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Collaborative Research to Optimize Warfighter Nutrition (CROWN)

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**13. SUPPLEMENTARY NOTES**
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 the Pennington Biomedical Research Center (PBRC) since 1988.

Objective: The Collaborative Research to Optimize Warfighter Nutrition (CROWN) project supports communication and interaction between USARIEM and PBRC scientists in the design, execution, analysis and translation of research projects categorized in three thematic areas: 1) Metabolism and Physical Performance, 2) Stress and Inflammation, and 3) Micronutrients and Resilience. The PBRC contribution includes provision of high quality analytical laboratories, nutrition databases, and metabolic support for military nutrition clinical research protocols.

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.

Subject Terms: Metabolism, nutrition, energy expenditure, readiness, performance, warfighter

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INTRODUCTION

The modern Warfighter faces numerous physiological challenges during training and combat. The Collaborative Research to Optimize Warfighter Nutrition (CROWN) project is designed to conduct and support research in nutrition, metabolism and human physiology in an attempt to discover novel strategies that promote Warfighter resilience, improve Warfighter combat readiness, and assure optimal Warfighter performance. Specifically, the CROWN project represents an ongoing effort, based upon a 23-year history of collaborative research between the 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). The PBRC has unparalleled expertise and facilities to conduct nutrition research, which complement and expand the specialized expertise and laboratories at the Military Nutrition Division at the United States Army Research Institute of Environmental Medicine (USARIEM). The two collaborating institutions involved in the CROWN project are the PBRC and the Military Nutrition Division at USARIEM.

CROWN supports the three branches of the military. Over the 26 years of collaboration, PBRC has supported field studies for Army, Navy (including Marines), and Air Force both a military installations at home, abroad, and aboard ship. The unsurpassed resources at PBRC support nutrition research and the specific focus of USARIEM on Warfighter nutrition are combined in CROWN to address nutritional neuroscience, stress, physical and mental performance, and garrison feeding. This research portfolio fulfills the unique needs of the US Military. The collaboration of these two entities takes advantage of the unique strengths of each partner. The military could not duplicate the assets the PBRC brings to the project. PBRC benefits by the opportunity to collaborate with USARIEM on scientific questions of broad importance. The research supported by CROWN has implication for improving Warfighter nutrition, and it also provides the opportunity to broadly impact the nutritional health of Americans.

Throughout the 26 years of affiliation, the PBRC and USARIEM have operated in a unique collaborative relationship that has resulted in the support of 98 projects (see appendix B) and more than 100 scientific publications (see appendix C). PBRC personnel have provided high quality analytical laboratory, nutrition database, and metabolic unit support for DOD nutrition related research programs. Additionally, the PBRC has conducted research that complements and extends USARIEM’s intramural program in areas of nutritional neuroscience, stress, physical, and mental performance, and garrison feeding. Though funded through earmarks, the PBRC program has been periodically successfully peer reviewed by an external panel from the Committee on Military Nutrition Research (CMNR), Institute of Medicine (1988, 1990, 1996, and 2002). This joint effort of PBRC and military researchers has led to significant improvements of operational rations, better understanding of Warfighter energy and nutritional requirements, and modifications in garrison feeding.

This proposal is aimed at continuing a research program that has been in place since 1988. The proposal is for the fourth specially funded cooperative agreement series that began in 1988. The previous cooperative agreements are listed below:

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The primary advantages of the longstanding relationships are first, to avail military researchers of the outstanding nutrition research facilities and faculty at PBRC and, second, to allow long-term planning for the military research agenda. PBRC has invested in equipping the laboratories, with the following estimated equipment expenditures:

- Clinical Chemistry Laboratory
- Stable Isotope Laboratory
- Dietary Assessment Laboratory

**BODY**

PBRC provides high quality support of military nutrition clinical research protocols. PBRC and the US Army Research Institute of Environmental Medicine (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 the year of this report, PBRC supported 9 projects directed by USARIEM and PBRC investigators. The projects are listed below.

2. H12-09 - Optimizing vitamin D status during initial military training: A randomized, double-blind, placebo-controlled trial. (PI: James McClung, Ph.D.)
5. H12-43 - Effect of Tyrosine on Behavioral, Physiological and Nutritional Status during Survive, Evade, Resist, Escape (SERE) School (PI: Harris Lieberman, Ph.D.)
6. H13-14 Encouraging healthy food choices with an environmental intervention in military dining facilities (Jenna Scisco, R.D.)
7. H13-26 - Testing the effectiveness of nutritional interventions in a military dining facility serving an isolated population (PI: Aaron Crombie, Ph.D.)
8. H14-02- Effect of protein supplementation on lean body mass recovery and physiological resilience following Survive, Evade, Resist, Escape (SERE) School (PI: Stefan Pasiakos, Ph.D.)
9. Marine Study – Measurement of Total Body Water and Total Daily Energy Expenditure in Marines in Afghanistan (PI:

The clinical laboratory provides support for military nutrition research by providing the following services:

a. assistance with protocol development
b. sample collection and processing on-site or in a field setting  
c. sample analysis  
d. new method development  
e. assistance with manuscript publication  

The laboratory is accredited by the Health Care Financing Administration (HCFA) and the College of American Pathologists (CAP) and participates in the lipid standardization program of the Centers for Disease Control. Good Laboratory Practices guidelines are being followed in the laboratory. The Clinical Research Laboratory is staffed by licensed medical technologists, phlebotomists, and accessioners.  

The laboratory is well-equipped for performing routine and specialized tests on clinical subjects. In 2013 over 275 different analytical procedures involving 550,000 tests were performed by the lab. The laboratory is comprised of 5 departments: chemistry, special chemistry, point of care testing, hematology, and urinalysis. Testing is performed on a variety of specimen types including blood, urine, sweat, saliva, and feces.  

The research conducted by the Stable Isotope Laboratory is in the area of energy and water requirements, and changes in body water, of soldiers, often under harsh environmental conditions. The method used to determine energy requirements is the doubly labeled water (DLW) technique, which involves oral administration of water labeled with the stable isotopes, $^2$H and $^{18}$O. Saliva and urine samples are then obtained for periods of four to 14 days, longer with redosing. Water intake can be determined using only the $^2$H labeled water. The use of doubly labeled water for measurement of energy expenditure was developed as a field technique for use in small animals. The method is based on the premise that after a loading dose of $^2$H$_2^{18}$O, $^{18}$O is eliminated as CO$_2$ and water, while deuterium is eliminated from the body as water. The rate of CO$_2$ production, and, hence, energy expenditure, is calculated from the difference of the two elimination rates. The only requirement of subjects is to give urine and saliva specimens before and after drinking an initial dose of $^2$H$_2^{18}$O, and then return in one to two weeks to give a final urine specimen. During the period between the two urine and saliva samplings, subjects are free to carry out their normal activities and are not required to maintain extensive diaries. The doubly labeled water method has been extensively validated in humans under controlled settings, but there are confounding factors that need to be considered in field studies, particularly in Army Field Studies. Among these are changes in location or food and water supply immediately preceding, or during an energy expenditure study. These changes may cause a change in baseline isotope abundance and, therefore, interfere with the accuracy of the energy expenditure measurement. This has occurred in a previous field training exercise involving the study of the MRE and RLW rations. This is a particular problem with studies such as the Ranger Training Studies, in which soldiers are moved to different parts of the country during the study. Therefore, a group not receiving labeled water must be followed to make any corrections in baseline isotope shifts.  

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

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

In addition to the DLW studies, the PBRC Stable Isotope lab has provided analytical and technical support to examine protein turnover and gluconeogenesis using stable isotope tracer technology in a clinical protocol examining the effects of protein supplementation during caloric restriction (Project 1). The purpose of the study is twofold: 1) to determine if, during increased energy expenditure, physically fit individuals exhibit a smaller, larger, or equivalent increase in protein requirement as sedentary individuals
and 2) to determine if, during periods of heavy physical activity and inadequate intake, an increase in protein intake will enhance conservation of whole-body protein content.

The lab determined the appropriate methods to quantitate glucose appearance, disappearance (6,6 d2 glucose) and gluconeogenesis (2-13C glycerol). The laboratory also decided on the appropriate tracers to quantitate protein synthesis (15N phenylalanine, 2,3,5,6 D4 tyrosine, and 15N tyrosine).

Assessing dietary intake is essential in determining the soldier’s nutritional needs and how those needs interface with other aspects of military performance. PBRC currently participates in field studies planned and conducted by the Military Nutrition Division of USARIEM by providing assistance with and analysis of dietary intakes collected during military field studies. That participation includes the following:

- Support for USARIEM field studies requiring data collection and data entry needs
- Support for PBRC in-house Military Nutrition Tasks
- Continued programming efforts directed toward meeting computer needs of both USARIEM and PBRC Military Nutrition Tasks

The Nutrient Database Integration Laboratory provides essential services for military operations. This involves the oversight of the database containing nutrient information for all operational rations, in addition to the USDA Standard Reference Foods and the USDA Food Survey Database food files. We also provide critical support to studies which seek to improve soldier nutrition in a variety of field settings.

Personnel at PBRC are proficient in all aspects of nutritional intake assessment, including the Food Frequency Questionnaire, Food Diary Analysis, and Dietary Recall and have been trained in the USDA multiple pass methodology.

**Key Research Accomplishments**

   a. Preparation of barcoded specimen collection tubes and specimen aliquot cryovials
   b. Completion of urine nitrogen assays (n=92)
   c. Completion of serum amino acid profiles consisting of the following: alanine, arginine, asparagine, aspartate, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, serine, threonine, tryptophan, tyrosine, and valine (5,244 assays)
   d. Completion of urine 3 methyl histidine (92 assays)
   e. Completion of serum gut hormones including active ghrelin, glp-1, insulin, pancreatic polypeptide, and PYY (1,980 assays)
   f. Completion of glucose and leptin during the meal test (672 assays)
   g. Completion of chemistry profiles including cortisol, DHEA-s, insulin, BHBA, free fatty acids, glucose, triglycerides, and soluble leptin receptor (2,208 assays)
   h. Transmission of all results electronically to USARIEM principal investigator

2. **H12-09 - Optimizing vitamin D status during initial military training: A randomized, double-blind, placebo-controlled trial. (PI: James McClung, Ph.D.)**
   a. Preparation of barcoded specimen collection tubes and specimen aliquot cryovials
   b. Field support for specimen collection and processing – 3 employees from Pennington Biomedical traveled to San Antonio for pre and post specimen collection for iteration 3 and 4 for this project
c. Completion of serum amino acid profiles consisting of the following: alanine, arginine, asparagine, aspartate, cystine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, serine, threonine, tryptophan, tyrosine, and valine (6,289 assays)

d. Completion of chemistry assays including % iron saturation, albumin, calcium, cholesterol, estradiol, ferritin, homocysteine, HDL cholesterol, hsCRP, insulin, LDL cholesterol, phosphorus, progesterone, PTH, soluble transferrin receptor, total iron binding capacity, triglycerides, 25 OH vitamin D, hepcidin, testosterone, 1,25 di-OH vitamin D, bone alkaline phosphatase, carboxy-terminal telopeptide of type I collagen, FGF-23, tartrate resistant acid phosphatase, osteocalcin, and procollagen type I N-terminal propeptide (16,100 assays)

e. Completion of urine assays including calcium, creatinine, and phosphorus (258 assays)

f. Completion of serum cytokine and bone panels including IL-1b, IL-6, TNFa, DKK, GM-CSF, IFNg, IL-10, IL12p70, ILK-13, IL-2, IL-4, IL-5, IL-7, IL-8, OPG, RANKL, SOST, and Leptin. (8,748 assays)

g. Completion of serum fatty acid profiles 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. (3,740 assays)

h. Transmission of all results electronically to USARIEM principal investigator


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

b. Completion of serum amino acid profiles consisting of the following: alanine, arginine, asparagine, aspartate, cystine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, serine, threonine, tryptophan, tyrosine, and valine (9,680 assays)

c. Completion of serum chemistry and cytokine profiles including the following cortisol, DHEA-s, ferritin, growth hormone, hsCRP, insulin, myoglobin, free fatty acids, glucose, lactate, albumin creatine, kinase, lactate dehydrogenase, prealbumin, soluble transferrin receptor, IGF-1, testosterone, hepcidin, GM-CSF, IFNg, IL-1b, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12p70, IL-13, and TNFa. (8,128 assays)

d. Completion of all stable isotope measurements in muscle samples for 2H5 phenylalanine

e. Transmission of all results electronically to USARIEM principal investigator


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

b. Completion of red blood cell fatty acid profiles 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. (2,156 assays)

c. Completion of serum fatty acid profiles 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. (2,002 assays)

d. Completion of serum chemistry assays including % iron saturation, albumin, cholesterol, iron, HDL cholesterol, LDL cholesterol, magnesium, total iron binding capacity, triglycerides, hepcidin, igf-1, cortisol, hsCRP, IL-1b, IL-1ra, IL-6, PTH, sex hormone binding globulin, soluble transferrin, testosterone, TNFa, and 25 OH vitamin D. (2,376 assays)

e. Transmission of all results electronically to USARIEM principal investigator
5. H12-43 - Effect of Tyrosine on Behavioral, Physiological and Nutritional Status during Survive, Evade, Resist, Escape (SERE) School (PI: Harris, Lieberman, Ph.D.)
   a. Preparation of barcoded specimen collection tubes and specimen aliquot cryovials
   b. Completion of saliva assays including cortisol DHEA-s, estradiol, testosterone, and neuropeptide Y (3,280 assays)
   c. Completion of serum assays including albumin, cortisol, DHEA-s, estradiol, ferritin, hsCRP, prolactin, SHBG, soluble transferrin receptor, testosterone, hepcidin, and neuropeptide Y. (3,504 assays)
   d. Completion of serum amino acid profiles consisting of the following: alanine, arginine, asparagine, aspartate, cystine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, serine, threonine, tryptophan, tyrosine, and valine (6,490)
   e. Transmission of all results electronically to USARIEM principal investigator

6. H13-14 Encouraging healthy food choices with an environmental intervention in military dining facilities (Jenna Scisco, R.D.)
   The purpose of the project was to introduce a MyPlate intervention to increase intakes of foods from the five food groups. USARIEM personnel implemented a digital photography method to do this, estimated the quantities of foods consumed, and sent the data to PBRC for analysis and computation of nutrient content. These food collections were matched to USDA food composition data and nutrient files were generated containing the nutritional content of those consumed foods, including the computation of the Healthy Eating Index 2010. All data was transmitted electronically to the Principal Investigator.

7. H13-26 - Testing the effectiveness of nutritional interventions in a military dining facility serving an isolated population (PI: Aaron Crombie, Ph.D.)
   Pennington Biomedical Research Center’s Dietary Assessment Team assisted USARIEM in a study at Camp Mackall, North Carolina which occurred in three phases in 2014. This project was similar to the Fort Bragg project “Healthy initiatives in food service production” conducted during 2009-2010. The purpose of the project was to evaluate the energy and nutrient intake of Special Forces Soldiers at all meals consumed in the Dining Facility (DFAC) via food photography. This involved initially setting up HD video cameras in the DFAC facility on the Army base at Camp Mackall. Food standards of all the foods being served were weighed prior to the meal. Photos were taken of the soldier’s food tray before and after meals were consumed. These photos were compared to the photographed standards and used to determine actual food intake of each soldier. The purpose of this research study was to determine whether the garrison DFAC provided sufficient nutrition to match the activities performed during this intense training [Small Unit Tactics (SUT) iteration of the Special Forces Qualification Course.
   Phase 1 occurred the week of February 3, Phase 2 occurred the week of April 28, and Phase 3 occurred the week of August 4. Food photos from soldiers participating in the study were estimated by the Pennington team after their return. Currently, the photos from Phase 1 have been completed and approximately 95% of those from Phase 2 have been estimated, however only about 15% of those from Phase 3 have been estimated. Once all photos are estimated, we will begin the process of evaluating the assessments of the photos, adjudicating the mismatches (this process already completed for Phase 1) and delivering the final nutrient information to the Principal Investigator.
8. H 14-02- Effect of protein supplementation on lean body mass recovery and physiological resilience following Survive, Evade, Resist, Escape (SERE) School (PI: Stefan Pasiakos, Ph.D.)

   a. Preparation of barcoded specimen collection tubes and specimen aliquot cryovials.
   b. Completion of red blood cell fatty acid profiles 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. (440)
   c. Completion of serum fatty acid profiles 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. (1,320 assays)
   d. Transmission of all results electronically to USARIEM principal investigator

9. Marine Study
   a. Completion of urine assays for deuterium and oxygen 18 to calculate total body water and total daily energy expenditure. (17 participants)
   b. Transmission of all results electronically to USARIEM principal investigator

Reportable Outcomes

Publications:


Abstracts:


Conclusions

The unsurpassed resources at PBRC support nutrition research and the specific focus of USARIEM on Warfighter nutrition are combined in CROWN to address nutritional neuroscience, stress, physical and mental performance, and garrison feeding. This research portfolio fulfills the unique needs of the US Military. The collaboration of these two entities takes advantage of the unique strengths of each partner. The military could not duplicate the assets the PBRC brings to the project. PBRC benefits by the opportunity to collaborate with USARIEM on scientific questions of broad importance. The research supported by CROWN has implications for improving Warfighter nutrition, and it also provides the opportunity to broadly impact the nutritional health of Americans.

We continue to position ourselves to expedite efforts on our end to incorporate state of the art technology for use in field trials in the Military Nutrition Division of USARIEM. We have developed a system that can meet the needs of field experiments in a timely manner and we are ready to support future studies with very little lead time. We are also expanding our collaboration with USARIEM and having PBRC investigators serving as lead investigators on projects.