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14. ABSTRACT

Background: The project's overarching objective is to discover novel strategies that promote Warfighter resilience, improve Warfighter combat readiness, and assure optimal Warfighter performance. Specifically, this project provides 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. This project continues and sustains the collaborative alliance that has been forged between USARIEM and Pennington Biomedical Research Center (PBRC) since 1988.

Objective: CROWN II conducts research in nutrition, metabolism and human physiology to discover novel strategies that promote Warfighter health and resilience, improve Warfighter combat readiness, and sustain Warfighter performance. Projects are conducted in each of four focus areas: Metabolism and Physical Performance, Stress and Inflammation, Nutrients and Resilience, and Healthy Eating and Behavior. Research will begin with 1 or 2 projects in each focus area. Additional projects may be added following the annual planning meeting between PBRC and USARIEM.

Study Design: This is an ongoing relationship that sets an annual research agenda in consultation between the Military Nutrition Division and PBRC over the three years of the award. The research projects conducted under this award require PBRC to provide critical capabilities that do not exist in house, but are needed to fulfill the Army Surgeon General's responsibility to provide nutritional research support to the Department of Defense.

Relevance: Studies of metabolism, stress and inflammation, and of nutritional status responses to extreme environmental circumstances can be applicable to the public at large and to workers who must endure environmental stress.

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) project is an extension of the previously funded CROWN project which conducts research in nutrition, metabolism and human physiology to discover novel strategies that promote Warfighter health and resilience, improve Warfighter combat readiness, and sustain Warfighter performance. Specifically, the CROWN II project continues a 28-year history of collaborative research between Pennington Biomedical Research Center (PBRC) and the Department of Defense (DoD). This relationship has evolved from a series of specially funded cooperative agreements between the PBRC and the U.S. Army Medical Research and Materiel Command (USAMRMC). PBRC has unparalleled expertise and facilities to conduct nutrition research, which complement and expand the specialized expertise and laboratories at the Military Nutrition Division (MND) at the United States Army Research Institute of Environmental Medicine (USARIEM). The two collaborating institutions involved in the CROWN II project are PBRC and the MND at USARIEM.

CROWN II proposes research to address objectives of all branches of the military. Over the 28 years of collaboration, PBRC and USARIEM have conducted laboratory and field research studies for the Army, Navy (including Marines), and Air Force at military installations at home, abroad, and aboard ship. The collaboration of these two entities takes advantage of the unique strengths of each partner. The military could not duplicate the assets the PBRC contributes to the project. PBRC benefits by the opportunity for collaboration with USARIEM on scientific questions of broad importance. The unsurpassed resources at PBRC supporting nutrition research and the specific focus of USARIEM on Warfighter nutrition are combined in CROWN II to address the unique needs of the U.S. military and it also provides the opportunity to broadly impact the nutritional health of Americans. CROWN II proposes research in nutrition and metabolism to develop better models to assess resilience or performance capacity. Additionally, this work may allow us to better predict dietary requirements needed by military units.

The overall hypothesis of the CROWN II project is that novel strategies can enhance Warfighter resilience, improve Warfighter combat readiness, and sustain optimal Warfighter health and performance during all phases of the deployment cycle. The CROWN II project enables USARIEM and PBRC scientists to work together on the design, execution, analysis, and translation of research project categories in four thematic areas:

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

Our hypothesis is that through the use of state of the art technologies and metabolic profiling, we will be able to develop better models to assess resilience or performance capacity and these may allow us to better predict dietary requirements needed by military units. This multi-year proposal allows us to develop an initial project in each area and then build upon that work in subsequent years. All projects will be conducted in collaboration between PBRC and USARIEM.

KEYWORDS

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

ACCOMPLISHMENTS

Scientists at PBRC and the MND at USARIEM cooperate in this specially funded agreement to assess and evaluate novel ways to sustain warfighter performance during high intensity missions at home and abroad. During this year, projects were initiated in each of the four focus areas: Metabolism and Physical Performance, Stress and Inflammation, Nutrients and Resilience, and Healthy Eating and Behavior.

Administration:

During this year, we continued work on 11 projects in the focus areas. The titles and principal investigators are listed below.

Pennington Biomedical led projects

1. 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

2. 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

USARIEM led projects

3. 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 Pasiakos

4. 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

5. 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

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

7. 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

8. 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 Pasiakos

9. 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

10. PBRC IRB # 2016-013
    Title: H14-14: Nutritional Practices of Soldiers Consuming Meals in Military Dining Facilities (DFAC-Nutrition Study)
    PI: Asma Bukhari

11. PBRC IRB # 2016-015
    Title: H16-02: Substrate utilization, exercise performance, and skeletal muscle response to energy deficit and altitude acclimatization
    PI: Stefan Pasiakos

During this year, Dr. Jennifer Rood and Dr. Tiffany Stewart traveled to Washington, D.C., to meet with Dr. Scott Montain, Dr. James McClung, and Dr. Roy Vigneulle to discuss project status for CROWN II. Current projects and future direction were discussed and additional projects were planned to meet the goals/initiatives of the overall project.

Dr. Rood submitted all active protocols to the PBRC IRB for a determination of human subject research. All IRB determination letters were sent to Brigit M. Ciccarello, M.A., who served as the Science Officer for this award. Copies of the IRB determinations were included in the quarterly report documents.

Dr. Rood submitted her protocol "Physiological and psychological effects of testosterone during severe energy deficit and recovery: a randomized, placebo controlled trial" to both the PBRC IRB and to the Army HRPO and received approval from both entities. Copies of the approvals were included in the quarterly reports.

Thematic Area 1. Metabolism and Physical Performance

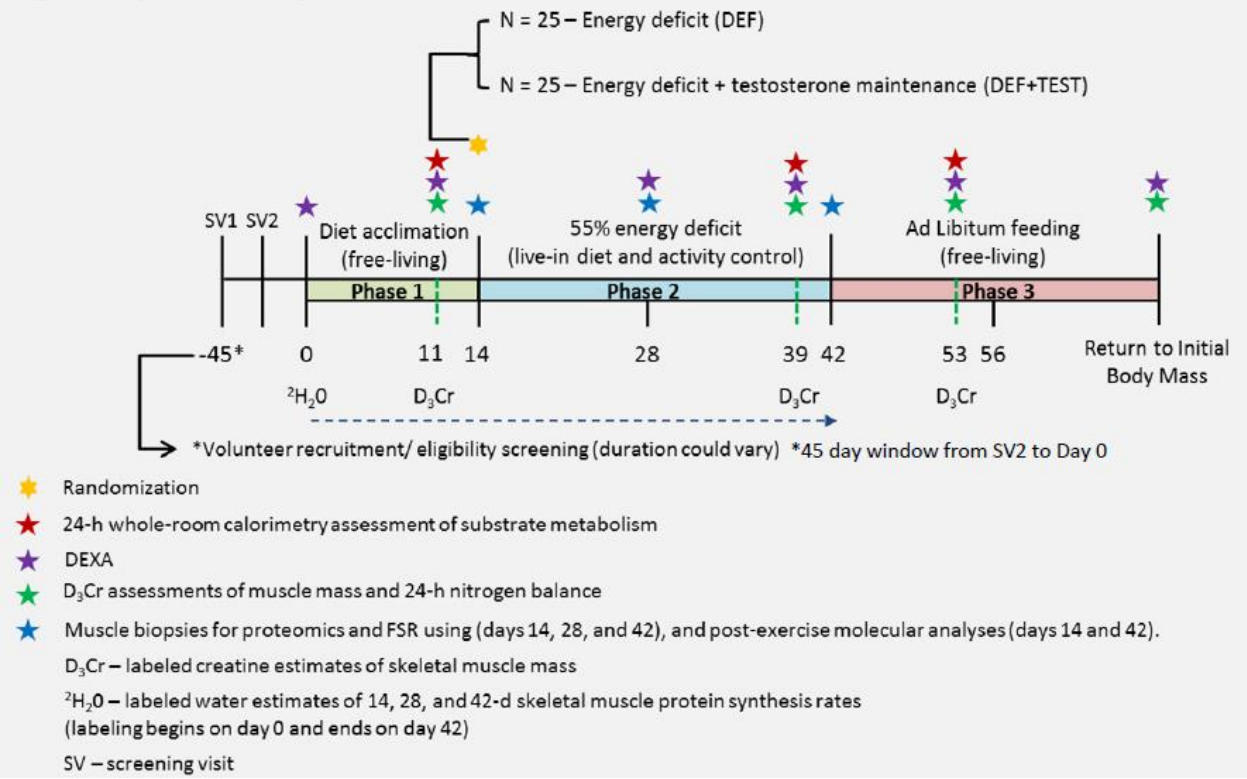
Projects in this area focus on metabolically regulated physiological functions that affect performance and susceptibility to illness, infection, and injury. These findings will be used to

support simulation modeling and will be incorporated into dietary policy and military ration development to promote Warfighter health, resilience, and recovery. In the statement of work, the initial plan 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 of this project to build upon the previous knowledge we have learned. The first project in this area examines the effect of androgen status on skeletal muscle mass during severe energy deficit and recovery. The project is titled "Physiological and psychological effects of testosterone during severe energy deficit and recovery: a randomized, placebo controlled trial."

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.

The objective of this study is 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 will enroll up to 60 physically active men in a 3-phase, randomized, placebo-controlled study. 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 will be randomized to one of two experimental groups and undergo 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) will be assessed after completing phase 2 to determine when body mass has been recovered within ± 2.5% of initial body mass (duration will vary, 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 will be used to assess physiological and psychological responses to energy restriction and recovery feeding while in a hypogonadal versus eugonadal state. Results of the proposed experiments will provide a basis for future studies aimed at improving Warfighter resiliency to and recovery from negative energy balance.

The experimental design of the project is shown in the figure below.



The study is being conducted at PBRC. PBRC will provide 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. Details of the daily schedule for all three phases of the study are shown below:

Phase 1: Screening and Diet Acclimation (Adjustment)

			Days													
	SV1	SV2	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Informed Consent	X																
Blood Pressure, Pulse	X	X															
Height	X																
Weight	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Neck/Waist Circumference	X																
Medication Use	X	X															
ECG (Electrocardiogram)		X															
3 Day Food Record		X															
Medical History	X																
Physical Exam		X															
Head Circumference		X															
Blood Draw including Archives		X	X							X							X
Urine Collection		X												X		X	X
Activity Log		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Questionnaires	X	X								X							X
Heavy Water			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Food Intake Test with blood draws										X							
Accelerometry		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
DXA Scan			X											X			
fMRI*								X				X			X		
Cognitive Testing			X		X		X	X	X		X		X			X	
Exercise Test (VO_2 Max Test)			X														
Muscular Strength Test			X													X	
Adverse Event Assessment		X	X							X							X
In-House Meal & Pick-Up			X	X	X	X	X	X	X	X	X	X	X		X	X	
24 Hour Stay														X			X
Stool Collection														X			
D_3-creatine (D_3Cr) Consumed														X			
Muscle Biopsy																	X
24 hr Metabolic Chamber														X			
Randomization																	X
Cycle Session																	X
Sugar Substitute Test														X			
Saliva Collection						X				X				X			
Bike Familiarization Ride+				X													

*The fMRI may occur on any day between Day 1 and Day 13. +The Bike Familiarization Ride will occur on any day between Day 1 and Day 8.

Phase 2: Energy Deficit

	Days																										
	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42
Blood Pressure, Pulse	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Weight	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Blood Draw Including Archives														x														x
Urine Collection																									x		x	x
Question-naires																												x
Heavy Water	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Acceler-ometry	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
DXA Scan														x											x			
fMRI																						x	x	x				
Cognitive Testing	x					x		x					x		x					x		x				x	x	
Muscular Strength Test																											x	
Adverse Event Assessment							x							x							x							x
24 Hour Stay	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Stool Collection											x														x			
D_3-creatine (D_3Cr)																									x			
Muscle Biopsy														x														x
Injection	x						x							x							x							
Cycle Session																												x
Exercise Sessions	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Sugar Substitute Test																									x			
Saliva Collection	x				x				x				x				x				x				x			
24 hr Metabolic Chamber																									x			

Phase 3:(Ad Lib Feeding) (Free-Living)

	Days																			
	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	Weekly until return to initial body weight	EOS	EOS +1	EOS +2	EOS +3	PRN
3 day food record*										x	x	x					X *	X *	X *		
Blood Pressure, Pulse																					
Weight	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
Medication Use																	x				
Testosterone Check																					x
Blood Draw Including Archives														x			x				
Urine Collection											x		x	x			x		x	x	
Questionnaires																	x				
Food Intake Test with Blood Draws	X																	X			
Accelerometry	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		x	x	x	x	
DXA Scan											x						x				
fMRI													x	x	x						
Cognitive Testing												x					x				
Muscular Strength Test													x				x				
Adverse Event Assessment																	x				
24 Hour Stay											x										
Stool Collection																	x				
D_3-creatine (D_3Cr)											x						x				
Metabolic Chamber											x										
Sugar Substitute Test																	x				

*Participant will be asked to complete a 3-day food record upon return to approximately 3% of initial body weight prior to EOS.

Key accomplishments for this protocol are described below:

a. IRB and HRPO approval obtained
b. Detailed information about the study was uploaded to www.clinicaltrials.gov
c. Study began screening and enrolling participants. Recruitment is proceeding according to schedule.

d. 268 people have been screened for this trial via the phone and 133 were eligible after screening
e. 76 participants completed an on-site screening visit and 13 are waiting to be scheduled for the initial screening visit
f. 19 participants have enrolled in the trial
g. 9 participants have completed the trial, 8 participants are in progress, and 2 participants have dropped
h. DSMB annual meeting was held on April 12, 2016.
i. DSMB received quarterly update on July 13, 2016.

This project has not been completed and therefore no results are presented in this report.

A second project was begun in this focus area. The H16-02 study examines substrate utilization, exercise performance, and skeletal muscle response to energy deficit and altitude acclimatization. This project was conducted at Pikes Peak, Colorado, and was led by Stefan Pasiakos.

The purpose of this research is to obtain new information that can 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 will specifically evaluate 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 will also evaluate 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 will take place at the USARIEM research labs in Natick, MA (50 m), and the acute and chronic HA portions will be conducted at the summit of Pikes Peak, CO (USARIEM John Maher Altitude Laboratory, 4300 m).

During the 22-d sea level phase, volunteers will receive dietary counseling to maintain baseline weight and consume 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) will be prepared and provided to volunteers by research staff. Changes in total body, lean body, and fat mass will be 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 will be 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 will be used to assess total, exogenous, and endogenous carbohydrate oxidation and performance capacity. Sub-aims of the study include 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 will be conducted by USARIEM investigators. PBRC will provide specimen collection materials and biomarker analysis. Additionally, PBRC will provide 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

Key accomplishments for this protocol are described below:

a. PBRC prepared labeled vials for blood and urine collection and will provide specimen analysis for this study.
b. All specimens were collected and shipped to PBRC for analysis.
c. Sample analysis is in progress for the following analytes: lactate, free fatty acids, glucose, glycerol, total protein, iron, total iron binding capacity, % iron saturation, insulin, testosterone, sex hormone binding globulin, cortisol, DHEA-s, ferritin, osmolality, IL-6, hepcidin, soluble transferrin receptor, active ghrelin, cholecystokinin, GLP-1, GLP-2, LBP, claudin-3, I-FABP, leptin, and glucagon.

Analysis is continuing at this time and no final results are available for this project.

Thematic Area 2. Inflammation and Stress

Projects in this area will focus on the inflammatory and stress-related response to military operational stress. These projects will evaluate nutritional countermeasures to operational stress and assess whether functional performance (e.g., physical and psychological) can be maintained using anti-inflammatory directed nutrition. There were no projects conducted in this area in the past year.

Thematic Area 3. Nutrients and Resilience

Projects in this area will examine the role that nutrition plays in recovery and resilience following military stress. As listed in the statement of work, the initial project (H14-02) 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 Corps (MARSOC) SERE training. Evidence from recent studies, including those conducted by PBRC and USARIEM, suggests consuming high protein diets or supplemental tyrosine confers protection and promotes recovery from extreme physiological and psychological stress. Whether supplemental protein, in particular high-quality protein with high levels of tyrosine, enhances skeletal muscle and physical and behavioral recovery from "real-world" military stress has 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.

Key accomplishments for this protocol are described below:

a. During the first year of this project, specimen collection materials were prepared, samples were collected, and sample analysis was begun.
b. During this year, all sample analysis was completed. 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

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

d. 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.

To build upon the knowledge we obtained from this project and to complement the work we have already concluded, we consulted with investigators at USARIEM and five additional projects were added to this focus area.

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.

This project examines the efficacy of a calcium and vitamin D fortified food product in improving bone microarchitecture in response to Army basic combat training. This project is led by Erin Gaffney-Stomberg, Ph.D. (USARIEM) and is occurring 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. Dr. Rood and members of her team traveled to Ft. Jackson to assist with blood collection, sample processing, and testing at the site.

Key accomplishments for this project are described below:

a. Preparation and shipment of barcoded specimen collection tubes and specimen aliquot cryovials.
b. 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.
c. All data was sent to the principal investigator at USARIEM electronically via the secure website https://safe.amrdec.army.mil/safe/.

The investigators are in the process of analyzing the initial data.

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.

This project will conduct a prospective study of factors associated with career success among soldiers in Special Forces training. This study will be led by Emily Farina, Ph.D. (USARIEM) and will be conducted at Camp Mackall, NC. This is an observational study and will 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. As part of this protocol, samples will be shipped to PBRC for biomarker analysis.

Key accomplishments for this project are described below:

a. Preparation and shipment of barcoded specimen collection tubes and specimen aliquot cryovials.

b. Testing has been completed for the first 6 iterations and includes 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.
c. All RBC fatty acid assays were completed for the first 9 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.
d. Data was sent to the principal investigator at USARIEM electronically via the secure website https://safe.amrdec.army.mil/safe/.

This project is ongoing and samples are still being collected. Data analysis will begin at the completion of sample collection and analysis.

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

This project will examine the effects of MRE consumption on gut health. This study will be led by J. Philip Karl, Ph.D. (USARIEM) and will occur at the U.S. Army Natick Soldier Systems Center in Natick, MA. The primary objective of this study is to determine the impact of consuming MREs as a sole source of subsistence for 21 days on gut barrier integrity. As part of this protocol, samples will be shipped to PBRC for biomarker analysis. Specimen collection and subsequent sample analysis is in progress.

Key accomplishments for this project include the preparation and shipment of barcoded specimen collection tubes and specimen aliquot cryovials.

This project is ongoing and specimens are being collected. No results are available at this time.

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

This project will evaluate calcium and vitamin D supplementation as a method to optimize bone health during Marine Corps recruit training. The project is led by Erin Gaffney-Stomberg, Ph.D. (USARIEM) and will take place at Parris Island, SC. As part of this protocol, PBRC provided support for sample processing, and samples were shipped to PBRC for biomarker analysis.

Key accomplishments for this project are described below:

a. Preparation and shipment of barcoded specimen collection tubes and specimen aliquot cryovials.
b. A specimen processing team (n=3) from PBRC traveled to Parris Island, SC, to assist with blood and urine processing and performing blood assays on-site.

c. Sample testing was completed for both iterations of the study. 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.
d. Data was sent to the principal investigator at USARIEM electronically via the secure website https://safe.amrdec.army.mil/safe/.

Data analysis is in progress now. Results will be generated for publication in the next quarter.

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

This project examines energy balance and physiological responses to the U.S. Marine Corps Forces Special Operations Command (MARSOC) Individual Training Course (ITC). This project is directed by Dr. Stefan Pasiakos. As part of this protocol, Pennington Biomedical will provide doubly labeled water for the measurement of total daily energy expenditure during the different phases of training. PBRC will also provide specimen collection supplies and biochemical assays at timepoints throughout the study.

Key accomplishments for this project are described below:

a. Preparation and shipment of barcoded specimen collection tubes and specimen aliquot cryovials.
b. Preparation and shipment of doubly labeled water to measure total daily energy expenditure. All urine collection supplies were shipped to USARIEM.
c. Specimen collection for all 4 phases of the study were completed.
d. Urine specimens were analyzed for isotopic enrichment to calculate total daily energy expenditure and total body water.
e. Urine data was sent to the principal investigator at USARIEM electronically via the secure website https://safe.amrdec.army.mil/safe/.
f. Blood sample analysis was completed for glucose, hepcidin, soluble transferrin receptor, igf-1, high sensitivity C reactive protein, FSH, ferritin, insulin, LH, sex hormone binding globulin, and testosterone. A total of 1,298 assays were completed. Additional blood testing is in process.
g. Completed blood testing results were sent to the principal investigator at USARIEM electronically via the secure website https://safe.amrdec.army.mil/safe/.

Biomarker analysis for this project is ongoing and results have not been compiled for publication.

Thematic Area 4. Healthy Eating Behavior

As described in the statement of work, PBRC initially proposed two projects in this area:

The first project (H15-04) assessed the quality of dietary intakes of Soldiers using food photography. The purpose of this project 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 consume meals at the Tactical Human Optimization Rapid Rehabilitation and Reconditioning ($THOR^3$) modified SWCS dining facility (DFAC), Fort Bragg, NC, were compared to Soldiers consuming meals at a standard Fort Bragg DFAC without extensive $THOR^3$ intervention. The digital photography method is being used to quantify food selections and intake of enrolled study participants. At each meal, foods and beverages selected by the Soldiers prior to and after eating will be photographed using digital video cameras. As part of this project, PBRC is piloting the use of a second camera at baseline in order to evaluate whether this simplifies and enhances accuracy of estimations.

All 4 phases of the Fort Bragg study testing 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 have been completed and the data is being written up for publication. 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, 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.

Additional conversations between LTC Cole, Catherine Champagne and administrative staff from SWCS and 2BCT have been ongoing, mainly focused on both reviewing the final data from SWCS. There have also been follow up conversations with 2BCT to evaluate Go For Green (G4G), a healthy eating initiative in place at a number of Army DFACs nationwide. G4G, although implemented, has not been evaluated as of this time. LTC Cole and Champagne continue to await a decision on a proposed G4G study, assistance required, and set up of timeline as a follow up to their March visit to Fort Bragg.

In the second project in this area, PBRC will test the efficacy of an intensive intervention to assist Soldiers in meeting standards for body fat and fitness. The intervention will include: 1) the Internet/Smartphone program for weight management and weight gain prevention developed during previous DoD research (*H.E.A.L.T.H.);* 2) integrated remote monitoring technology/devices, e.g. smart scales, *fitbit*; and 3) individualized weight management and exercise recommendations based on data collected from web/mobile app/devices will be delivered via web/mobile app/Smartphone; participants will not travel to a clinical facility for services. The H.E.A.L.T.H. II protocol was finalized, submitted to the IRB and received approval. It was also submitted to the Army HRPO and received approval. This study is currently in progress.

After consultation with investigators at USARIEM, a new project (H14-14) examining the nutritional practices of soldiers consuming meals in military dining facilities was begun. This project is led by LTC Asma Bukhari, Ph.D. (USARIEM) and is being conducted at the Natick Soldier Systems Center dining facility. The purpose of this study is to examine the efficacy of a dietary intervention to improve soldiers' omega-3 fatty acid status in a garrison feeding environment. As part of this project, blood samples are being collected and will be shipped to PBRC for the measurement of fatty acids, lipids, and markers of inflammation. Dr. Champagne continues as an associate investigator on this project serving in an advisory role and assisting with data analysis.

Key accomplishments in this focus area are described below:

H15-04: Dining satisfaction and diet quality of soldiers eating at two Fort Bragg DFACS.
PI: Renee Cole, Ph.D.

a. All data collection was completed and data analyzed.
b. Results were compiled and are being prepared for a technical report and subsequent publication.

H.E.A.L.T.H. II. Healthy Eating, Activity, & Lifestyle Training Headquarters.
PI: Tiffany Stewart, Ph.D.

a. Protocol was finalized; received IRB and HRPO approval.
b. Screening and enrollment of participants began.
c. 32 Soldiers have been randomized. Of those, 31 have completed Baseline assessment and are in progress (26 males and 5 females). Of those 31, 16 were assigned to the Active Group and 15 were assigned to the Control Group. 13 Soldiers (11 male and 2 female) currently have spouses participating with them in the study. One participant is currently scheduled for baseline. In sum, we have 32 Soldiers enrolled (13 with spouses), with one participant in the pipeline.

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

PI: Asma Bukhari

a. Barcoded specimen collection tubes and specimen aliquot cryovials were prepared and shipped.
b. Blood assays were completed, including the following: cholesterol, homocysteine, HDL cholesterol, hsCRP, LDL cholesterol, and triglycerides. (222 assays)
c. 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)
d. Results were sent to the principal investigator at USARIEM electronically via the secure website https://safe.amrdec.army.mil/safe/.

IMPACT

The goal of this series of projects is to assess and evaluate novel ways to sustain warfighter performance during high intensity missions at home and abroad. During this year, a total of 11 projects were initiated in four focus areas: Metabolism and Physical Performance, Stress and Inflammation, Nutrients and Resilience, and Healthy Eating and Behavior. This work is 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 this past year. All publications are listed further in the report and provide additional detail.

1. Many times warfighters are faced with intermittent periods of severe energy deficit which results 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.

CHANGES/PROBLEMS

Nothing to Report

PRODUCTS

Abstracts:

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

Scott JM, Gaffney-Stomberg E, Palmer JP, Daigle R, Kazman JB, McClung JP, and Gasier HG. Vitamin D supplementation augment SIgA secretion rates in Marine Corps basic trainees. American College of Sports Medicine Annual Meeting, June 2016

Orr JS, Sepowitz JJ, McClung HL, Berryman CE, McClung JP, and Pasiakos SM. Predictors of the Testosterone Response to Negative Energy Balance. Military Health Symposium, August 2016

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 Expo, October 2016

Cole RE, Bukhari AS, Champagne CM, McGraw SM, Hatch AM, Logan CM, Spanbauer SM, and Montain SJ. Adequate sleep is associated with healthy eating, physical activity, and time spent inactive. Military Health System Research Symposium, August 2016

Cole RE, Bukhari AS, Champagne CM, McGraw S, Hatch AM, Logan CM, Spanbauer SM, and 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

Sepowitz JJ, McClung HL, Berryman CE, Armstrong NH, Ferrando AA, Lieverman HR, McClung JP, and 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

Berryman CE, McClung HL, Sepowitz JJ, Armstrong NJ, Lieberman HR, McClung JP, and Pasiakos SM. Greater diet quality and physical activity do not protect lean body mass during military training. American College of Sports Medicine Annual Meeting, June 2016

Pasiakos SM, Berryman CE, Margolis LM, Sepowitz JJ, McClung HL, Lieverman HR, McClung JP, and Ferrando AA. Changes in protein turnover, hormonal status, and body composition during physiologically demanding military training. Experimental Biology, April 2016

Karl JP, Margolis LM, Murphy NE, Martini S, Gundersen Y, Castellani JW, Carrigan CT,Teien HK, Madslien EH, Montain, SJ, and Pasiakos SM. Increased Gastrointestinal Permeability During Prolonged Physical Stress is Associated with Lower Energy Intakes but not Dietary Macronutrient Composition. Experimental Biology, April 2016

Margolis LM, Murphy NE, Martini S, Gundersen Y, Cstellani JW, Karl JP, Carrigan CT, Teien HK, Madslien EH, Montain SJ, and 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

Derosier A, Berryman CE, Karl JP, Wilson M, Young AJ, and Pasiakos SM. Higher-protein intake during sustained negative energy balance attenuates elevations in resting energy expenditure elicited by high altitude (4300 m) exposure. Experimental Biology, April 2016

Kenefick RW, Luippold AJ, Bradbury KE, Young AJ, Deroser AN, Wilson MA, Berryman CE, and Pasiakos SM. No Impact of Carbohydrate Supplementation and Altitude Acclimatization on Aerobic Exercise Performance. American College of Sports Medicine, June 2016

Karl JP, Margolis LM, Murphy NE, Martini S, Gundersen Y, Castellani JW, Carrigan CT, Teien HK, Madslien EH, Montain SJ, and Pasiakos SM. High Energy Expenditure and Negative Energy Balance Modulate Composition and Metabolism of the Gut Microbiota. Experimental Biology, April 2016

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 2016

Berryman CE, Karl JP, Cole RE, Kenefick RW, Margolis LM, Carbone JW, Ferrando AA, Lieberman HR, Young AJ, and Pasiakos SM. Effects of a higher-protein diet on body composition responses to sustained negative energy balance during prolonged high altitude (4300 m) exposure. Experimental Biology, April 2016

Young AJ, Berryman CE, Derosier A, Kenefick RW, and Pasiakos SM. Effects of acclimatization to high altitude on exogenous carbohydrate oxidation during steady-state exercise. Medicine &Science in Sports & Exercise, June 2016

Karl JP, Cole RE, Berryman CE, Kominsky MT, Radcliffe PN, Margolis LM, Young AJ, and Pasiakos SM. Higher Protein Diets Suppress Appetite at High Altitude. Experimental Biology. April 2016.

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

Manuscripts

Lutz LJ, Gaffney-Stomberg E, Williams KW, McGraw SM, Niro PJ, Karl JP, Cable SJ, Cropper TL, and McClung JP. Adherence to the dietary guidelines for americans is associated with psychological resilience in young adults: A cross-sectional study. J Acad Nutr Diet. 2016 Nov 5. pii: S2212-2672(16)31161-3.

Karl JP, Smith TJ, Wilson MA, Bukhari AS, Pasiakos SM, McClung HL, McClung JP, and Lieberman HR. Altered metabolic homeostasis is associated with appetite regulation during and following 48-h of severe energy deprivation in adults. Metabolism. 2016 Apr;65(4):416-27.

Margolis LM, Murphy NE, Martini S, Gundersen Y, Castellanie HW, Karl JP, Carrigan CT, Teien H, Madslien E, Montain SJ, and 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.

O'Connor KL, Scisco JL, Smith TJ, Young AJ, Montain SM, Price LL, Lieberman HR, and Karl JP. Altered appetite-mediating hormone concentrations precede compensatory overeating after severe, short-term energy deprivation in healthy adults. J Nutr. 2016 Feb;146(2):209-17.

Pasiakos Sm, Margolis LM, Murphy NE, McClung HL, Martini S, Gundersen Y, Castellanie JW, Karl JP, Teien H, Madslien E, Stenberg PH, Young AJ, Montain SM, and McClung JP. Effects of exercise mode, energy and macrontirient interventions on inflammation during military training. Physiol Rep. 2016 Jun;4(11).

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

Name	Role	% Effort
Allen, Ray	Computer Programmer	26%
Blumberg, Kate	Research Dietitian	10%
Conner, Bridget	Research Associate – laboratory	85%
Davis, Allison	Research Associate – psychology	16%
Efferson, Nathan	Research Associate – computer	46%
Harris, Melissa	Research Associate – project manager	60%
Hymel, Valery B	Research Associate – laboratory	85%
Lee, Stephen P	Research Associate – laboratory	85%
Rood, Jennifer C	Principal Investigator	60%
Roussel, Stacey H	Research Associate – laboratory	85%
Savoie, Jonathan J	Research Associate – laboratory	85%
Tuminello, Jamie H	Research Associate – laboratory	100%
White, Edie	Administrative Support	50%

SPECIAL REPORTING REQUIREMENTS

Nothing to Report

APPENDIX A

Quad Chart

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:** $7,305,717.00

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 categories in four thematic areas:

- Metabolism and Physical Performance
- Stress and Inflammation
- Nutrients 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
Initial Study Design and Execution for each Focus Area			
Follow Up Studies			
Biospecimen Analysis/Field Support			
Budget (M)	$2.377	$2.437	$2.490

Updated: (04/12/2017)

Milestones:
Demonstrated the following:

- Leucine-enriched supplemental nutrition optimized skeletal muscle anabolic and whole-body anti-proteolytic responses during steady-state, load carriage exercise.
- Consuming supplemental carbohydrate- and protein-based food products during short-term, arctic military training produced protein-sparing, less inflammation, and reduced gut permeability
- A calcium and vitamin D fortified food product improved bone microarchitecture in response to Army basic combat training.
- Strategic food placement, performance-based menu enhancement, and point-of-service labeling can enhance nutritional intake and dining satisfaction of Special Operations Forces (SOF) Soldiers subsisting in garrison dining facilities.

FY 16: 11 projects are in progress and on track to be completed on time and within budget.

Budget Expenditure to Date
Actual Expenditure: $2,106,084.18