards

(1) One of each of the following options offered each meal
(i) One pureed vegetable soup, preferably a colored vegetable as primary base (e.g. tomato, carrot-ginger, pumpkin, cauliflower, etc.) or creamy soup made with ≤30% total fat, ≤10% saturated fat

(ii) One broth type mixed soup with vegetables, rice, pasta, beans, chicken, turkey, stew, chili type

b. Guidelines
   (1) Offer at least one soup at lunch based on customer demand
   (2) One trans-fat free baked product to accompany soup option

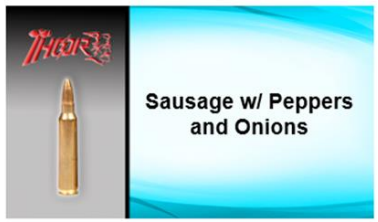
13. Desserts
   a. Standards
      (1) One cut up fresh fruit
      (2) One trans-fat free baked product
   b. Guidelines
      (1) Fresh fruit served on all dessert bars should be cut up and ready to eat
      (2) Offer one ≤10% saturated fat dessert
      (3) Offer one trans-fat free product (e.g. custard, pudding, coffee cake, cookies, ice cream, frozen yogurt, parfaits, etc.)

Appendix B: HPP THOR^3 Point-of-Service Label Examples

USASOC HPP Point-of-Service Labeling Examples for Foods

Green = Quality Carbohydrates
Blue = Lean Proteins
Orange = Performance Fats
Purple = Combination Foods

Quality Rating:
3 Bullet = Best Choice
2 Bullet = Good Choice
1 Bullet = Limit Intake



Appendix C: Demographic & Lifestyle Survey

Dining Satisfaction and Diet Quality of Soldiers Eating at Two Fort Bragg DFACs (15-04-HC)

Demographics & Lifestyle Information

Thank you for participating in this study. Please answer the following questions by filling in the circles that corresponds with your answer. All of the information you provide be kept confidential. Thank you.

MARKING INSTRUCTIONS	VOLUNTEER NUMBER	FILL IN TODAY'S DATE
• Use a No. 2 pencil only. • Do not use ink, ballpoint, or felt tip pens. • Make solid marks / fill the response completely • Erase cleanly any marks you wish to change. • Make no stray marks on this form. CORRECT: ● INCORRECT: ⊘⊠◒◔		MONTH DAY YEAR 20__

1. Gender:
   - M Male
   - F Female

2. What is your age today?

AGE years	

3. Your height in inches? (without shoes/boots)

HEIGHT inches	

for reference:
5 feet = 60 inches
6 feet = 72 inches

4. Your weight in pounds? (without clothing)

WEIGHT pounds		

5. What is your ethnic background?
   - 1 Hispanic or Latino
   - 2 Not Hispanic or Latino

RACE_OTHER

6. What is your racial background? (select all that apply)
   - 1 White or Caucasian
   - 2 Black or African American
   - 3 Native American/Alaskan Native
   - 4 Asian
   - 5 Native Hawaiian/Pacific Islander
   - 6 Other:_____

7. Please indicate the HIGHEST level of education you have completed (pick only one)
   - 1 Some high school (no GED or diploma)
   - 2 High school graduate (GED or diploma)
   - 3 Some college courses
   - 4 Associate degree (two-year college)
   - 5 Bachelors degree (four-year college)
   - 6 Graduate degree

8. What is your rank?

Enlisted	1 2 3 4 5 6 7 8 9
Officer	1 2 3 4 5 6 7 8 9
WO	1 2 3 4 5

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9. Are you a SWCS student in the Special Forces Qualification Course (SFQC)?

1 Yes
2 No

10. What is your primary MOS? Description:_____

11. How long have you been in the Armed Services?

Active Duty

1 Less than one year **OR** If a year or more, please fill in the number of years (start with leading zero's when needed).

12. During the past 7 days, which meals and snacks did you eat on a regular basis (at least 5 times per week)?
Please fill in one response for each line:

Yes	No	
Y	N	A. Early morning snack
Y	N	B. Breakfast
Y	N	C. Morning snack
Y	N	D. Lunch
Y	N	E. Afternoon snack
Y	N	F. Dinner
Y	N	G. Evening snack

13. During the past 7 days, where did *MOST* of your meals and snacks come from? Select ONLY ONE choice for each meal and snack.

	EARLY MORNING SNACK	BREAKFAST	MORNING SNACK	LUNCH	AFTERNOON SNACK	DINNER	EVENING SNACK
Do not eat a meal/snack at this time	1	1	1	1	1	1	1
Home/Barracks (current residence)	2	2	2	2	2	2	2
Military dining facility	3	3	3	3	3	3	3
Fast food restaurants	4	4	4	4	4	4	4
Restaurants (table service)	5	5	5	5	5	5	5
Cafeterias (non military)	6	6	6	6	6	6	6
All you can eat buffets	7	7	7	7	7	7	7
Vending machines	8	8	8	8	8	8	8
Convenient stores	9	9	9	9	9	9	9
Other_____	10	10	10	10	10	10	10

Q13_oth

For questions 14-17, during the last 7 days, think about only those physical activities that you did continuously for at least 10 minutes at a time. On how many days did you do each type of activity and on the average, for how long?

14. VIGOROUS physical activity
(makes you breathe much harder than usual with heavy sweating; e.g. lifting weights, aerobics, or fast running / bicycling)?

Number of days per week: 0 1 2 3 4 5 6 7 X

TIME per day: Hr. | Min.

15. MODERATE physical activity
(makes you breathe somewhat harder than usual; e.g. jogging, carrying light loads, or bicycling at a regular pace)?

Number of days per week: 0 1 2 3 4 5 6 7 X

TIME per day: Hr. | Min.

16. WALK for at least 10 minutes at a time?
This includes walking at work and at home, walking to travel from place to place, and other walking that you did solely for recreation, sport, exercise or leisure.

Number of days per week: 0 1 2 3 4 5 6 7 X

TIME per day: Hr. | Min.

17. How much time in total did you usually spend SITTING on a week day?
This includes while at work or home, while doing course work and during leisure time, sitting at a desk, visiting friends, reading, traveling in a vehicle, and sitting or lying down to watch television.

TIME per day: Hr. | Min.

Use the table below to indicate how many hours during a typical day (24 hrs), during the week and then again during the weekends, you spend engaged in electronic activities.

18. DURING WEEKDAYS							
WATCHING TV		VIDEO GAMES		USING COMPUTER FOR WORK OR SCHOOL		USING COMPUTER FOR LEISURE	
0	0	0	0	0	0	0	0
1	1	1	1	1	1	1	1
2	2	2	2	2	2	2	2
3	3	3	3	3	3	3	3
4	4	4	4	4	4	4	4
5	5	5	5	5	5	5	5
6	6	6	6	6	6	6	6
7	7	7	7	7	7	7	7
8	8	8	8	8	8	8	8
9	9	9	9	9	9	9	9

19. DURING WEEKEND							
WATCHING TV		VIDEO GAMES		USING COMPUTER FOR WORK OR SCHOOL		USING COMPUTER FOR LEISURE	
0	0	0	0	0	0	0	0
1	1	1	1	1	1	1	1
2	2	2	2	2	2	2	2
3	3	3	3	3	3	3	3
4	4	4	4	4	4	4	4
5	5	5	5	5	5	5	5
6	6	6	6	6	6	6	6
7	7	7	7	7	7	7	7
8	8	8	8	8	8	8	8
9	9	9	9	9	9	9	9

20. How would you rate your physical readiness for military training or combat at this time?

1. Best physical shape in my life
2. Good physical shape
3. Neither good nor bad physical shape
4. Bad physical shape
5. Worst physical shape in my life

21. APFT score

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

22. Number of pushups

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

23. Number of sit ups

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

24. 2 MILE RUN TIME

minutes		/ seconds	
		/	
0	0		0
1	1		1
2	2		2
3	3		3
4	4		4
5	5		5
6	6		6
7	7		7
8	8		8
9	9		9

25. Alternate APFT event:

1. Bike
2. Walk
3. Swim
4. Other_____

26. DATE OF LAST APFT		
MONTH		0 1
		0 1 2 3 4 5 6 7 8 9
DAY		0 1 2 3
		0 1 2 3 4 5 6 7 8 9
YEAR 20__		0 1 2
		0 1 2 3 4 5 6 7 8 9

SLEEP

27. During the last 7 days, how would you rate your sleepiness during the day?

1. Feeling active, vital, alert, or wide awake
2. Functioning at high level, but not at peak; able to concentrate
3. Awake but relaxed; responsive but not fully alert
4. Somewhat foggy; let down
5. Foggy; losing interest in remaining awake; slowed down
6. Sleepy, woozy, fighting sleep; prefer to lie down
7. No longer fighting sleep, sleep onset soon; having dream-like thoughts

28. During the last 7 days, on average how many hours of sleep did you get in a 24-hour period? ***(to nearest ½ hr)***

Hours per day	
Hr.	Min.
0 0	0 0
1 1	1 1
2 2	2 2
3	3 3
4	4 4
5	5 5
6	6
7	7
8	8
9	9

PERFORMANCE

29. During the last 7 days, did your food choices in the dining facility have an effect on:

	NEVER	SOMETIMES	MOST OF THE TIME	ALWAYS
Feeling energized throughout the day?	1	2	3	4
Improving your mood during the day?	1	2	3	4
Feeling satisfied for several hours after meals (not over hungry nor over full)?	1	2	3	4
Improving your mental performance (e.g. ability to think clearly, focus, learn, and ability to recall information during the day)?	1	2	3	4
Improving your level of physical performance (e.g. during workout or military training)?	1	2	3	4
Improving your ability to sustain physical performance longer?	1	2	3	4
Feeling good about yourself?	1	2	3	4
Improving your recovery after a vigorous physical activity OR workout? *(recovery refers to how quickly your muscles and cardiovascular systems rebound after a workout or physical activity)*	1	2	3	4
Improving your recovery after a moderate physical activity OR workout? *(recovery refers to how quickly your your muscles and cardiovascular systems rebound after a workout or physical activity)*	1	2	3	4
Reducing injury?	1	2	3	4
Improving your sleep quality?	1	2	3	4
Improving your response to emotional or psychological stress?	1	2	3	4

Appendix D: Dining Facility Satisfaction Survey

Dining Facility Satisfaction Survey

MARKING INSTRUCTIONS	VOLUNTEER NUMBER	FILL IN TODAY'S DATE
• Use a No. 2 pencil only. • Do not use ink, ballpoint, or felt tip pens. • Make solid marks / fill the response completely • Erase cleanly any marks you wish to change. • Make no stray marks on this form. CORRECT: ● INCORRECT: ⊘ ⊠ ◒ ◔		MONTH DAY YEAR 20__

Thank you for participating in this study. Please answer the following questions by filling in the circles that reflect your experience with the food service. All of the information you provide be kept confidential. Thank you.

Complete by marking the boxes that reflect your experience with our Food service.

		STRONGLY AGREE	MODERATELY AGREE	AGREE	UNDECIDED	DISAGREE	MODERATELY DISAGREE	STRONGLY DISAGREE
1.	Appearance of the food is pleasing	1	2	3	4	5	6	7
2.	Flavor and taste of the food is good	1	2	3	4	5	6	7
3.	Choices available are adequate	1	2	3	4	5	6	7
4.	Availability of healthy foods is adequate	1	2	3	4	5	6	7
5.	Availability of performance foods is adequate	1	2	3	4	5	6	7
6.	Portion sizes are appropriate	1	2	3	4	5	6	7
7.	Availability of fresh fruit is adequate	1	2	3	4	5	6	7
8.	The salad bar offers a variety of fresh vegetables	1	2	3	4	5	6	7
9.	The main dishes served are healthy and performance-based	1	2	3	4	5	6	7
10.	The side dishes are served without added fat (ex: butter)	1	2	3	4	5	6	7
11.	Healthy and performance-based dessert choices are available	1	2	3	4	5	6	7
12.	Temperature of food (ex: hot food is hot) is just right	1	2	3	4	5	6	7
13.	Vegetarian food choices are available	1	2	3	4	5	6	7
14.	I find the DFAC nutrition labels easy to use	1	2	3	4	5	6	7
15.	Nutrition labels provide knowledge to make performance based choices	1	2	3	4	5	6	7
16.	I use the DFAC nutrition labels to chose healthy foods	1	2	3	4	5	6	7
17.	I use the DFAC nutrition labels to chose performance foods	1	2	3	4	5	6	7

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Appendix E: PreSLIM Scale

Appendix D1 - Pre-SLIM scale

MARKING INSTRUCTIONS	VOLUNTEER NUMBER	FILL IN TODAY'S DATE
• Use a No. 2 pencil only. • Do not use ink, ballpoint, or felt tip pens. • Make solid marks / fill the response completely • Erase cleanly any marks you wish to change. • Make no stray marks on this form. CORRECT: ● INCORRECT: ⊘⊠◒◔		MONTH DAY YEAR 20__

TIME (24 HR FORMAT)	PRE-MEAL ONLY WHAT WAS YOUR LAST MEAL	*IF A SNACK, WHAT DID YOU EAT?
	① Early morning snack* ② Breakfast ③ Morning snack* ④ Lunch ⑤ Afternoon snack* ⑥ Dinner ⑦ Evening snack*	_____ _____ _____

Please indicate your level of hunger or fullness right now by placing a hash mark (/) on the scale below.

GREATEST IMAGINABLE FULLNESS
EXTREMELY FULL
VERY FULL
MODERATELY FULL
SLIGHTLY FULL
NEITHER HUNGRY NOR FULL
SLIGHTLY HUNGRY
MODERATELY HUNGRY
VERY HUNGRY
EXTREMELY HUNGRY
GREATEST IMAGINABLE HUNGER

Appendix F: PostSLIM Scale

Appendix D2 - Post-SLIM scale

MARKING INSTRUCTIONS	VOLUNTEER NUMBER	FILL IN TODAY'S DATE
• Use a No. 2 pencil only. • Do not use ink, ballpoint, or felt tip pens. • Make solid marks / fill the response completely • Erase cleanly any marks you wish to change. • Make no stray marks on this form. CORRECT: ● INCORRECT: ⊘⊠◒◔	4 boxes, each 0 1 2 3 4 5 6 7 8 9	MONTH: 0 1 DAY: 0 1 2 3 YEAR 20__: 0 1 2 0 1 2 3 4 5 6 7 8 9

TIME (24 HR FORMAT)	POST-MEAL ONLY
4 boxes, each 0 1 2 3 4 5 6 7 8 9	1. Was today's meal: 1 Shorter than usual 2 Typical 3 Longer than usual 2. Did you have enough time to eat what you want? Y Yes N No 3. How would you describe the speed you ate? 1 Very slow 2 Relatively slow 3 Medium 4 Relatively fast 5 Very fast

Please indicate your level of hunger or fullness right now by placing a hash mark (/) on the scale below.

- GREATEST IMAGINABLE FULLNESS
- EXTREMELY FULL
- VERY FULL
- MODERATELY FULL
- SLIGHTLY FULL
- NEITHER HUNGRY NOR FULL
- SLIGHTLY HUNGRY
- MODERATELY HUNGRY
- VERY HUNGRY
- EXTREMELY HUNGRY
- GREATEST IMAGINABLE HUNGER