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TITLE: Warfighter Recovery Nutrition: Optimizing Protein Quantity, Quality, and
Combat Ration Delivery Systems

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CONTRACTING ORGANIZATION: University of Arkansas for Medical Sciences

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Fort Detrick, Maryland 21702-5012

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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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1. **INTRODUCTION:**

The primary goal of Study 2 is to determine the best matrix for optimal delivery of EAA on resting and post-whole-body exercise skeletal muscle and whole-body protein kinetics after a 5-d, 30% energy deficit.

2. **KEYWORDS:**

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

3. **ACCOMPLISHMENTS:**

○ **What were the major goals of the project?**

- The primary goal of Study 2 is 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.

○ **What was accomplished under these goals?**

Major Activities:

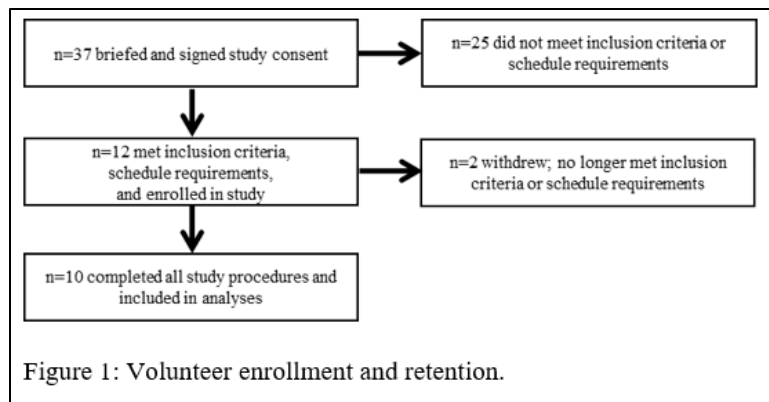
- Study 1 manuscript has been accepted for publication: Gwin JA, Church DD, Hatch-McChesney A, Howard EE, Carrigan CT, Murphy NE, Wilson MA, Margolis LM, Carbone JW, Wolfe RR, Ferrando AA, Pasiakos SM. Effects of high versus standard essential amino acid intakes on whole-body protein turnover and mixed muscle protein synthesis during energy deficit: a randomized, crossover study. *Clinical Nutrition*. <https://doi.org/10.1016/j.clnu.2020.07.019>.
- 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.
- Manuscript for Study 2 is in preparation.

Specific Objectives:

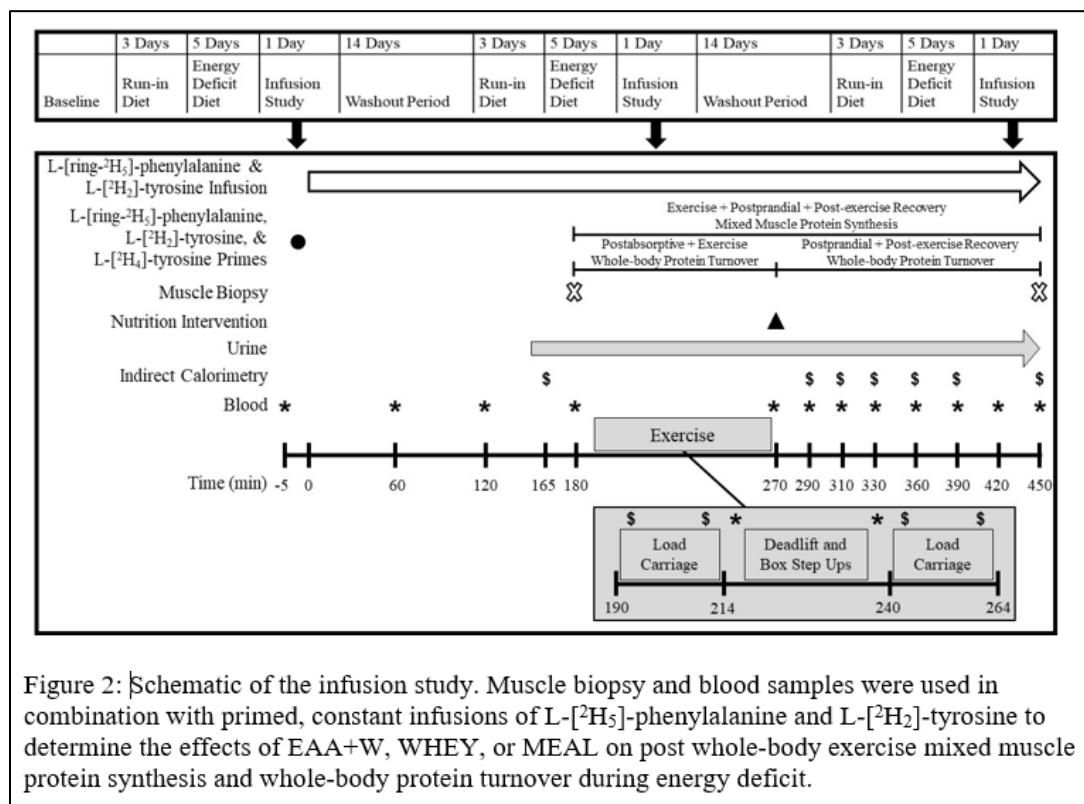
- The primary outcomes for this study were whole-body protein turnover responses to ingesting 3 treatments: EAA+W, WHEY, and MEAL and mixed-MPS responses across the exercise plus postprandial recovery period.
- Secondary outcomes included blood EAA, leucine, phenylalanine, tyrosine, and insulin concentrations over time and incremental area under the curve (iAUC) following EAA+W, WHEY, and MEAL ingestion.

Methodology:

- *Participants:* Ten healthy, young (18-25 y), non-obese (body mass index, < 30.0 kg/m²), resistance exercise-trained (≥ 2 sessions/week for previous 6 months) males and females (mean±SD; 21±4y; 25.7±1.7 kg/m²) completed all study procedures and were included in the final analyses, as shown in CONSORT **Figure 1**.



- Experimental Design:** Volunteers underwent a randomized, double-blind crossover study consisting of three, 5 d-controlled periods of diet-induced energy deficits ($-30 \pm 3\%$ of total energy requirements), separated by 14 d (**Figure 2**). Immediately following each energy deficit, stable isotope infusion studies were used to determine whole-body protein synthesis (PS), protein breakdown (PB), and net balance (NET) in response to post-exercise ingestion of an EAA-enriched, low dose of whey protein isolate (EAA+W; 35 g protein) or iso-nitrogenous amounts of whey protein isolate (WHEY) or protein in a mixed-macronutrient meal (MEAL). Mixed-MPS was also assessed for the entire exercise plus postprandial recovery period. Exercises consisted of load carriage treadmill walking, deadlifts, and box step-ups.

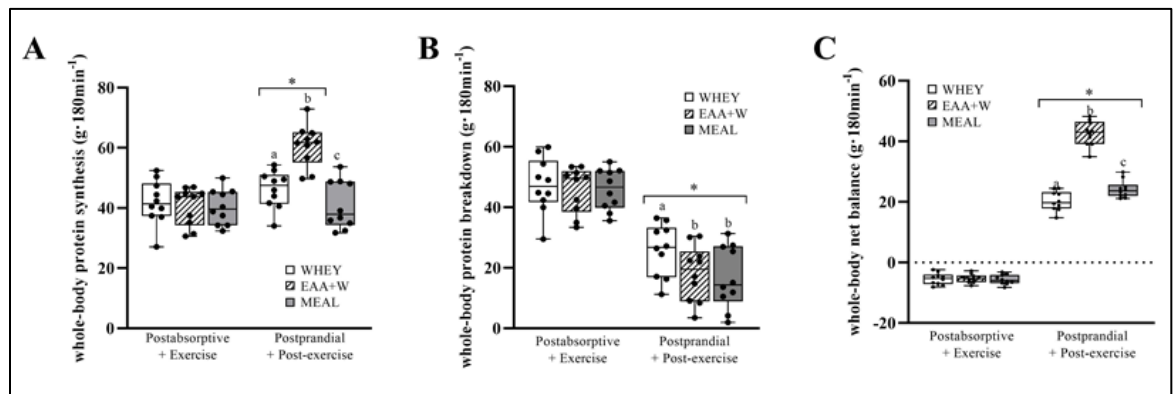


- Diet Intervention:** Dietary protein was provided at 1.6 g/kg/d, carbohydrate comprised 50-55% of total energy, and fat provided the remaining energy. The 30% energy deficit was achieved by reducing carbohydrate and fat intakes while maintaining protein intake at 1.6 g/kg/d. Registered Dietitians developed individualized menus (Food Processor SQL, v.11.3.2; ESHA Research, Salem, OR, USA) consisting primarily of military combat rations (Meal, Ready-to-Eat; menu 37; Ameriquel, Evansville, IN, USA), supplemented with commercial products (e.g., frozen sandwiches, yogurt, snack foods).

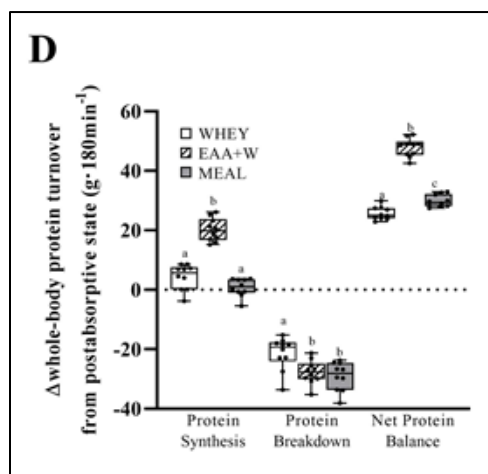
- *Stable isotope infusion studies:* Infusion studies were conducted the morning (after ≥ 8 h overnight fast) following each 5-d energy deficit to determine whole-body protein turnover and mixed-MPS. Intravenous catheters were placed in each arm for the continuous isotope infusions and serial blood draws. Following the baseline blood sample, primed, constant infusions of L-[ring- $^2\text{H}_5$]-phenylalanine and L-[3,3- $^2\text{H}_2$]-tyrosine were started and maintained for the next 450 min. Two muscle biopsies were collected from the vastus lateralis using a single incision on one leg during each infusion study to assess mixed-MPS. The first muscle biopsy was performed 10 min prior (180 min after infusion initiation) to a bout of whole-body exercise. Plasma and muscle samples were processed and analyzed for phenylalanine and tyrosine enrichments using gas chromatography-mass spectrometry (models 7890A/5975; Agilent Technologies, Santa Clara, CA). Plasma EAA concentrations were determined using the internal standard technique. Serum insulin concentrations were measured using a Siemens Immulite 2000XPI (Siemens Medical Solutions USA, Inc., Malvern, PA). Whole-body PS and PB rates were calculated based on the determinations of the rate of appearance (R_a) into the plasma of phenylalanine and tyrosine and the fractional R_a of endogenous tyrosine converted from phenylalanine. Whole-body protein turnover was calculated by dividing kinetic values of phenylalanine by its fractional contribution to protein. Whole-body net balance was calculated as PS-PB. The precursor-product model was used to determine mixed-MPS (i.e., fractional synthetic rate).
- *Exercise:* The exercise bout consisted of 24 min of load carriage (LC) followed by 18 min of alternating trap bar deadlifts (70% 1RM) and box step-ups followed by another 24 min of LC. Volunteers were given 4 min of rest before and after the bout of deadlifts and step-ups. All LC was performed by walking on a treadmill while wearing a weighted pack equivalent to 30% of each individual's baseline body mass. Speed and grade were adjusted throughout the LC to achieve 1 min intervals of low to moderate intensity ($55\pm 5\%$) and moderate to vigorous intensity ($70\pm 5\%$) work based on $\dot{V}\text{O}_{2\text{peak}}$ determined at baseline and confirmed during each washout period. If the volunteer was unable to complete the prescribed workload, the speed of the treadmill was reduced until the participant could complete the work. Every effort was made to match LC bouts between infusion studies and the bouts were nearly identical between all trials for all volunteers. For each set of trap bar deadlifts and box step-ups, volunteers completed 5 repetitions of deadlift immediately followed by 16 step-ups (8 per leg) totaling to ~ 1 min of work. The volunteers then rested for 1 min before completing the next set. In total, 9 sets were performed.
- *Post-Exercise Supplementation:* Within ~ 5 min of completing the exercise bout (270 min), volunteers consumed either EAA+W dissolved in 200 mL of water as a bolus, WHEY dissolved in 200 mL of water as a bolus (BiPro Elite Whey Protein Isolate; BiPro USA, Eden Prairie, MN) or MEAL (Chili and Beans Entrée, Meal, Ready-to-Eat; menu 37; Ameriqual, Evansville, IN, USA). Volunteers were given an additional 300 mL and 500 mL of water to consume with the beverages and MEAL, respectively. The study treatments and additional water were consumed within 5 min. Thereafter, volunteers rested for the remaining 180 min recovery period while blood samples were continually collected until the final biopsy was performed at 450 min.
- *Statistical Analysis:* Linear mixed models, with participant treated as a random effect, were used to determine the effects of treatment (EAA+W, WHEY, and MEAL), condition (postabsorptive and postprandial), and their interaction (treatment-by-condition) on whole-body protein kinetics and phenylalanine hydroxylation. A one-way repeated measure analysis of variance (ANOVA) was used to determine the effects of treatment (EAA+W, WHEY, and MEAL) on change in (Δ postabsorptive + exercise/postprandial + recovery) whole-body protein kinetics and mixed-MPS. Two-way repeated measures ANOVA were used to determine the effects of treatment (EAA+W, WHEY, and MEAL), time (min), and their interaction (treatment-by-time) on plasma EAA, leucine, phenylalanine, tyrosine, and insulin concentrations. EAA, leucine, phenylalanine, tyrosine, and insulin were also calculated using iAUC and one-way repeated measures ANOVA were used to evaluate whether iAUC differed between EAA+W, WHEY, and MEAL. All statistical analyses were performed with IBM SPSS software (version 26; IBM Corp. Armonk, NY, USA). Significance was set at $P < 0.05$ and data are presented as means \pm SD.

Significant Results:

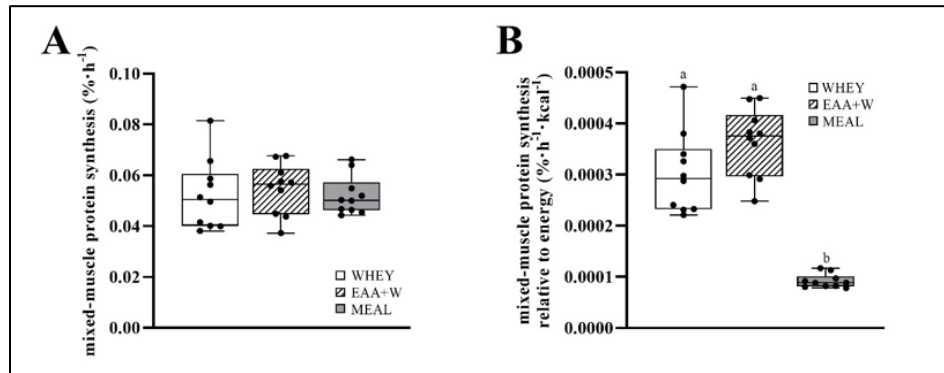
- Postabsorptive whole-body PS, PB, and NET did not differ (all, $P > 0.5$) between treatments (**Figure 4A-C**). A treatment-by-condition interaction ($P = 0.001$) was observed for whole-body PS such that postprandial PS was greater than postabsorptive PS for WHEY and EAA+W ($P = 0.008$ and $P = 0.001$, respectively), but not for MEAL ($P = 0.6$). Postprandial PS for EAA+W was greater than both WHEY and MEAL [14.3 g/180min (10.6, 18.4); $P = 0.001$, and 19.7 g/180min (15.8, 23.6); $P = 0.001$, respectively] and postprandial WHEY was greater than MEAL [5.2 g/180min (1.3, 9.1); $P = 0.006$, **Figure 4A**]. A treatment-by-condition interaction ($P = 0.011$) was observed for whole-body PB such that postprandial PB was lower than postabsorptive PB in all treatments. In the postprandial state, PB was lower for EAA+W and MEAL versus WHEY [-7.8 g/180min (-12.3, -3.2); $P = 0.001$, and -9.1 g/180min (-13.7, -4.5); $P = 0.001$, respectively], but did not differ between EAA+W and MEAL ($P = 1.0$, **Figure 4B**). A treatment-by-condition interaction ($P = 0.001$) was observed for NET such that postprandial versus postabsorptive NET was increased in all treatments. Postprandial NET was greater in EAA+W versus WHEY [22.3 g/180min (20.2, 24.4); $P = 0.001$] and versus MEAL [18.4 g/180min (16.3, 20.5); $P = 0.001$] and was also greater in MEAL versus WHEY [3.9 g/180min (1.8, 6.0); $P = 0.001$, **Figure 4C**].



- Changes in whole-body PS were greater for EAA+W than WHEY and MEAL [15.8 g/180min (9.8, 21.9); $P = 0.001$, and 19.4 g/180min (14.8, 24.0); $P = 0.001$, respectively], but did not differ between WHEY and MEAL ($P = 0.09$, **Figure 4D**). Reductions in whole-body PB were greater for EAA+W than WHEY [-6.3 g/180min (-11.5, -1.18); $P = 0.02$] and for MEAL than WHEY [-7.7 g/180min (-11.9, -3.6); $P = 0.002$], but did not differ between EAA+W and MEAL ($P = 0.37$, **Figure 4D**). As a result, change in NET was more positive for EAA+W than WHEY and MEAL [22.1 g/180 min (20.5, 23.8); $P = 0.001$, and 18.0 g/180 min (16.5, 19.5); $P = 0.001$, respectively, **Figure 4D**]. Change in NET was also more positive for MEAL than WHEY [4.2 g/180min (2.7, 5.6); $P = 0.001$, **Figure 4D**].



- Mixed-MPS did not differ ($P = 0.68$) between WHEY, EAA+W, and MEAL (**Figure 5A**). Mixed-MPS relative to the energy content of the treatments did not differ ($P = 0.063$) between WHEY and EAA+W, but both were greater than MEAL [0.00021%/h (0.00013, 0.00029); $P = 0.001$, and 0.00027%/h (0.00022, 0.00033); $P = 0.001$, respectively, **Figure 5B**].



Discussion of findings:

- The purpose of this study was to determine the effects of various EAA/protein delivery formats on post-exercise whole-body protein balance in healthy, young adults after a 5 d, 30% energy deficit. Mixed-MPS responses to the combined effects of exercise and recovery feeding were also determined. The primary finding of this work was that post-exercise NET was greatest after EAA+W ingestion. The superior postprandial NET response following EAA+W was related to a greater increase in peripheral EAA, and a greater increase in PS compared to the other treatments. In addition, there was a greater reduction in PB with EAA+W compared to WHEY. Regardless of differences in NET, mixed-MPS was the same across treatments. However, EAA+W and WHEY had a greater anabolic response when normalizing mixed-MPS to total energy intake, suggesting a greater efficiency of these formats in maintaining MPS. The data suggest that consuming high-quality intact protein enriched with free-form EAA elicits enhanced whole-body protein balance compared to the other iso-nitrogenous formats. Therefore, the combined EAA/protein delivery format may be an effective strategy to offset body protein loss during the catabolic stress of energy deficit.
- The marked increase in NET after ingesting EAA+W confirms our hypothesis that changes in NET reflect increases in circulating EAA concentrations. These findings extend our previous study which demonstrated greater NET after ingesting EAA-enriched whey compared to an iso-nitrogenous whey-based recovery product. In the current study, greater NET after ingesting EAA+W was due to a robust increase in PS and concomitant reduction in PB. Based upon previous work, it is likely that the resultant NET was largely driven by the circulating EAA profiles. The enhanced peripheral EAA concentrations induced by the free-form and whey-derived components of EAA+W provide the required EAA to initially stimulate PS, as well as the non-EAA for a sustained increase of PS. In addition, a dose-dependent inhibition of PB by EAA has been demonstrated in the splanchnic region, independent of any insulin-specific effects. Lastly, the non-EAA component of the EAA+W likely also provides for a greater use of exogenous EAA for PS, rather than their conversion to non-EAA.
- In addition to measuring whole-body protein turnover, our intent was to determine whether the free-form EAA component of each EAA/protein format would impact mixed-MPS. Despite the enhanced peripheral EAA concentrations with EAA+W, mixed-MPS was equally stimulated between treatments during a combined exercise and recovery period. Lack of an effect in mixed-MPS despite differences in peripheral EAA availability is in agreement with our previous work, showing no difference in mixed-MPS responses to post-exercise ingestion of two doses of free-form EAA during energy deficit. While we cannot report the extent to which mixed-MPS was stimulated in the current study, the lack of differences between treatments is likely due to that fact that during energy deficit rapidly absorbed exogenous amino acids, both EAA and non-EAA, are prioritized centrally (i.e., liver, splanchnic region) to meet whole-body amino acid and energy (i.e., provision of carbon

skeletons) requirements. This metabolic prioritization is reflected in the demonstrated changes in whole-body protein turnover between formats despite no difference in mixed-MPS. However, despite similar absolute mixed-MPS, an important finding was that EAA+W and WHEY resulted in greater mixed-MPS when normalized to total energy intake. The greater anabolic response achieved by the less energy dense formulations highlights the efficiency of these EAA/protein formats for supporting MPS compared to MEAL.

Conclusion:

- In conclusion, EAA-enriched whey enhanced NET versus iso-nitrogenous amounts of whey isolate and a mixed-macronutrient meal during 30% energy deficit. NET was achieved through an increase in PS and an attenuation of PB. In addition, EAA-enriched whey stimulated mixed-MPS to the same extent as the other treatments, though the stimulation of MPS was more efficient for the given energy intake. These findings indicate that protein-containing food formats that have a high EAA content and achieve rapid and sustained peripheral EAA concentrations can enhance whole-body protein status and efficiently support MPS during the catabolic stress of underfeeding.
- **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?**
 - Manuscript with results from the first study is accepted for publication: 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. *Clinical Nutrition*. <https://doi.org/10.1016/j.clnu.2020.07.019>.
 - Manuscript from this study (Study 2) is being prepared.
- **What do you plan to do during the next reporting period to accomplish the goals?**
 - Manuscript preparation for Study 2 is ongoing. We intend to have these results published prior to the next (annual) reporting period.
 - The next reporting period entails analyzing and consolidating the results from Study 3, scheduled to begin in August 2020.

4. **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.
 - The impact of these 2 studies indicates that development of a specialized product is necessary to offset the detriments and physiological alterations associated with caloric deficit. This is currently being pursued at USARIEM in the Combat Feeding Directorate.
- **What was the impact on other disciplines?**

- These findings have an impact on food delivery and/or format, in particular future combat rations/feeding.
- The findings of an increased EAA requirement and ideal food matrices and/or delivery require careful consideration of current food delivery and/or rations. Further, these findings have clinical applications in intensive care profiles where caloric intake is limited, and the physiology is stressed by pathology.
- **What was the impact on technology transfer?**
 - Nothing to Report at this time, though the development of a specialized product will have civilian/clinical applications.
- **What was the impact on society beyond science and technology?**
 - Nothing to Report at this time, but as mentioned, future product development would have application in clinical and particularly elderly populations, where caloric intake is limited.

5. **CHANGES/PROBLEMS:**

- **Changes in approach and reasons for change.**
 - 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.
- **Actual or anticipated problems or delays and actions or plans to resolve them.**
 - Nothing to Report
- **Changes that had a significant impact on expenditures**
 - Nothing to Report
- **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents.**
 - Nothing to Report
- **Significant changes in use or care of human subjects.**
 - Nothing to Report
- **Significant changes in use or care of vertebrate animals.**
 - 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

6. **PRODUCTS:**

- **Publications, conference papers, and presentations.**
 - **Journal publications.**

Study 1 is accepted (see above). Manuscript preparation is underway for Study 2.
 - **Books or other non-periodical, one-time publications.**
 - A review concerning these findings is in revision: Muscle protein synthesis and whole-body protein turnover responses to ingesting essential amino acids, intact protein, and protein-containing mixed meals with considerations for energy deficit. Jess A. Gwin, David D. Church, Robert R. Wolfe, Arny A. Ferrando, and Stefan M. Pasiakos. *Nutrients*.

- **Other publications, conference papers, and presentations.**

Data and results from this project will be presented on Wednesday, 5 August at the 2020 USSOCOM Performance Nutrition Summit at the United States Military Academy.

- **Website(s) or another 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

7. 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:	4 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:	12 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):	None
Nearest person month worked:	6 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):	None
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 the scheduling of travel arrangements for both the PI and Fellow. Also assists 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

8. SPECIAL REPORTING REQUIREMENTS

- **COLLABORATIVE AWARDS:** None to Report
- **QUAD CHARTS:** None to Report

9. APPENDICES: None to Report

ADDITIONAL NOTES: Unlimited Distribution A