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TITLE: Warfighter Recovery Nutrition: A Strategic Partnership with USARIEM to Optimize Protein Quantity, Quality, and Combat Ration Delivery Systems

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14. ABSTRACT The overarching goal of this strategic, collaborative plan is to provide evidence-based nutritional recommendations for the development of new combat ration food products designed to maintain performance during SUSOPS. The following specific aims address the proposed studies within this plan: #1. Determine resting and post-resistance exercise skeletal muscle and whole body protein kinetic responses to graded EAA intake (Study 1); #2. Determine resting and post-resistance exercise skeletal muscle and whole body protein kinetics responses to various formats of EAA intake in response to acute, moderate energy deficit (Study 2); #3. Determine the effects of various protein-containing food matrices on skeletal muscle and whole body protein kinetics with combined military-type activities and acute, moderate energy deficit (Study 3); #4. Test the ration prototype during a 5-d simulated SUSOPS (Study 4) on whole-body protein kinetics and performance outcomes; and #5. Validate the efficacy of a protein-containing combat ration during a "real-world" training exercise (Study 5). There is a critical need for effective and feasible interventions that sustain and optimize Warfighter health and performance during real-world operations. Development of combat ration items for optimal protein delivery will spare muscle and whole body protein and promote recovery from operational stress.					
15. SUBJECT TERMS Protein turnover, muscle, essential amino acids (EAA), energy deficit, exercise, net protein balance, protein synthesis.					
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Table of Contents

Front Cover	1
Standard Form (SF 298).....	2
Table of Contents.....	3
Introduction	4
Keywords.....	4
Accomplishments.....	4
Impact.....	6
Changes/Problems.....	6
Products	7
Participants	8
Other Collaborating Organizations	9
Special Reporting Requirements	9
Appendices	9
Additional Notes.....	9

1. INTRODUCTION:

While Study 1 and Study 2 provide the initial biomedical evidence needed to devise nutrition formulations for the development of a protein-centric, recovery-based combat ration product, our results clearly indicate that additional work is needed to optimize the formulation to ensure the best possible muscle and whole-body anabolic responses are achieved. This includes addressing the potential value of adding non-EAA/protein components to support energy demands. Providing additional non-protein energy to an EAA-enriched whey protein formulation may reduce the proportion of exogenous amino acids directed towards energy production thereby preserving its use for muscle protein synthesis. During severe energy deficit (total intake of 300 or 500 kcal/d for 7-d), providing additional energy in the form of carbohydrate reduces amino acid oxidation to a greater extent than isocaloric amounts of either additional amino acids or fat. However, whether suppressing exogenous amino acid oxidation by providing additional carbohydrate allows for a greater muscle protein synthetic stimulus during moderate energy deficit (- 30% total energy requirements) is unknown. Lastly, Study 1 and Study 2 were not intended to discern changes in the anabolic response to EAA intake between energy balance and energy deficit. Short-term energy deficit results in negative whole-body protein balance and downregulates muscle protein synthesis. Therefore, Study 3 will also assess the magnitude of change in muscle protein synthesis and whole-body protein turnover between energy balance and energy deficit and evaluate the extent to which postprandial anabolic responses in these conditions are altered by ingesting EAA-enriched whey protein with carbohydrate or additional EAA.

KEYWORDS:

Protein turnover, muscle, essential amino acids (EAA), energy deficit, exercise, net protein balance, protein synthesis.

2. ACCOMPLISHMENTS:

- **What were the major goals of the project?**
- The primary goal of Study 2 was to execute a human clinical research study in order to determine the best matrix for optimal delivery of EAA on resting and post-resistance exercise skeletal muscle and whole-body protein kinetics after a 5-d, 30% energy deficit.
- The primary goal of Study 3 is to execute a human clinical research study to determine whether additional energy provided as carbohydrate or additional EAA provides the best energy substrate to support the effects of the EAA-whey protein matrix derived from Study 2 on post-exercise muscle protein synthesis and whole-body protein turnover during energy balance and a 30% energy deficit.
- **What was accomplished under these goals?**
 - Study 2 Summary: Ten adults representative of active duty military personnel completed this randomized, double-blind crossover study consisting of three, 5 d energy-deficit periods (- 30±3% of total energy requirements), separated by 14 d washout periods. Whole-body protein synthesis, breakdown, and balance were determined at rest and in response to combination exercise consisting of load carriage treadmill walking, deadlifts, and box step-ups at the end of each energy deficit using L-[2H5]-phenylalanine and L-[2H2]-tyrosine stable isotope infusions. Muscle protein synthesis was measured during exercise through recovery using a L-[2H5]-phenylalanine stable isotope infusion. Immediately following the exercise bout, volunteers consumed iso-nitrogenous amounts of EAA-enriched whey protein, standard whey protein, and an MRE entrée during each respective testing day. Blood and muscle samples were collected throughout the stable isotope infusions.
 - Study 3 key updates:
 - Study 2 was completed on December 13, 2019. Ten subjects have been studied during 3 interventions.
 - All analyses associated with this study have been completed.
 - Data has been consolidated and kinetic calculations have been accomplished.

- The primary manuscript is published in a peer-reviewed journal.
 - Study 3: Summary: In Study 3 we will test the effects of EAA-enriched whey protein plus carbohydrate versus EAA-enriched whey plus additional EAA using a randomized, cross-sectional longitudinal study design. Thirty healthy, functional and aerobic exercise trained adults (≥ 2 d/wk for the past 6 mo) will complete two 5-d controlled feeding phases, one maintaining energy balance and one inducing energy deficit in random order and separated by at least a 7d washout period. The difference in post-exercise (whole-body exercise model) muscle protein synthesis and whole-body protein turnover between the study treatments will be determined the day after each 5-d controlled feeding phase.
 - Study 3 key updates:
 - Study 3 received IRB approval on 8 June 2020.
 - Initial recruitment began 21 August 2020.
 - Initial recruitment/studies delayed due to COVID-19 pandemic. Thirteen subjects were enrolled, and 8 ongoing in Fall 2020 were withdrawn due to COVID.
 - Recruitment and data collection again underway beginning in February 2021. Seven subjects have been completed at the time of this report, with an additional 2 undergoing the scheduling process to begin data collection. Our corrective actions to COVID-19 delays in enrollment have been effective and continue to support progress with this project (i.e., expanding the recruitment pool for this project and leveraging surrounding Universities and human research volunteer recruitment avenues). Due to these actions, we expect continued progress with enrollment in Fall 2021.
- **What opportunities for training and professional development has the project provided?**
 - The project supports a post-doctoral fellow at UAMS and provides training and professional development for a post-doctoral fellow at both UAMS and USARIEM.
 - This project serves as the primary conduit for the fellow's education and experience in the assessment of protein metabolism in response to various physiological interventions. The fellow learns the background rationale, methodology, study conduct, sample preparation and analyses, data analysis, data consolidation and interpretation, data presentation, and manuscript publication.
- **How were the results disseminated to communities of interest?**
 - Results from the first and second study have been disseminated as peer-reviewed publications.
 - **Study 1:** Gwin JA, Church DD, Hatch-McChesney A, Howard EE, Carrigan CT, Murphy NE, Wilson MA, Margolis LM, Carbone JW, Wolfe RR, Ferrando AA, Pasiakos SM. Effects of high versus standard essential amino acid intakes on whole-body protein turnover and mixed muscle protein synthesis during energy deficit: A randomized, crossover study. *Clin Nutr.* 2020 Jul 22;S0261-5614(20)30384-8. doi: 10.1016/j.clnu.2020.07.019. Epub ahead of print. PMID: 32768315.
 - **Study 2:** Gwin, J.A., Church, D.D., Hatch-McChesney, A. et al. Essential amino acid-enriched whey enhances post-exercise whole-body protein balance during energy deficit more than iso-nitrogenous whey or a mixed-macronutrient meal: a randomized, crossover study. *J Int Soc Sports Nutr* 18, 4 (2021). <https://doi.org/10.1186/s12970-020-00401-5>.
- **What do you plan to do during the next reporting period to accomplish the goals?**
 - Participant recruitment and data collection for Study 3 is ongoing. Our plan is to study 30 subjects. Seven individuals have completed the study. Two individuals are undergoing the clearance and scheduling phase. We expect another group of HRV (human research volunteers from military) will be recruited for study participation this summer through the fall (approximately 8 -10 individuals). We have expanded the recruitment pool for this project to

leverage surrounding universities and other human research volunteer recruitment avenues. Recruitment and enrollment will continue through these additional avenues.

- The next reporting period entails an update on study participants/completions due to the delay by COVID-19. Recruitment and data collection have resumed (following regulatory approval for return to research processes). Although we will make every effort to keep this project on time, we do anticipate a ~6 month delay in completion due to COVID-19 related disruptions in human research processes.

3. **IMPACT:**

- **What was the impact on the development of the principal discipline(s) of the project?**
 - Thus far, the data indicate that a significant intake of EAA are required to offset the combined physiological impact of a caloric deficit and exercise.
 - Data from Study 2 demonstrate that a combination product of free-form EAA and intact whey protein resulted in an optimal delivery of EAA and greatest response of whole-body protein kinetics.
 - Data from Study 2 indicate that simpler matrices/ingestion forms of EAA are most beneficial to both muscle and whole-body protein homeostasis.
- **What was the impact on other disciplines?**
 - These findings may impact food delivery and/or format, in particular future combat rations/feeding. Plans are underway to project final product usage in a field training environment.
 - The findings of an increased EAA requirement and ideal food matrices and/or delivery require careful consideration of current food delivery and/or rations.
- **What was the impact on technology transfer?**
 - Nothing to Report
- **What was the impact on society beyond science and technology?**
 - Nothing to Report

4. **CHANGES/PROBLEMS:**

- **Changes in approach and reasons for change.**
 - **Study 2:**
 - One subject withdrawn due to inability to adhere to study schedule or study diet. One subject withdrawn due to medical ineligibility caused by an unrelated medical procedure.
 - **Study 3:**
 - Expansion of recruitment avenues to overcome COVID-19 -related reductions in the number of individuals participating in the Natick Soldier Systems Center Human Research Volunteer Program. Expansion includes greater recruitment in the surrounding community, as well as the HRV subject pool.
- **Actual or anticipated problems or delays and actions or plans to resolve them.**
 - We experienced a complete halt to human research processes at USARIEM from March 2020 through ~July 2020. Thereafter we were able to successfully recruit, enroll, and begin data collection on 8 Natick Soldier Systems Center (NSSC) Human Research Volunteers. However, this series of data collection was halted October 2020 due a COVID-19 outbreak within the NSSC Human Research Volunteer cohort. We were unable to resume data collection on these individuals due to the decision by NSSC Command to outprocess all NSSC Human Research Volunteers.

- We were approved to return to recruitment and data collection following the October 2020 halt beginning in February 2021. Seven subjects have been completed at the time of this report, with an additional 2 undergoing the scheduling process to begin data collection. Our corrective actions to COVID-19 delays in enrollment have been effective and continue to support progress with this project. Due to these actions, we expect continued progress with enrollment in Fall 2021.
 - Corrective actions to overcome delays in enrollment have included expanding the recruitment pool for this project and leveraging surrounding Universities and human research volunteer recruitment avenues. Upcoming volunteers entail a combination of surrounding population and HRV participants.
 - There is always potential for another COVID-19-related delay. Mitigation plans are in place and the tempo of experimental testing may be adjusted accordingly. We will make every effort to completing this experiment on time and on budget. However, we anticipate an approximate 6 month delay due to COVID-19-related disruptions in human research processes.
- **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.**
 - No animal use research was performed to complete the Statement of Work.
- **Significant changes in use of biohazards and/or select agents.**
 - Nothing to Report

5. PRODUCTS:

- **Publications, conference papers, and presentations.**
 - **Journal publications.**
 - **Study 1:** Gwin JA, Church DD, Hatch-McChesney A, Howard EE, Carrigan CT, Murphy NE, Wilson MA, Margolis LM, Carbone JW, Wolfe RR, Ferrando AA, Pasiakos SM. Effects of high versus standard essential amino acid intakes on whole-body protein turnover and mixed muscle protein synthesis during energy deficit: A randomized, crossover study. Clin Nutr. 2020 Jul 22:S0261-5614(20)30384-8. doi: 10.1016/j.clnu.2020.07.019. Epub ahead of print. PMID: 32768315.
 - **Study 2:** Gwin, J.A., Church, D.D., Hatch-McChesney, A. et al. Essential amino acid-enriched whey enhances post-exercise whole-body protein balance during energy deficit more than iso-nitrogenous whey or a mixed-macronutrient meal: a randomized, crossover study. J Int Soc Sports Nutr 18, 4 (2021). <https://doi.org/10.1186/s12970-020-00401-5>
 - **Books or other non-periodical, one-time publications.**
 - Nothing to Report
 - **Other publications, conference papers, and presentations.**
 - Nothing to Report

- **Website(s) or other Internet site(s.)**
 - Nothing to Report
- **Technologies or techniques.**
 - Nothing to Report
- **Inventions, patent applications, and/or licenses.**
 - Nothing to Report
- **Other Products.**
 - Nothing to Report

6. **PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**

- **What individuals have worked on the project?**

Name:	Arny A. Ferrando, PhD
Project Role:	Principal Investigator
Researcher Identifier (e.g. ORCID ID):	0000-0003-2916-7878
Nearest person month worked:	3 months
Contribution to Project:	Involved in all aspects of study design, conduct, data collection, and data consolidation and interpretation. Dr. Ferrando serves as the primary interface with USARIEM and Dr. Pasiakos. Further, he supervises the individual analytical aspects and laboratory personnel involved in this project.
Funding Support:	USAMRAA
Name:	Robert R. Wolfe, PhD
Project Role:	Co-Investigator
Researcher Identifier (e.g. ORCID ID):	0000-0003-3425-8889
Nearest person month worked:	1 month
Contribution to Project:	Involved in study design and data interpretation. His expertise in isotope methodology and metabolic studies provides unique and valuable insight for each study.
Funding Support:	USAMRAA
Name:	David Church, PhD
Project Role:	Postdoctoral Fellow
Researcher Identifier (e.g. ORCID ID):	0000-0003-4918-5037
Nearest person month worked:	3 months
Contribution to Project:	Works directly with Dr. Ferrando on all aspects of this project. Assists USARIEM and Dr. Pasiakos' group with data collection and study conduct. Assists the Research Associate in sample preparation and analyses. Performs kinetic calculations and consolidates data for publication.
Name:	Rick Williams, MS
Project Role:	Research Associate
Researcher Identifier (e.g. ORCID ID):	-
Nearest person month worked:	3 months
Contribution to Project:	Responsible for processing muscle and blood samples for GCMS analyses. Also responsible for LCMS analyses, including the determination of tracer enrichment and amino acid concentrations.
Funding Support:	USAMRAA

Name:	Deborah Viane
Project Role:	Project Specialist
Researcher Identifier (e.g. ORCID ID):	-
Nearest person month worked:	3 months
Contribution to Project:	Responsible for monthly project expenditures and resolution of project-related costs. Responsible for acquisition of study-related materials and scheduling and reconciliation of all travel arrangements for both the PI and Fellow. Also assists the PI in required project reporting.
Funding Support:	USAMRAA

- **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?**
 - US Army Research Institute of Environmental Medicine (USARIEM)
 - Organization Name: Headquarters, U.S. Army Medical Research and Materiel Command (HQ USAMRMC)
 - **Location of Organization:** Natick, MA
 - **Partner's contribution to the project:**
 - **Financial support:** N/A
 - **In-kind support:** N/A
 - **Facilities:** Human studies are conducted at USARIEM; USARIEM recruits volunteers
 - **Collaboration:** Staff, fellow, and PI collaborations
 - **Personnel exchanges:** Fellow travels to Natick/USARIEM to perform metabolic studies.
 - **Other:** N/A

7. SPECIAL REPORTING REQUIREMENTS

- **COLLABORATIVE AWARDS:**
 - Nothing to Report
- **QUAD CHARTS:**
 - Nothing to Report
- **APPENDICES:**
 - Nothing to Report