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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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1. **INTRODUCTION:**

Study 3 is ongoing; Study 4 is complete, though analyses is ongoing. Note, the rationale and design of Study 3 and 4 have not changed from previous quarterly reports:

While Study 1 and Study 2 provided the initial biomedical evidence needed to devise nutrition formulations for the development of a protein-centric, recovery-based combat ration product, our results indicated 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 versus their utilization in the stimulation of body 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. Importantly, 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 a 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 either carbohydrate or additional EAA.

The scientific objectives of the original research plan for Studies 4 and 5 were combined to best address the research question in a highly-relevant artic field training environment. Given the unique opportunity to test a product developed through this research program in a very physiologically demanding environment, we combined Study 4 (product development) and Study 5 (test in field training environment) which was accomplished in conjunction with the Norwegian Army.

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?**
  - Overall scientific goal:
    - Study 3 is a human clinical research study to determine whether additional energy, provided as carbohydrate or additional EAA, supports 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. This work is sequential in nature in that Study 3 builds upon the evidence derived from Studies 1 and 2. This information will be transifitioned to US Army Combat Capabilities Development Command, Combat Feeding Division and used to inform the development of future generation combat rations. **Analyses for the first 13 subjects is complete. Additional subjects are being**

recruited and interim statistical analysis is being conducted to determine an actual subject number required to accomplish the scientific objectives, given the COVID-19 delays in subject recruitment.

- **Study 4 was a field study conducted during soldier training to investigate the effects of our derived nutritional product on proteostasis during a demanding winter training course. Dietary records have been held up in US Customs and have recently been released. Analyses are currently ongoing.**

- Summary of status:
  - Study 3 is testing the effects of EAA-enriched whey protein plus carbohydrate versus EAA-enriched whey plus additional EAA using a randomized, cross-sectional longitudinal study design. The original study design was to test thirty healthy, functional, and aerobically trained adults (≥ 2 d/wk for the past 6 mo) during two 5-d controlled feeding phases; one maintaining energy balance and one inducing energy deficit (randomized) 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.

- Techical goals:
  - In light of post-COVID-19 research environment, a main technical goal during this quarter was to continue to adapt to the current research environment and continue to evaluate and overcome setbacks in research timing and study participant recruitment.

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

- Note: Due to the disruptive nature of COVID-19 and subsequent alterations in study timelines, we have included all accomplished goals related to this project which span several reporting periods. These are included to better communicate the status of the project, our efforts to keep the project on time and on-budget, and maintain transparency.

- 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 were withdrawn in Fall 2020 due to COVID-19 related shutdowns within the USARIEM human research program.
  - Recruitment and data collection were again underway beginning in February 2021. Thirteen subjects have been completed at the time of this report. Analyses for these 13 subjects has been completed in Dr. Ferrando's laboratory. Data interpretation is ongoing.
  - 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 reserach volunteer recruitment avenues).
  - **The uptick in COVID-19 cases related to the Omicron variant caused further delay to the arrival of the USARIEM Human Research Volunteer 2022 class, a primary avenue for volunteer recruitment. However, these volunteers arrived late March 2022**

and were briefed and recruited. Although three volunteers from the Human Research Volunteer Spring 2022 class were enrolled in the study, the volunteers withdrew due to scheduling or external reasons. In alignment with our corrective actions, we recruited and enrolled an additional 3 volunteers for a total of 3 volunteers actively participating and expected to finish by mid July 2022. We continue to expect progress with recruitment and enrollment in 2022 using other avenues previously mentioned.

- Study 4 received IRB approval on 03 August 2021.
  - **The study was executed 14-24 March 2022 in Kirkenes, Norway.**
  - **96 volunteers were enrolled in the study, 68 volunteers completed the study. Our nutritional supplement, developed from data derived from Studies 2 and 3, was tested in a real-world and demanding military training scenario.**
  - **Following international shipping delays, all study samples and materials were returned from Norway and data analysis is ongoing and will continue through Summer 2022.**

- **What opportunities for training and professional development has the project provided?**
  - The project continues to support a post-doctoral fellow at UAMS and provides training and professional development for post-doctoral fellows 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 mass spectrometry analyses, data analysis, data consolidation and interpretation, data presentation, and manuscript publication.
  - Given the human research environment during the COVID-19 pandemic. This project has also provided unique exposures to logistical management and professional problem-solving to the fellows involved.

- **How were the results disseminated to communities of interest?**
  - Note: The following results have been previously reported.
    - Results from the first and second study have been disseminated as peer-reviewed publications and presented within the greater scientific communicate at Scientific Society Meetings. **Publications have been updated with complete reference information.**
      - **Study 1 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. Clin Nutr. 2020 Jul 22:S0261-5614(20)30384-8. doi: 10.1016/j.clnu.2020.07.019. PMID: 32768315. **Clin Nutr. 2021 Mar; 40(3):767-777. doi: 10.1016/j.clnu.2020.07.019. Epub 2020 Jul 22.PMID: 32768315.**

- **Study 1 Abstracts Presented at Scientific Society Meetings.**
- **Study 2 Publication:** 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. **J Int Soc Sports Nutr. 2021 Jan 7; 18(1):4. doi: 10.1186/s12970-020-00401-5.PMID: 33413462.**
- **Study 2 Abstract Presented at Scientific Society Meetings.**

- Results from the third study will be disseminated as peer-reviewed publications and again presented at scientific society meetings.

- **What do you plan to do during the next reporting period to accomplish the goals?**

- **Participant recruitment and data collection for Study 3 remains ongoing. Our current plan remains to study 30 subjects. Thirteen individuals have completed the study. Three individuals are enrolled with data collection expected to finish mid July 2022. We have expanded the recruitment pool for this project to leverage surrounding universities and other human research volunteer recruitment avenues. Recruitiment and enrollment will continue through these additional avenues.**

- Please note that although approvals for recruitment and data collection have resumed (following human research protections regulatory approval for return to research processes) at USARIEM, the Omicron and other variants have created case-by-case delays. We are making every effort to keep this project on time, we anticipated a ~8-10 month delay in completion due to COVID-19 related disruptions in human research processes. We will continue to communicate the progress of the project and the nature of COVID-19-related delays within our quarterly reports.

- **Data analysis for Study 4 in ongoing. Due to delays in international shipping, our goals for the next reporting period are extended from the last reporting period and are to complete data analysis and begin assimilation of the knowledge products.**

3. **IMPACT:**

- **What was the impact on the development of the principal discipline(s) of the project?**

- Note: Due to the nature of this project, Studies 1 and 2 have had direct impacts on Study 3.
  - To date, 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 benefitical to both muscle and whole-body protein homeostasis.

- Transition/translation of information gained from the project (Studies 1 & 2) has already begun. Specifically, the Combat Feeding Division within the US Army Combat Capabilities

Development Command has pursued a proposal through the CFREP program to **begin testing proto-type ration products which include shelf-stability tests of formulations from Studies 1 & 2.**

- **What was the impact on other disciplines?**
  - **These findings may impact food delivery and/or format, in particular future combat rations/feeding. Specificially, Studies 1 and 2 have been shared with the Natick Soldier Systems Center Development Command Combat Feeding Division. Food Scientists and Technologist within the Combat Feeding Division have used the scientific knowledge products to inform and underpin ongoing food technology and ration research and development, to include additional food technology/stability experiments.**
- **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 3**:

    Expansion of recruitment avenues to overcome COVID-19 -related reductions in the number of individuals participating in the US Army Combat Capabilities Development Command (formerly Natick Soldier Systems Center) Human Research Volunteer Program. Expansion includes greater recruitment in the surrounding community, as well as the HRV subject pool.

  - **Studies 4/5**:

    The scientific objectives of the original research plan for Studies 4 and 5 have been combined to best address the research question in a highly-relevant artic field training environment. Given the unique opportunity to test a product developed through this research program in a very physiologically demanding environment, we have combined Study 4 (product development) and Study 5 (test in field training environment) which will be accomplished in conjunction with the Norwegian Army.

    Study 4 Rationale for Study Protocol and summary of previous data:

    Optimizing protein quality and quantity to protect muscle and whole-body protein status during periods of unavoidable energy deficit. The magnitude of muscle and body protein lost during strenuous military training and operations can be attributed in large part to the severity and duration of the energy deficit, the amount and quality (i.e., EAA content, digestibility/absorption kinetics) of protein-containing food products consumed, and the resulting effects on skeletal muscle and whole-body protein turnover. Given limitations to the volume of food a Warfighter appears willing to carry and consume, manipulating quantity and quality of dietary protein represent an additional strategy to attenuate the consequences of energy deficit on protein balance. In general, the increase in peripheral EAA delivery dictates whole-body and muscle anabolic responses to dietary protein. Existing ration meals and snacks are consumed in a mixed-macronutrient format with mixed-protein sources. These food sources blunt the anabolic stimulus and diminish the anti-catabolic protective effect of protein consumed by requiring protein digestion and absorption which blunts peripheral EAA

delivery. Therefore, a combat ration item optimizing EAA content and delivery format (i.e., free-form EAA plus intact protein versus mixed-meal) to better stimulate protein synthesis and limit endogenous protein breakdown, may enhance net protein balance during energy deficit.

Our Team has conducted a series of collaborative studies to identify an optimal protein-based recovery ration product that meets the needs of Warfighters during training and combat operations. The first study of this series compared the effects of standard (0.1 g/kg/dose) versus high (0.3 g/kg/dose) free-form EAA intakes on resting and post-exercise muscle protein synthesis following 5 days of energy deficit. We found that consuming more EAA post-exercise than traditionally recommended (0.3 versus 0.1 g/kg/dose) facilitated whole-body and muscle protein recovery by suppressing whole-body protein breakdown and maintaining muscle protein synthesis during energy deficit. These findings suggest consuming greater amounts of EAA during military operations and energy deficits may offset protein losses and optimize recovery. However, isolated free-form EAA are difficult to provide in rations due to poor palatability and challenges associated with shelf-stablility. Alternatives include food products containing high-quality isolated intact proteins rich in EAA (i.e., whey protein) or isolated intact proteins enriched with EAA (i.e., EAA-enriched whey protein). Therefore, our follow-up work examined post-exercise MPS and net protein balance in response to three protein delivery formats [whey, EAA-enriched lower-dose whey, and a current combat ration entrée derived from the Meal Ready-to-Eat (MRE)] following 5 days of energy deficit. This work demonstrated that an easily digested product combining free-form EAA and whey protein provides a superior metabolic response (i.e., enhanced peripheral delivery of EAA [**Figure 1**], greater net whole-body protein balance [**Figure 2**]) than iso-nitrogenous amounts of either whey protein alone or protein provided by a mixed-macronutrient combat ration meal.

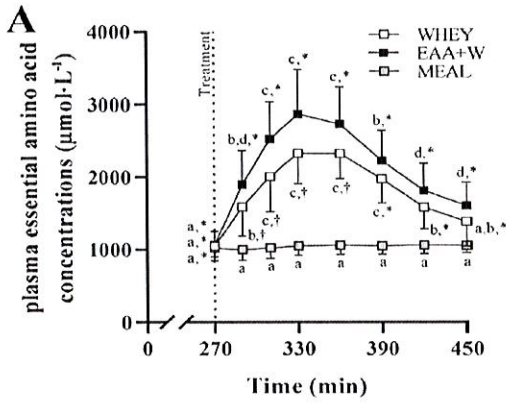

Figure 1: Comparison of postprandial plasma EAA concentrations following isonitrogenous amounts of whey, EAA-enriched whey, and a mixed-meal combat ration during energy deficit (derived from Study 2).

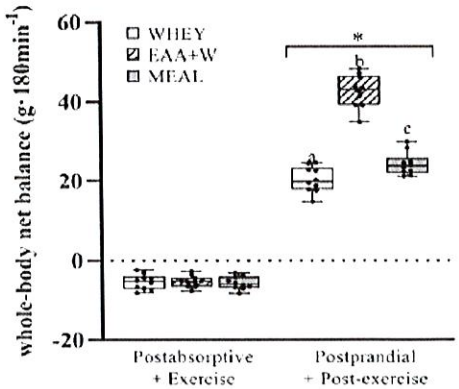

Figure 2: Comparison of whole-body net protein balance following isonitrogenous amounts of whey, EAA-enriched whey, and a mixed-meal combat ration during energy deficit (derived from Study 2).

Our findings suggest that, unlike the whey protein-based snack bars provided to Soldiers during winter military training in our previous work, an optimal protein-based recovery ration product should include a mixture of free-form EAA and whey protein in a rapidly digestible, easy to prepare and consume product. If consumed as intended (i.e., after exercise, between meals, on-the-go nutrition), an EAA-enriched whey protein food product should offset the catabolic effects of unavoidable energy deficit and promote a robust anabolic stimulus that enhances net protein balance.

Study 4 Deliverables: The proposed study will allow us to test strategies for mitigating the catabolic effects of energy deficit. This work extends the data published from our real-world field assessments conducted in collaboration with the Norwegian Defence Research Establishment and will leverage this existing partnership to study Soldiers participating in strenuous field/artic training. This study is the final project in our systematic approach (MOMRP 20670, 20790, 223360, and CAA W81XWH1820021) to provide the Combat Feeding Directorate (CFD) within the U.S. Army Combat Capabilities Development Command Soldier Center (DEVCOM Soldier Center) additional scientific evidence to optimize next generation combat rations (T.MRIEM.2018.4 and T.MRIEM.2019.4; Performance Nutrition For An Expeditionary Force – Phase II).

- **Actual or anticipated problems or delays and actions or plans to resolve them.**

Note: The following section has been previously reported, but included in the present report to maintain communication and transparency regarding COVID-19 related disruptions in the human research environment.

- Study 3: 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 US Army Combat Capabilities Development Command Human Research Volunteers. However, this series of data collection was halted October 2020 due a COVID-19 outbreak within the Human Research Volunteer cohort. We were unable to resume data collection on these individuals due to the decision by the Combat Capabilities Development Command to halt all military Human Research Volunteers.
- Study 3: We were approved to return to recruitment and data collection following the October 2020 halt beginning in February 2021. Thirteen subjects have been completed at the time of this report. 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 winter/spring 2022.
- Study 3: 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.
- Study 3: 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.

- **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**
  - **Review Articles previously published but not reported. See Appendices.**

    #1 Appendix: Church DD, Gwin JA, Wolfe RR, Pasiakos SM, Ferrando AA. Mitigation of Muscle Mass in Stressed Physiology: Military Relevance. Nutrients. 2019 Jul 24;11(8):1703. doi: 10.3390/nu11081703. PMID:31344809 Review.

    #2 Appendix: Gwin JA, Church DD, Wolfe RR, Ferrando AA, Pasiakos SM. 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. Nutrients. 2020 Aug 15;12(8):2457. doi: 10.3390/nu12082457.PMID: Review.
  - **Books or other non-periodical, one-time publications.**
  - Nothing to Report
  - **Other publications, conference papers, and presentations.**
  - **Church, DD. Presentation at 2022 July ISSN 19th Annal ISSN Conference and Expo, Fort Lauderdale Beach, FL. Warfighter Recovery Nutrition – Optimizing Protein Quantity, Quality, and Combat Ration Delivery Systems.**
- **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 Development Command (HQ USAMRDC)

    - **Location of Organization:** Natick, MA
    - **Partner's contribution to the projec**t:
      - **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:**
  - **Item #1**
  - **Item #2**

nutrients #1 Appendix MDPI

Communication

Mitigation of Muscle Loss in Stressed Physiology: Military Relevance

David D. Church [1,*], Jess A. Gwin [2,3], Robert R. Wolfe [1], Stefan M. Pasiakos [2] and Arny A. Ferrando [1]

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2 Military Nutrition Division, U.S. Army Research Institute of Environmental Medicine, Natick, MA 01760, USA
3 Oak Ridge Institute for Science and Education Supporting the Military Nutrition Division of the US Army Research Institute of Environmental Medicine, Natick, MA 01760, USA
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Received: 21 June 2019; Accepted: 22 July 2019; Published: 24 July 2019

Abstract: Military personnel may be exposed to circumstances (e.g., large energy deficits, sleep deprivation, cognitive demands, and environmental extremes) of external stressors during training and combat operations (i.e., operational stressors) that combine to degrade muscle protein. The loss of muscle protein is further exacerbated by frequent periods of severe energy deficit. Exposure to these factors results in a hypogonadal state that may contribute to observed decrements in muscle mass. In this review, lessons learned from studying severe clinical stressed states and the interventions designed to mitigate the loss of muscle protein are discussed in the context of military operational stress. For example, restoration of the anabolic hormonal status (e.g., testosterone, insulin, and growth hormone) in stressed physiological states may be necessary to restore the anabolic influence derived from dietary protein on muscle. Based on our clinical experiences, restoration of the normal testosterone status during sustained periods of operational stress may be advantageous. We demonstrated that in severe burn patients, pharmacologic normalization of the anabolic hormonal status restores the anabolic stimulatory effect of nutrition on muscle by improving the protein synthetic efficiency and limiting amino acid loss from skeletal muscle. Furthermore, an optimal protein intake, and in particular essential amino acid delivery, may be an integral ingredient in a restored anabolic response during the stress state. Interventions which improve the muscle net protein balance may positively impact soldier performance in trying conditions.

Keywords: skeletal muscle; military; protein metabolism; amino acids; testosterone; androgens

1. Introduction

The skeletal muscle system is most often a topic of discussion in relation to physical function. In addition, muscle serves as the body's primary protein reserve. Muscle mass and strength are crucial for health, physical function, and athletic performance in all populations [1]. In healthy trained athletes, increased muscle strength and mass are often important goals for achieving a maximal performance. With increasing physiological stress, the maintenance of muscle mass becomes a primary determinant of both quality of life and survival [2]. In stressed physiological states, muscle serves as a metabolic reserve by providing essential amino acids (EAA) for gluconeogenesis, as well as splanchnic, immunological, and wound-healing protein synthesis requirements [3,4]. In a clinical setting, muscle represents a key metabolic component required to combat the prevailing pathology. These roles of muscle are also relevant to circumstances such as multi-stressor military operations, in which health physiology is

significantly challenged. Contrary to this clinical scenario, there are certain circumstances, such as multi-stressor military operations, where health physiology is significantly challenged. This situation provides a unique opportunity to study the effects of severe stress on muscle mass in otherwise healthy individuals. For military personnel, understanding the physiological consequences of severe stress on muscle mass is critical for the design, assessment, and implementation of pharmacological and/or nutritional strategies to conserve muscle mass and, most importantly, function.

Exposure to catabolic states is common in some military personnel, including light infantry and special operations forces (SOF), who, at times, may be exposed to a wide range of diverse stressors during sustained training and combat operations (SUSOPS). For example, energy deficits of 2500–4000 kcal·day^{-1} have been reported during operational situations [5–8]. Furthermore, Norwegian men undergoing 7 days of field training lost an average of 4 kg of fat-free mass [9]. The loss of body protein during simulated combat operations is the result of altered protein kinetics that favor catabolism [10–12], which in turn may compromise performance [13]. We will use our previous investigations of critical care and severe stress to discuss potential mechanisms and strategies to combat muscle loss in military personnel during SUSOPS.

2. Lessons Learned from Clinical Stress States

Various clinical stress states have been studied and documented in the literature [14]. One of the most severe is the hypermetabolic and catabolic state that occurs in response to severe burn injury. Burn injury induces anabolic resistance in muscle, as muscle transitions from metabolic maintenance to catabolism and the provision of constituent amino acids (AA) for higher priority tissues. This alteration in muscle metabolism can persist for up to a year after the injury has fully healed [15]. Burn patients display some of the largest negative net protein balances (NB) reported in the literature [14,16], and although the catabolic state is a response to an injury, interventions that can ameliorate the prevailing catabolism may be relevant to stressed states in healthy individuals. For example, certain physiological perturbations are present in both burn patients and military personnel, such as elevated metabolic rates and altered hormonal secretion and/or influence. In burn patients, the rate of muscle protein breakdown (MPB) exceeds that of muscle protein synthesis (MPS), leading to a catabolic state that, if not corrected, results in large losses of skeletal muscle mass [4]. Even though MPS is elevated in these patients, the greater increase in MPB indicates that the majority of muscle protein is being broken down to supply the higher priority areas of the body with AA [3]. The only means of reducing the dramatic loss of muscle protein, and its constituent AA, with burn injury is to restore the ability of muscle to respond to anabolic stimuli. In addition, the stressed states generally suppress anabolic hormone production and/or elicit an anabolic resistance to those hormones. Overall, the greater the stressed physiology, the greater the propensity for the loss of muscle protein.

3. Role of Muscle in the Stressed State

The fundamental mechanism mediating changes in muscle mass is the NB between MPS and MPB (i.e., NB = MPS − MPB). A net gain in muscle protein occurs when NB is positive, indicating an anabolic state. A net loss, or catabolic state, occurs when MPB exceeds MPS. In the catabolic state, there is a significant acceleration of protein breakdown relative to the rate of synthesis in the post-absorptive state in both skeletal muscle and the whole body [14]. AA cannot be "stored" in the body in the same manner as lipids and carbohydrates. Incorporation of AA into proteins, enzyme function in metabolic pathways, conversion of their carbon skeleton to fatty acids, and excretion as urea or ammonia serve as the possible fates of AA. The lack of AA storage dictates that muscle protein is the sole source of EAA for the rest of the body. Once AA concentrations decrease in response to prolonged starvation/energy deficit, the MPB rate is increased in order to maintain blood AA concentrations until food or protein is provided. This mechanism is effective at maintaining blood AA concentrations until body protein is exhausted, at which point the individual dies [1].

In the absence of the dietary intake of protein, muscle protein serves as the body's only source of precursor AA, particularly the EAA. As the cumulative stress on an individual increases in the absence of food, muscle protein NB becomes more negative. With increasing stress, the metabolic priority of muscle is to deliver AA to higher priority functions necessary to facilitate survival. These include the support of acute-phase protein synthesis, immune function, wound healing, energy production, etc. [3,17]. During periods of high stress, the maintenance of muscle mass and the continued supply of EAA become essential for health and survival. For example, obese humans and animals die after the depletion of body protein when subjected to prolonged starvation, even when energy stores have not been depleted [18,19]. During starvation, the body's main metabolic focus is the maintenance of body protein. In fact, a small amount (7.5 g) of carbohydrate intake can reduce urinary nitrogen loss by ~40%–50% [20]. Notably, this effect is not further enhanced with the provision of 15–25 g or 54–108 g of carbohydrate [17,20]. Taken together, these data indicate that muscle protein is likely the main determinate of long-term survival in humans, particularly in response to stress [2].

The magnitude of stress affects both the direction and magnitude of NB. For example, resistance exercise increases protein turnover and results in a less negative NB in healthy, normal humans [21]. Oral protein and AA ingestion following an acute bout of resistance exercise creates positive NB [22]. However, with greater physiological stress comes a greater attenuation of the anabolic stimulus to ingested protein/AA, even when given in abundance [23]. When the physiological stress results in skeletal muscle anabolic resistance, nutrition alone is generally not able to match the body's requirement for AA. In this regard, elevated rates of MPB are required to provide the needed precursor AA for splanchnic (acute-phase), immunological, and wound healing protein synthesis (Figure 1) [4]. While this counter-regulatory response of muscle protein is beneficial to survival in the short term, a prolonged catabolism of muscle protein leads to continued muscle mass loss. The challenge is to develop strategies that counter this physiological phenomenon.

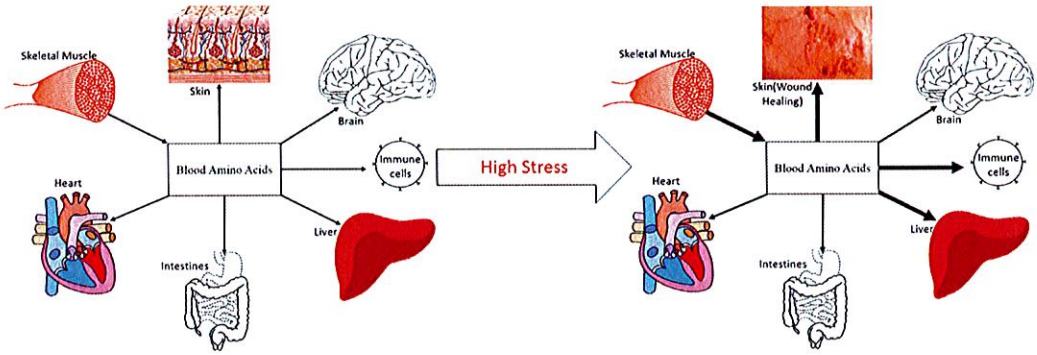

Figure 1. High stress induces muscle catabolism, resulting in the release of amino acids into circulation to be used for higher priority survival processes (representative example; not an exhaustive list; not drawn to scale).

4. Amelioration of Muscle Loss in Clinical Stress

In a stressed state such as critical illness, burn, severe injury, and major surgery, the energy requirement to accommodate the resulting hypermetabolism is reasonable. In studies in severely burned (growing) children, measurement of the total energy expenditure with doubly-labelled water indicated only an 18% increase above resting energy expenditure [24]. The delivery of a larger number of kilocalories to patients via parenteral and enteral nutrition does little to ameliorate the loss of muscle mass. In fact, it can be counterproductive and, perhaps, harmful, as the delivery of large amounts of energy can contribute to lipid accumulation in the liver [25]. This suggests other factors which accompany the hypermetabolic response are responsible for the loss in muscle mass, namely the increased whole-body requirement for AA, which are derived from muscle (Figure 1). The body's AA

requirements for acute phase protein synthesis and the synthesis of proteins related to immune function and wound healing are so great that these patients are in a catabolic state despite constant enteral feeding [23]. In fact, MPB in a postprandial burn patient is more than twice that of a fasted, healthy subject [16]. Muscle protein turnover is influenced by hormonal stimuli in a healthy person, a process which serves to rebuild and replace proteins to preserve muscle function and protein homeostasis. During periods of substantial physiological stress, the accompanying neuroendocrine changes are directed towards survival. The hormonal environment in burn patients is characterized by marked hypercortisolemia and hypoandrogenemia [23]. The combination of prolonged elevations in cortisol and the absence of the anabolic influence from testosterone, growth hormone, and insulin, serves as a persistent signal for MPB. The persistent negative NB not only occurs in the post-absorptive state, but also in response to exogenous AA [23]. These physiological alterations are not unique to burn injury, as other clinical stressors, such as sepsis, lead to an increased rate of whole-body protein catabolism, regardless of the nutritional support status [26]. Therefore, despite the presence of an abundance of AAs in circulation, their inward transport into skeletal muscle is compromised and not sufficient to offset the negative NB across muscle. In this circumstance, it may be difficult to restore NB without restoration of the normal anabolic hormonal influence. A variety of anabolic agents have been investigated in burn patients, and it has been demonstrated that the administration and/or normalization of testosterone and/or its oral analogue, oxandrolone, growth hormone (GH), insulin, insulin-like growth factor (IGF)-1, and IGF-1 plus IGF-1 binding protein-3 (IGFBP3), will all improve muscle NB and mitigate the efflux of muscle amino acids [4]. Not surprisingly, these hormones have been shown to be altered by SUSOPS and military training [5,6,10,27], indicating a systemic amelioration of anabolic influence on skeletal muscle with increasing physiological stress. Despite the clinical success of the restoration and/or normalization of various hormonal effects, testosterone administration remains the most pragmatic, safe, and logistically feasible paradigm for consideration in military personnel.

5. Applications to Military Personnel

Studies in military personnel conducting multi-stressor SUSOSP (Figure 2) provide a unique opportunity to investigate an otherwise healthy, non-pathological physiology subjected to unavoidable stressors that disrupt metabolism and muscle and whole-body protein homeostasis. These stressors include prolonged periods of strenuous physical work, limited access to food in hot and cold conditions, and exposure to a high altitude (i.e., hypobaric hypoxia).

Independent of the environmental, physical, and nutrition-related stress, military personnel are exposed to other stressors, including sleep deprivation and significant cognitive demands that may exacerbate the consequences of any of the perturbative stressors inherent to SUSOPS. For example, one study involved a simulated reconnaissance and support mission which required SOF to stealthily infiltrate a wooded area to observe a potential weapon sale [28]. The mission required soldiers to remain undetected in a prone position for an 8-day period, during which a high degree of mission stress was maintained (presence of guard dogs and helicopters). Soldiers could only move when crawling supine to an observation point in close proximity to the resting point. The soldiers were provided with 3 liters of water and standardized field rations (3567 kcal, 84g protein, 547g carbohydrates, and 116g fat) each day [29]. Despite the provision of what appeared to be adequate food, lean mass decreased by 5%. The loss of lean mass was accompanied by a reduced performance, as the training operation produced an 8.2% decrease in the vertical jump height, a 9.2% decrease in the maximal force, a 21.9% decrease in the rate of force development, and alterations in the muscle fiber type [28,29]. Energy intake was not tracked, so an energy deficit, which alone can attenuate MPS [30], was not ruled out; however, these studies highlight that even without high energy expenditure and physical activity levels, military personnel can experience decrements in muscle mass, morphology, and function.

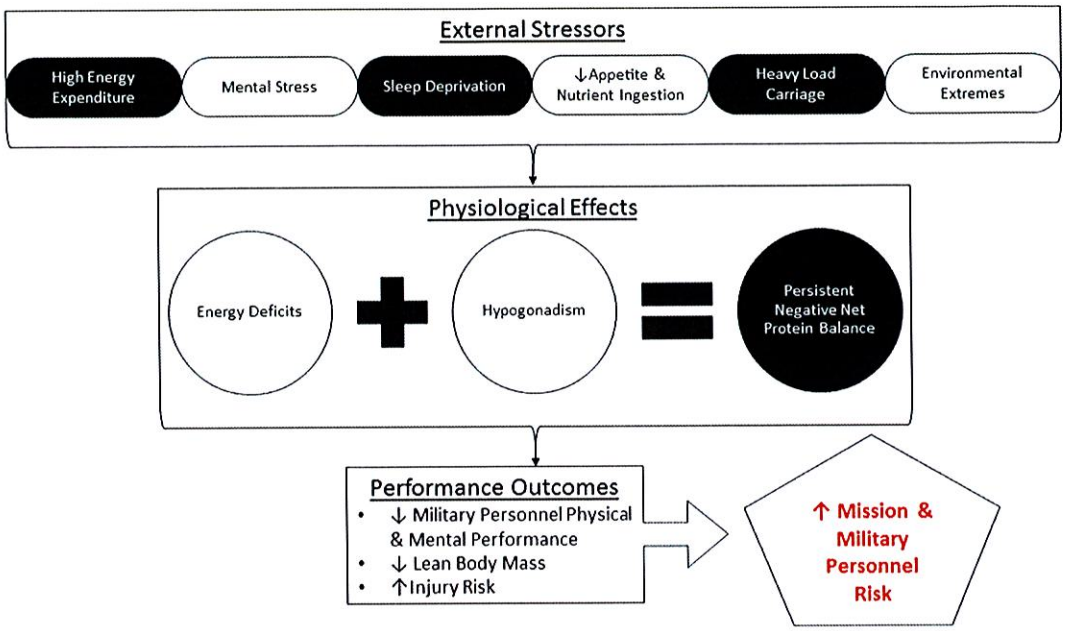

Figure 2. Potential stressors that military personnel may be exposed to during strenuous sustained training and combat operations (SUSOPS).

6. Military Operational Energy Deficits

Combat rations are designed to meet the average nutritional requirements during most operational scenarios. Energy expenditure, measured via doubly labelled water, from SOF personnel during 12 different SOF training scenarios ranged from 3700 to 6300 kcal·day^{-1} (average of ~4500 kcal·day^{-1}). These data indicate that even if the provided rations were consumed in their entirety, SOF personnel would still be in an energy deficit. Furthermore, the SOF courses that produced the highest energy expenditures also had the lowest energy intakes, and therefore, the largest energy deficits [7]. This is likely because missions with higher physical activity demands provide not only less time to eat, but less motivation to do so. Field operations place a high premium on activities that compete with eating. Tactical operations dictate that military personnel choose the mission over eating. In the US Air Force Survival Course, students consumed only 60% of the rations provided [31]. More recently, Margolis [11] demonstrated that soldiers will consume ration items containing extra protein or carbohydrate, but will compensate for the extra energy by eating less of the standard ration components. In the same study, soldiers who were able to consume enough of their rations to minimize their energy deficits to ~39% displayed a positive whole-body NB, though only 20.5% of participants were able to do so. In this regard, it is important to note that an increased protein intake is necessary to help retain body nitrogen during energy deficit [32]. Military personnel also traditionally carry large loads in the field, and this has only increased over time [33]. This often includes rations and occupational items required to carry out the mission. However, given the choice of items to carry, military personnel may be more tempted to trade food for necessities such as dry clothing [34]. As a result, military personnel often deliberately choose to "field-strip" their rations for a mission, thereby sacrificing their energy intake to improve the pack weight or to maintain essential personal or operational items [34]. Therefore, the issue of energy deficit during military operations is not only an issue of energy delivery to meet elevated energy demands, but also one of energy consumption. While the majority of these studies have been short-term, a further exacerbation of outcomes would be expected with prolonged energy deficient states.

7. Strategies to Combat Energy Deficient States

Given the prevalence of energy deficits in military personnel during SUSOPS, several strategies have been evaluated for their efficacy to reduce the resultant loss of muscle mass. Increasing dietary protein intake to double and triple the Recommended Dietary Allowance (RDA, 1.6 and 2.4 g protein·kg^{-1}·d^{-1}) has been shown to maintain the anabolic response to feeding and preserve lean mass during 21 days of moderate (40%) energy deficit when compared with protein intakes at the RDA (0.8 g protein·kg^{-1}·d^{-1}) [35]. Protein supplementation following 2 weeks of a 28–34% energy deficit was able to restore MPS to rates observed during energy balance [36], as energy deficits normally attenuate MPS [30]. The muscle protein sparing effects of higher-protein diets during energy deficient states are enhanced with the addition of resistance-type exercise. The addition of resistance exercise and high-intensity interval training to 2.4 g·kg^{-1}·d^{-1} protein intake during 4 weeks of 40% energy deficit resulted in an actual gain in lean mass in healthy young men [37]. The increase in lean mass appears to be attributed to resistance exercise, as consuming the same amount of protein in another study only attenuated lean mass loss when the exercise intervention that was primarily performed was aerobic in nature [35]. The combined stimulatory effect of dietary protein and resistance exercise during energy deficit has been demonstrated in a variety of populations [38]. Taken together, resistance exercise and high protein intakes help restore and provide the necessary anabolic stimulus to spare muscle mass during energy deficits of up to approximately 40% of the total energy requirements.

These studies have been useful; however, they assess a single stressor (energy deficits) in a controlled laboratory setting. These studies cannot replicate the influence of constant mental and physical stress during real-world military operations. The dilemma of supplying soldiers with adequate and appropriate nutrition has been recognized by military leaders for centuries [39]. The efficacy for maintaining the anabolic stimulus to muscle during a period of energy deficit appears to have a limit. When energy deficits are above 40%, high protein intakes do not appear to restore muscle anabolism to rates observed during energy balance. In this regard, AA become an oxidizable energy source to meet the energy demand [38]. This is a paramount issue for military personnel exposed to severe energy deficits (> 40% of total energy requirements) during SUSOPS. The MRE is designed to meet energy and nutrient requirements established by Army Regulation 40–25 if consumed as directed (i.e., 3 MRE per day, ~3900 kcal, 127 g protein, 507 g carbohydrate, 152 g of fat; [40,41]). However, despite the ration's design and intended use, energy deficits and a suboptimal nutrient intake, as well as their associated consequences on muscle mass and whole-body protein, are largely inevitable during strenuous SUSOPS [10–12,39,42]. To date, several strategies have been employed to prevent energy deficits during military operations, including the provision of supplemental carbohydrate or protein [11], increasing the size and energy content of certain ration components (unpublished data), and pre-operational nutrition education [8]. A recent study was conducted during a 70% energy deficit while at altitude. One group received a high-protein diet (2.0 g protein·kg^{-1}·d^{-1}), while the other was on a standard protein diet (1.0 g protein·kg^{-1}·d^{-1}), for 21 days at 4300 m. The high-protein diet group displayed a more negative post-absorptive whole body protein net balance as almost all (0.95 ± 0.32 g protein·kg^{-1}·d^{-1} more than the standard protein group) the additional protein was oxidized for energy [10]. In addition, the muscle was resistant to protein (25 g of whey protein) after aerobic exercise, as indicated by the inhibition of mammalian target or rapamycin complex 1-mediated signaling [43]. Therefore, dietary strategies to this point have not been successful. However, the lack of an apparent benefit gained by simply consuming more protein makes sense; borrowing from our clinical experience, as physiological stress increases, nutrition alone is not capable of sparing muscle mass.

For this reason, efforts have focused on optimal EAA delivery, rather than the consumption of a defined amount of protein. We have demonstrated that the EAA are the prime drivers of muscle anabolism [44,45]. Furthermore, we have also demonstrated that in anabolic resistance states, such as aging [46,47] and post-operative joint arthroplasty [48,49], adjusting the amount and/or individual ratios [47] of EAA intake can restore the anabolic response in skeletal muscle. While our current efforts are focused on optimizing EAA delivery, this aspect is part of an overall strategy to restore both the

anabolic signal and the anabolic capacity of skeletal muscle in the stressed state. Little is known about the EAA requirement and optimal delivery format required to overcome the large hypocaloric state experienced by military personnel during field and combat situations. Whey protein [10,43,50] has been utilized in the past in hopes of ameliorating body protein losses; however, the energy deficit was such that protein/amino acid intake was directed towards energy production, rather than protein turnover. For this reason, current investigations are focused on determining the optimal EAA requirement for skeletal muscle and whole body protein during energy deficit.

8. Restoration of Hormonal Influence

The inability of nutrition to preserve muscle mass in military personnel on its own is similar to the anabolic resistance displayed in severe burn patients receiving enteral nutrition [16,51]. Like burn patients, military personnel demonstrate an inhibition of endogenous testosterone production and a demonstrated hypercortisolemia [5,6,10,27], though to a lesser degree. Interestingly, the restoration of testosterone concentrations [4] has improved NB in severe burn patients. Testosterone has been shown to maintain muscle mass through two primary mechanisms: 1) reinstating the anabolic response of muscle to AA and 2) improving the protein synthetic efficiency in the post-absorptive state [23,52–56]. As previously mentioned, MPS in the burn patient does not respond to feeding [16,51]. However, the normalization of testosterone concentrations in adults and the use of oxandrolone in children restored the anabolic effects of enteral nutrition [4]. The return of the anabolic stimulus from feeding has also been demonstrated with insulin, IGF-1, and GH, where the stimulation of MPS is closely related to an increased rate of inward AA transport [4,57]. Therefore, the systematic administration of these hormones can only stimulate MPS if AA are present [56]. In contrast, mitigation of the hypogonadal state with exogenous testosterone has the advantage of increasing the reutilization of intracellular AA, thereby improving the protein synthesis efficiency [23,52,55,56]. In other words, for a given quantity of muscle intracellular AA (particularly the EAA), testosterone normalization directs them toward the synthetic process, rather than outward transport for utilization elsewhere. This has been shown to result in an improvement in muscle NB [54,55]. This mechanism of action may be advantageous for military personnel often exposed to conditions that elicit a severe energy deficit and hypogonadism [5,58]. It is plausible that the restoration of a eugonadal state would route MPB-derived AA back into muscle protein (via improved synthetic efficiency), rather than releasing them into the blood. Therefore, hormonal normalization provides a potential pharmacological strategy for military personnel to attenuate muscle mass and, importantly, functional declines. Combined with an optimal nutrition strategy, restoration of the anabolic signal may restore the effects of EAA on skeletal muscle.

9. Preliminary Investigations Utilizing Testosterone

Efforts are currently underway to investigate the utilization of testosterone in military personnel exposed to strenuous SUSOPS [59]. We suspect that given their habitual energy deficient and hypogonadal state during SUSOPS, testosterone administration, based upon our clinical findings, would restore the anabolic response derived from protein/AA intake. In particular, we would hypothesize that the improved synthetic efficiency in muscle will mitigate the loss of AA in the fasted state. Additionally, the restoration of anabolic influence by returning to a eugonadal state will restore the anabolic effects of protein/AA on skeletal muscle. Efforts involve both the administration of testosterone enanthate every 7 days, as well as the use of other, long-term testosterone esters that can maintain testosterone concentrations within the normal physiological range for 8–10 weeks [60]. Both administration paradigms may have logistical and physiological merit for use in the context of multi-stressor SUSOPS.

10. Summary

Although the present review focused on the responses in men, the number of women involved in combat roles has continued to increase. At present, there is a dearth of work delineating the

effects of military operations on women. Future research is needed to determine the physiological and metabolic responses of women to the cumulative stress of SUSPOS, and to what extent these responses differ from men. Nonetheless, current evidence indicates that military personnel are, at times, exposed to multi-stressor environments for sustained periods that can decrease muscle and whole-body protein [5,6,9–12,33,59]. Borrowing from clinical stress examples, greater physiological stress is associated with an absence of anabolic influence on muscle, and further, the resistance of muscle to the effects of nutrition/protein. In order to restore the anabolic influence of AA, hormonal signaling must be restored and/or enhanced, potentially providing military personnel with a feasible strategy to maintain muscle mass and function during SUSOPS. While the restoration or enhancement of most hormonal signals in burn patients improves muscle NB with nutrition, testosterone administration is particularly noteworthy due to its economic, logistic, and metabolic advantages. In this regard, we propose the exploration of potential pharmacological solutions that would restore the anabolic potential in muscle and, when combined with a targeted nutritional strategy, mitigate the loss of muscle protein and function in military personnel in response to multi-stressor SUSOPS.

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Conflicts of Interest: Drs. Ferrando and Wolfe are co-inventors of an EAA formula which is patented by UAMS: Essential Amino Acid Supplementation for Recovery of Muscle Strength and Function during Rehabilitation. US Patent (9,364,463 B2; UAMS).

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nutrients

#2 Appendix

MDPI

Review

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

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Abstract: Protein intake recommendations to optimally stimulate muscle protein synthesis (MPS) are derived from dose-response studies examining the stimulatory effects of isolated intact proteins (e.g., whey, egg) on MPS in healthy individuals during energy balance. Those recommendations may not be adequate during periods of physiological stress, specifically the catabolic stress induced by energy deficit. Providing supplemental intact protein (20–25 g whey protein, 0.25–0.3 g protein/kg per meal) during strenuous military operations that elicit severe energy deficit does not stimulate MPS-associated anabolic signaling or attenuate lean mass loss. This occurs likely because a greater proportion of the dietary amino acids consumed are targeted for energy-yielding pathways, whole-body protein synthesis, and other whole-body essential amino acid (EAA)-requiring processes than the proportion targeted for MPS. Protein feeding formats that provide sufficient energy to offset whole-body energy and protein-requiring demands during energy deficit and leverage EAA content, digestion, and absorption kinetics may optimize MPS under these conditions. Understanding the effects of protein feeding format-driven alterations in EAA availability and subsequent changes in MPS and whole-body protein turnover is required to design feeding strategies that mitigate the catabolic effects of energy deficit. In this manuscript, we review the effects, advantages, disadvantages, and knowledge gaps pertaining to supplemental free-form EAA, intact protein, and protein-containing mixed meal ingestion on MPS. We discuss the fundamental role of whole-body protein balance and highlight the importance of comprehensively assessing whole-body and muscle protein kinetics when evaluating the anabolic potential of varying protein feeding formats during energy deficit.

Keywords: essential amino acids; protein; meal format; muscle protein synthesis; whole-body protein balance

1. Introduction

The dose-dependent relationship between supplemental dietary protein and resting and post-resistance exercise skeletal muscle protein synthesis (MPS) is well described [1–5]. Moore et al. [4] reported that post-resistance exercise MPS demonstrates saturation kinetics and plateaus after ingesting a 20 g solution of whole-egg protein, as compared to 0, 5, 10, and 40 g in healthy young adults.

Witard et al. [5] also demonstrated that, in healthy young adults, ingesting a 20 g solution of whey protein maximally stimulates resting and post-resistance exercise MPS, while ingesting 40 g increases protein oxidation and ureagenesis, without an additional anabolic stimulus. Similar work has been conducted in middle-aged adults [6], as well as older adults with anabolic resistance [7–10]. These well-designed dose-response studies clearly demonstrate that consuming 0.25–0.3 g and 0.4 g of protein/kg per dose maximally stimulates MPS in young and older adults, respectively [4,5,7]. As a result, these doses are promoted as effective post-exercise dietary strategies that optimize muscle adaptations to exercise when coupled with habitual protein intakes nearly twice the recommended dietary allowance [11–13]. Whether these recommendations derived from studies in healthy individuals during energy balance are adequate for individuals undergoing physiological stressors, including the catabolic stress of energy deficit, remains in question. Our work suggests these protein doses are not effective for mitigating lean mass (muscle) loss during military training that often results from energy deficit-mediated changes in MPS. We demonstrated no apparent stimulatory effect of feeding with 25 g of whey protein on MPS-associated anabolic signaling in individuals exposed to high altitudes (4300 m), before and after 22 days of acclimatization and severe energy deficit (40% energy deficit, −1766 kcal/day of energy balance) [14]. We also found no effect of providing four 20 g whey protein supplements per day on whole-body protein retention during a multi-day strenuous winter military training exercise that elicited a severe energy deficit (54% energy deficit, −3313 kcal/day of energy balance) [15]. These studies highlight the stressors and protein metabolism-related consequences routinely experienced by military personnel during strenuous training or combat operations, including high-energy flux, limited food availability, severe energy deficit, and, in some cases, environmental extremes. Establishing protein intake recommendations specific to scenarios that include energy deficit-induced catabolic stress may also benefit other healthy, primarily lean, populations that frequently perform under these conditions, such as weight-class sports athletes, ultra-endurance athletes, and wildland firefighters.

Several factors contribute to energy deficit-induced lean mass loss, including downregulated MPS [16–18], blunted MPS-associated anabolic signaling responses to feeding [14], and increased whole-body protein oxidation [19–21]. The downregulation of postabsorptive and postprandial MPS is further exacerbated during severe energy deficit because dietary protein is used as a readily available, oxidizable energy source to meet increased whole-body energy demands [14,22]. While recommendations based on MPS responses provide valuable guidance for prioritizing muscle mass accretion and maintenance in healthy individuals during energy balance, they may overlook the potential benefit of protein intake on whole-body protein balance (whole-body protein synthesis—whole-body protein breakdown) during energy deficit. Feeding strategies that optimize the effects of protein on both whole-body protein balance and MPS are needed to mitigate or attenuate lean mass loss during sustained periods of moderate to severe energy deficit.

Certain considerations should be acknowledged when identifying best practices to leverage the anabolic properties of dietary protein. First, the aforementioned dose-response studies, and a significant portion of the work on muscle protein turnover, only assess the effects of isolated intact proteins on MPS and do not evaluate other amino acid containing feeding formats. This is an important consideration, since peripheral essential amino acid (EAA) concentrations that directly stimulate MPS [23–25] are influenced by the feeding format. Therefore, eating varying formats such as supplemental free-form EAA, supplemental intact protein, or protein-containing mixed meals dictates postprandial peripheral EAA concentrations and MPS. Second, initial work suggests that the MPS saturation point following isolated intact protein feeding in healthy individuals is similar during energy balance and moderate energy deficit conditions. However, whether the MPS saturation point changes as the magnitude of energy deficit increases remains unknown [26]. Lastly, the studies used to inform current recommendations do not consider enhancing whole-body protein balance as a marker of optimal protein feeding amounts. An understanding of how feeding formats and related alterations in circulating EAA concentrations impact MPS and whole-body protein balance is required in order to expand the current recommendations and design feeding strategies for periods of energy-mediated

catabolic stress. While several recent, well-written reviews [27–30] cover similar concepts, including protein quantity and quality, we sought to review and highlight specific format considerations and relate them to individuals undergoing energy deficit-induced stress. The objectives of this review are to summarize what is known regarding the effects of supplemental free-form EAA, supplemental intact protein, and protein-containing mixed meals on MPS during energy balance and present the importance of simultaneous measures of MPS and whole-body protein balance during energy deficit.

2. Considerations for the Application of MPS Measures

MPS is most commonly assessed using the precursor-product model, which involves the injection or infusion of amino acid tracers (precursor) and measuring their direct incorporation into muscle tissue (product) [31]. The fractional synthetic rate, or the rate of incorporation into the product, is calculated by dividing the rate of increase in product by the average precursor enrichment over the incorporation period, and is used to report acute changes (i.e., over several hours) in MPS [31]. It is critical to understand that MPS measures reflect only one aspect of muscle protein turnover and do not reflect protein anabolism without a concurrent measure of muscle protein breakdown. Ideally, muscle protein breakdown and MPS would be measured simultaneously to attain the net muscle protein balance. However, muscle protein breakdown measures present methodological challenges and accuracy concerns, especially during non-steady states (i.e., acute feeding and exercise) [32]. In addition, whether acute increases in MPS directly translate to long-term increases in muscle mass has also been questioned [33]. While some studies have demonstrated a good qualitative agreement between acute MPS measures and longer-term measures of hypertrophy-related outcomes [34–36], others have not [37,38]. The variance in agreement is attributed to the timing, frequency, and duration of measures, exercise type, training status, training protocol, and nutrition status (to include acute and habitual dietary protein/EAA intake). Beyond hypertrophy, MPS also serves to enable skeletal muscle adaptation to exercise through repair and remodeling, particularly after resistance exercise [39]. In this regard, MPS may be elevated as a response to exercise-induced muscle damage, but may not result in hypertrophy. While important, further discussion of these variables is beyond the scope of this review, and we direct the readers to other publications [33,40,41].

There has been a significant reliance on MPS measures alone to assess protein feeding responses due to the notion that MPS is more responsive and constitutes a greater contributing factor to muscle protein balance than muscle protein breakdown [32]. Although generally accurate during energy balance, this perspective may be problematic when translated to studies of severe energy deficit and other catabolic conditions. In some catabolic conditions, such as burn injuries, MPS is generally elevated due to increases in protein breakdown-derived intracellular amino acid availability [42]. Characterizing anabolism based solely on MPS in this scenario suggests that muscle protein is gained when it is actually lost due to greater elevations in breakdown than synthesis and the inefficient intracellular recycling of amino acids as a result of efflux and oxidation [43]. Although a valuable tool, there is also an inherent variability in MPS measures due to the stable-isotope methodologies employed (reviewed elsewhere [31,44–46]), study design, and magnitude of individual responses. These issues combine to increase the difficulty of direct comparisons from study to study and between laboratories [46]. Lastly, 40% of total-body protein is comprised of skeletal muscle, thus it is estimated that 33–50% of whole-body protein turnover is attributable to muscle tissue; however, the environmental context may alter the contribution [47]. Therefore, concurrent measures of MPS and whole-body protein turnover may provide a more holistic understanding of how feeding interventions impact body protein status.

3. Supplemental EAA and Protein Feeding Format Effects on MPS

Amino acid feeding formats, which include supplemental free-form amino acid mixtures, supplemental isolated intact proteins (e.g., whey protein), whole-food proteins, and protein-containing mixed meals (Figure 1), differentially stimulate MPS. The varying effects of these formats on MPS are driven by the EAA content, digestion, and absorption kinetics of the particular format. These aspects,

in turn, influence postprandial EAA concentrations, which are primarily responsible for stimulating MPS (Figure 2). Orally consumed free-form EAA do not require digestion, so they are absorbed and enter peripheral circulation rapidly, resulting in robust increases in EAA concentrations [48]. In contrast, isolated intact proteins, whole-food proteins, and protein-containing mixed meals must be digested before amino acid absorption. The co-ingestion of other nutrients may also influence their digestion and absorption, resulting in variations in postprandial EAA concentrations. Very few studies have directly compared the effects of supplemental free-form EAA, supplemental isolated intact proteins, whole-food proteins, or protein-containing mixed meals on postprandial EAA concentrations and MPS, though they have been independently studied in great detail.

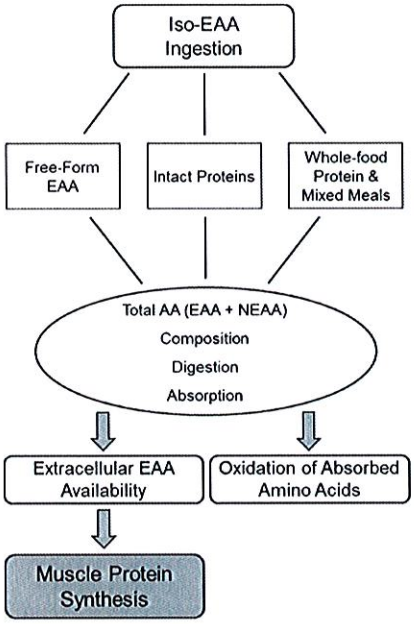

Figure 1. Factors moderating the effects of essential amino acids (EAA) on muscle protein synthesis.

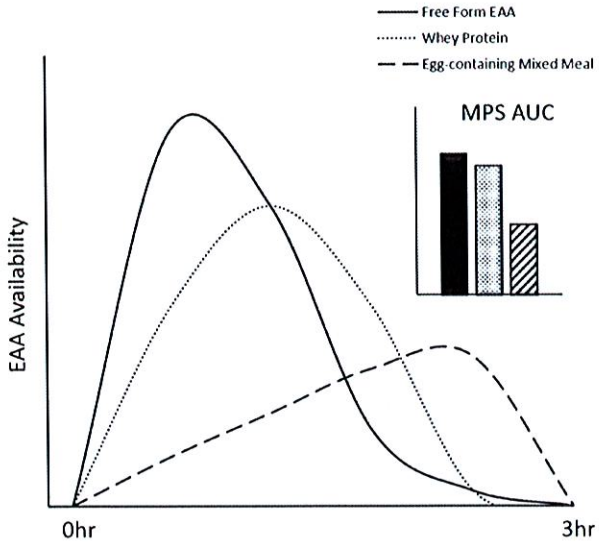

Figure 2. Theoretical comparison of magnitude changes in muscle protein synthesis (MPS) in response to the ingestion of supplemental free-form essential amino acids (EAA), whey protein, and a protein-containing mixed meal.

3.1. Total Amino Acid Content and Composition

The quantity and composition of EAA varies considerably between supplemental free-form EAA, supplemental intact proteins, and protein-containing mixed meals (i.e., free-form EAA mixtures, dairy, plant, non-dairy animal proteins; Figure 3). This influences the degree to which MPS is stimulated, as there is a dose-dependent relationship between the EAA quantity ingested, postprandial EAA concentrations in circulation, and MPS [4,5]. For this reason, feeding formats that contain higher EAA quantities per volume (i.e., a higher EAA density) may be consumed in smaller amounts than formats with lower EAA quantities (i.e., a lower EAA density), while achieving similar postprandial increases in EAA concentrations and MPS [49]. Beyond absolute quantity, the differences in EAA composition also influence circulating EAA concentrations.

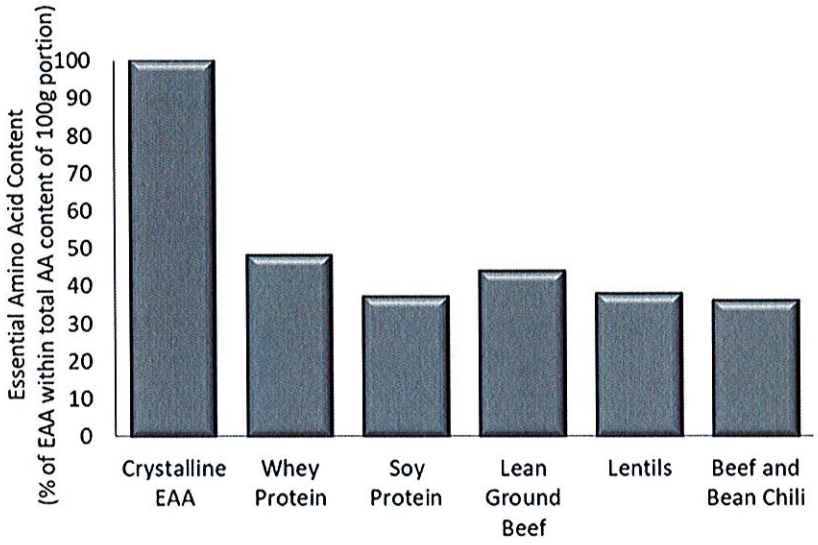

Figure 3. Relative essential amino acid content of free-form essential amino acids, various intact proteins, and a protein-containing mixed meal.

Regarding isolated intact proteins, Tang et al. [50] used a unilateral leg-resistance exercise model to compare resting and post-exercise MPS after ingesting 22 g of whey, casein, and soy protein. The treatments were iso-EAA (10 g), but differed in EAA composition (whey: 2.3 g leucine; casein: 1.8 g leucine; soy: 1.8 g leucine). The EAA concentrations 30 min post-ingestion were 80% higher than the postabsorptive concentrations after ingesting whey protein. The postprandial increase after ingesting whey was greater than that of soy (30%) and casein (16%), which were the same. After 60 min, the postprandial EAA concentrations continued to increase, and were greatest after ingesting whey (42%), followed by soy (30%), and then casein (14%). Resting MPS measured 3 h post-ingestion was the same after ingesting whey and soy, but both were greater than casein. However, post-exercise MPS was greatest after ingesting whey, followed by soy and then casein. These findings highlight the interactive effects of EAA composition, digestion, and absorption kinetics on MPS across different protein sources within a given protein/EAA format (i.e., isolated intact protein) that provides comparable amounts of EAA.

Blending isolated intact proteins may be one method to optimize MPS by leveraging variations in protein digestion and absorption kinetics. Reidy et al. [51] compared the effects of consuming whey (18 g protein; ~9 g EAA, ~1.9 g leucine) or an iso-nitrogenous soy-whey-casein blend (19 g protein; ~9 g EAA, ~1.8 g leucine) 1 h following exercise completion on post-exercise MPS. Whey protein elicited higher and earlier peak postprandial EAA concentrations, whereas the blended protein led to a delayed, gradual, and sustained increase in the EAA concentrations. Despite these differences,

both the treatments produced a >50% increase in the EAA concentrations over the 5 h post-exercise period. The MPS rates mimicked the EAA concentrations, such that MPS was the same between treatments across the entire 5 h period. Interestingly, the MPS rates in the latter 2–4 h period were only increased above rest by the blended protein. These data suggest that there are temporal differences in the postprandial EAA kinetics and MPS responses between single-source, isolated intact proteins and blended isolated intact proteins. The same laboratory conducted a follow-up study [52] in older men. While the total protein quantity was increased (30 g protein), the findings were the same, such that the increases in EAA concentrations and MPS were similarly elevated following whey or protein blend ingestion. Overall, these data support that similar increases in postprandial EAA concentrations stimulate and sustain MPS to a similar extent. These data may also support the co-ingestion of whole-food proteins (i.e., milk and eggs, beef and beans) as a practical way to achieve optimal EAA intakes.

The composition of amino acids provided in supplemental free-form EAA mixtures may vary to the same extent as (or more so than) the EAA compositions in intact proteins. The wide variation is almost certainly the result of intentions to develop the best combination of free-form amino acids to support MPS. For example, Glynn et al. [53] sought to determine whether manipulating the leucine content (1.8 (i.e., consistent with the proportion in whey protein) vs. 3.5 g leucine) within 10 g of free-form EAA had any greater benefit for stimulating resting MPS in young adults. The EAA mixtures produced similar postprandial EAA concentrations, which was in agreement with similar changes in MPS, although MPS was stimulated to a greater extent at 60 min by the mixture with the higher leucine content. These findings suggest that 10 g of EAA with a leucine content of 1.8 g is sufficient to achieve a maximal MPS response in young healthy adults at rest.

In addition, increasing the total EAA content of a feeding format by enrichment with one or more additional free-form EAA may augment MPS. Adding EAA to a low dose of whey protein (10 g) or to a protein source with lower EAA content (soy protein relative to whey protein [54]) may stimulate MPS to the same extent as a standard dose of whey protein (20 g). The enrichment of low-dose intact protein ensures an adequate amount of EAA to increase the EAA concentrations required to stimulate and sustain MPS. Churchward-Venne et al. [55] examined the effects of consuming low-dose, leucine-enriched whey (8.4 g protein, 5.14 g EAA, 3 g total leucine), low-dose, EAA-enriched whey (12.55 g protein, 9.29 g EAA, 0.75 g total leucine), or 25 g of whey (25 g protein, 11.54 g EAA, 3 g total leucine) on resting and post-resistance exercise MPS. Postprandial EAA concentrations followed similar patterns for the leucine-enriched whey and the EAA-enriched whey, which increased to peak concentrations by 1 h (both greater than ~75% above basal). In contrast, the 25 g whey led to a greater EAA peak (greater than ~90% above basal) from 1.3–2 h versus the other two groups. Resting and post-exercise 3h MPS increased similarly across all groups. However, post-exercise 3–5 h MPS remained elevated following 25 g whey, but not in the other groups, regardless of condition. The greater post-exercise 3–5 h MPS in the 25 g whey group may be attributed to the total circulating EAA concentrations, 30 min delay in the peak concentrations, and total amino acid quantity ingested. This study supports several key concepts. First, stimulating MPS over a 1–3 h postprandial period may be achieved by enriching lower quantities of isolated intact proteins with EAA, such that postprandial EAA concentrations are at least 50% greater than postabsorptive concentrations. Second, it seems that more total protein supports a sustained stimulation of MPS, particularly after exercise.

3.2. Digestion and Absorption

The protein digestion and absorption rates of intact proteins encompass the rate at which proteins are catabolized to peptides and free amino acids and the partitioning of these amino acids between the splanchnic and peripheral tissues [56]. Proteins can be broadly classified as fast (i.e., rapid digestion and absorption) or slow (i.e., prolonged digestion and absorption) based upon the peripheral EAA appearance, regardless of the amino acid composition [56]. Therefore, ingesting either fast or slow intact proteins or manipulating the timing of their ingestion may be methods to optimize postprandial

EAA concentrations and MPS. One approach to examine the impact of digestion and absorption on postprandial EAA concentrations and subsequent MPS, while controlling for differences between amino acid compositions, is to administer the same quantity of protein in varying portions at differing time points. Areta et al. [57] examined the effects of consuming 80 g of whey (~38 g EAA) as two large boluses (i.e., 40 g whey, ~19 g EAA), four intermediate pulses (i.e., 20 g whey, ~10 g EAA), or eight small pulses (i.e., 10 g whey, ~5 g EAA) across 12 h on EAA concentrations and MPS. The EAA concentrations in the first 6 h of recovery (~19 g of EAA delivered in all treatments) did not differ from the baseline after the intermediate and small pulse treatments. However, the bolus treatment increased EAA concentrations by 135%. During the final 6 h of recovery, EAA concentrations increased by greater than 50% across the treatments. Overall, the bolus treatment lead to a greater peak in EAA concentrations (~140%) compared to the intermittent (~66%) and small pulse treatments (~80%). The intermediate treatment only increased the EAA concentrations from 6–7 h. In contrast, the delayed increases in EAA concentrations following the small-pulse treatment were sustained throughout the 12 h. Regardless of the differences in circulating EAA, 4 h MPS increased similarly across treatments. However, between 4–6 h and 6–12 h, MPS was greater following the intermediate versus the small pulse and bolus treatments. The differences between the EAA concentrations and MPS in this study are in line with the concepts of an EAA concentration threshold and saturation point for stimulating MPS, such that any further provision of EAA has no further stimulatory effect [58,59]. These data suggest that the EAA concentrations following the small pulses did not reach the threshold to maximally stimulate MPS, and that the EAA quantities in the bolus perhaps saturated the MPS stimulatory mechanism. These findings support that feeding formats which augment MPS under energy balance conditions are those that provide adequate EAA (i.e., 20 g whey, ~10 g EAA) and have fast to moderate digestion and absorption kinetics.

The digestion and absorption kinetics of whole-food protein ingestion are also altered by the food form (solid versus liquid) and food matrix (non-amino acid components). A study by Burd et al. [60] examined the post-resistance exercise effects of beef versus milk (30 g protein and 13 g EAA) on MPS. Circulating phenylalanine concentrations were greater following beef versus milk from 1–2.5 h, while leucine concentrations rapidly increased after the milk (40%) and were greater than the beef (10%) 30 min post-ingestion. Yet, the beef led to greater peak and overall leucine concentrations (> 100%) from 1.5–2 h. The 2 h MPS was higher following the milk compared to the beef, whereas 5 h MPS was equal between foods. These data indicate that temporal differences in circulating EAA concentrations and MPS exist between whole-food proteins.

4. The Importance of Measuring MPS and Whole-Body Protein Turnover Together

The determination of optimal protein feeding strategies have been largely based on conventional myo-centric studies that only quantify MPS. This approach is suitable when the primary goal of protein feeding is to support muscle-specific adaptations. However, the use of MPS for evaluating an optimal feeding response fails to account for any further benefit on whole-body protein balance once MPS is saturated. EAA that remain in circulation after MPS is maximally stimulated may still be used to stimulate protein synthesis elsewhere in the body and reduce the reliance on precursor amino acids derived from energy deficit-induced upregulations in protein breakdown to support synthesis. The combination of increased whole-body protein synthesis and/or decreased whole-body protein breakdown is greater whole-body protein balance. Recent work from our laboratory highlights the value of simultaneous MPS and whole-body protein balance measures (62). Moreover, examining both muscle and whole-body measures is particularly important when developing recommendations for protein intake during catabolic stress, because MPS is not a priority amino acid-requiring process under these conditions. In contrast, life-sustaining processes, including acute-phase protein synthesis, immune function, wound healing, and energy production, are prioritized and upregulated during catabolic stress. Since skeletal muscle is the only labile amino acid source, an efflux of amino acids will be directed towards these important processes during this condition. There is also a greater

dependence on whole-body protein breakdown to supply the amino acid precursors needed to sustain these processes [42,61]. Thus, an attenuation of exacerbated protein breakdown is a principal goal of protein intake during catabolic stress.

Although several methodologies may be used to assess whole-body protein balance, our work compares the feeding effects of protein and free-form EAA feeding formats using the continuous infusion of EAA tracers. For a more detailed discussion of this method and others, we direct the readers to recent reviews [43,62]. The utility of whole-body protein turnover measures may be considered limited by the inability to determine the effects of feeding or training interventions on specific tissues. While technically correct, whole-body protein turnover measures do provide a holistic assessment of systemic changes in multiple body protein pools (i.e., skeletal muscle, organ, and splanchnic proteins), which is useful when assessing whether nutrition interventions meet total-body protein requirements. Perhaps most importantly, food is consumed at the whole-body level, and all nutritional guidelines have been based on whole-body responses (mainly nitrogen balance). Whole-body protein kinetics can be determined more rapidly than nitrogen balance, and can be performed using an experimental design that allows individuals to be used as their own control.

As previously stated, skeletal muscle contributes 40% of total-body protein and comprises ~33–50% of whole-body turnover measures [47]. It is important to consider how fluctuations in MPS are related to whole-body protein turnover and whether this relationship is moderated by exercise type. This is because the type and intensity of exercise stimulus dictates the MPS and hypertrophic response [63], and therefore may change the contribution of MPS to changes in whole-body protein turnover. To our knowledge, no studies have directly compared the effects of exercise type on the relationship between MPS and whole-body protein turnover. However, Koopman et al. [64], reported a correlation between post-resistance exercise MPS and whole-body protein balance following the ingestion of carbohydrate; carbohydrate and whey; or carbohydrate, whey, and leucine. Other work [8] from this laboratory also demonstrates a general agreement between the dose-dependent increases in post-resistance exercise MPS and increases in whole-body protein balance following protein ingestion. There also seems to be agreement between increases in MPS and increases in whole-body protein synthesis following endurance exercise [65]. Further investigation directly comparing this relationship is warranted and should emphasize a number of contributing factors, including training status, intensity, duration, age, and nutritional status.

The same format-related considerations for achieving the optimal quantity, composition, digestion, and absorption of protein and/or EAA are relevant for optimizing whole-body protein balance. For example, one of our studies [66] showed that consuming a mixed meal providing 70 g protein (~32 g EAA) results in greater whole-body protein balance compared to one providing 40 g of protein (~18 g EAA), despite comparable MPS rates. The enhanced whole-body protein balance was attributed to both the suppression of protein breakdown and the stimulation of protein synthesis. In addition to the quantity of protein/EAA, the composition of EAA within a meal format also has implications for enhancing whole-body protein balance. We recently demonstrated [67] the effects of consuming iso-nitrogenous, iso-caloric egg-based (26 g protein, ~10 g EAA), or cereal-based (26 g protein, ~7 EAA) mixed meals on whole-body protein turnover and MPS. Importantly, while iso-nitrogenous and iso-caloric, this was a comparison of whole-foods within formats typically consumed in the "real world". EAA concentrations were greater following the egg-based meal versus the cereal-based meal. Whole-body protein balance was also greater following the egg-based meal compared to the cereal-based meal and primarily due to a greater suppression of protein breakdown. MPS did not differ between the formats. These data indicate that protein-containing mixed meals with differing EAA compositions are capable of eliciting differences in whole-body protein balance, despite delivering a similar protein quantity as well as comparable MPS responses. Lastly, EAA-enriched intact protein formats may be a promising approach for enhancing whole-body protein balance and provide benefits beyond achieving optimal MPS. Our laboratory compared whole-body protein turnover and MPS following ingestion of low-dose EAA-enriched whey (5.6 g protein, 3.2g free-form EAA), high-dose

EAA-enriched whey (11.2 g protein, 6.4 g free-form EAA) and a commercially available whey-based supplement (12.6 g protein, 2.4 g EAA) [68]. Whole-body protein balance was greater following the high-dose EAA-enriched whey versus the other treatments due to a greater suppression of protein breakdown. Only the high-dose EAA-enriched whey increased MPS above basal values. While the increased MPS response is in agreement with the greater whole-body protein balance, the magnitude of these responses indicated the treatment led to greater benefit at the whole-body level. That is, if only the MPS response was evaluated to determine the efficacy of the high-dose EAA-enriched whey versus the other treatments, then it would appear rather modest. Whereas, when the MPS and whole-body protein balance responses are examined simultaneously, there is a robust feeding effect, clearly indicating the benefit of the high-dose EAA-enriched whey in supporting protein kinetics compared to the other treatments. Together, these data support the inclusion of simultaneous MPS and whole-body protein balance measures to assess the efficacy of feeding formats to support total-body protein status and that varying formats moderate feeding effects.

5. Maximizing MPS and Whole-Body Protein Status during Energy Deficit

Negative energy balance generally results in muscle mass loss, the extent of which corresponds to the degree and duration of the deficit incurred [69,70]. This relationship is concerning for healthy, non-obese individuals who have a greater proportion of body mass derived from fat-free mass. Military personnel are one example of an at-risk population that undergoes frequent and repeated exposures to energy deficits in both training and operational environments. Nutritional countermeasures to attenuate muscle mass loss and subsequent decrements in health and physical performance are crucial. There is general consensus that providing more than the recommended dietary allowance for protein (0.8 g/kg body mass/day) can counteract the detrimental effects of energy deficit on lean mass. Controlled laboratory studies consistently show that increasing dietary protein intake (1.6–2.4 g/kg body mass/day) during moderate energy deficit (≤ 40% total daily energy requirements) prevents lean mass loss by restoring MPS rates to those observed during energy balance and maintaining postprandial anabolic sensitivity to high-quality, protein-containing meals [26]. As mentioned, our recent work [15,71] demonstrates that a single dose of whey protein within the recommended amount, 20–25 g, is inadequate to support muscle mass during conditions when energy deficit is severe. The apparent diverging effects of higher protein feeding during moderate and severe energy deficit suggest that the magnitude of energy deficit is an important moderator of whether dietary protein is used to support protein synthesis and spare lean mass, or whether it is diverted towards oxidative energy metabolism.

There are limited studies examining MPS following acute supplemental free-form EAA or intact protein feeding in healthy populations exposed to energy deficit. The studies that have been conducted in our laboratory [16,72,73], and others [17,18,74–76] have largely focused on the interactions of energy deficit (i.e., typical weight loss or unavoidable, moderate to severe energy deficit) and supplemental free-form EAA or intact protein feeding on MPS. To our knowledge, only two have assessed acute MPS in response to varying quantities of supplemental free-form EAA or intact protein during moderate energy deficit [18,72]. Areta et al. [18] examined post-resistance exercise MPS after ingestion of 15 g (~7.5 g EAA) and 30 g (~15 g EAA) of whey protein. There was a dose-dependent increase in MPS; however, this increase only equated to the postabsorptive rates commonly observed during energy balance. Since quantities greater than 30 g of whey protein (15 g of EAA) were not included, the maximal stimulation of MPS could not be determined. Recent work [72] from our laboratory demonstrated that post-resistance exercise MPS was not further stimulated by ingesting 24 g (0.3 g/kg body weight) of free-form EAA when compared to 8 g (0.1 g/kg body weight). The discrepancies in MPS response between these two studies may be related to differences between the effects of whey protein and free-form EAA on MPS. For example, Areta et al. provided an intact protein which delivers both essential and non-essential amino acids and requires digestion before absorption, whereas we only provided rapidly absorbable free-form EAA. However, our findings coincide with a similar dose-response study examining free-form EAA [25] conducted under energy balance conditions.

These studies suggest 8–10 g of EAA [25,72] optimally stimulate MPS regardless of the energy balance and moderate energy deficit conditions. However, further study of these doses provided in other feeding formats (i.e., plant-based intact proteins or whole-food proteins in mixed meals) or under conditions of greater energy deficit severity (i.e., >30% energy deficit) is warranted.

In addition to MPS measures, our recent work [72] also included whole-body protein turnover measures. Despite no difference in MPS stimulation, the 24 g EAA dose increased whole-body protein synthesis and attenuated whole-body protein breakdown, which resulted in enhanced whole-body protein balance compared to the 8 g EAA dose. The enhanced whole-body protein balance following the 24 g EAA dose was consistent with greater postprandial circulating EAA concentrations compared to the standard dose. In this regard, there was no further benefit of greater circulating EAA concentrations for stimulating MPS. However, the elevations in circulating EAA provided precursor amino acids to meet whole-body protein synthesis requirements, which likely contributed to a reduction in the reliance on whole-body protein breakdown to supply precursor amino acids [77,78]. The effect on whole-body protein balance is particularly significant when considering the importance of preventing or attenuating extensive whole-body protein breakdown (i.e., beyond the breakdown required for normal tissue maintenance and repair) and related body protein loss during energy deficit [79]. As stated, muscle is not the priority tissue during catabolic stress. Instead, the body relies on muscle as a readily available supply of precursor amino acids used to meet the increased amino acid requirements for life-sustaining processes or to be oxidized as fuel to meet increased energy demands. Reliance on muscle protein breakdown may be increased further if amino acid precursors sourced from the diet are limited due to inadequate amount and/or quality of protein or EAA intake.

6. Knowledge Gaps Surrounding Protein and EAA Formats to Support Muscle Protein Synthesis and Whole-Body Protein Balance under Energy Deficit

The advantages and disadvantages of supplemental free-form EAA, supplemental intact protein, or protein-containing mixed meals depend on the populations and conditions in which they are consumed. Optimizing the feeding format-related effects on MPS, whole-body protein balance, and muscle mass may be less concerning for healthy individuals adhering to daily food intake patterns that meet optimal protein intake recommendations and daily energy requirements. Yet, feeding formats may have large implications for delivering the quantity and quality of protein and/or EAA, as well as total energy needed to support MPS and total body protein for individuals during energy deficit. Feeding strategies under these conditions must leverage formats that deliver optimal amino acid quantity and composition within a highly digestible and absorbable food format. As stated, military personnel are one example of an otherwise healthy population consistently exposed to multiple catabolic stressors including energy deficit [79]. Military personnel generally subsist on combat food rations during strenuous, sustained trainings and operations that elicit moderate to severe energy deficits. Current rations, while designed to provide adequate nutrition, are rarely consumed as intended due to logistical constraints and consumer acceptability (i.e., reduced appetite, high desire to carry fewer food items to reduce weight) [80]. In addition, the majority of protein-containing ration items contain protein sources with suboptimal EAA compositions and are primarily components of mixed-macronutrient meals, which may not optimally stimulate and sustain MPS or whole-body protein balance. While the current evidence provides initial direction for developing more optimal feeding formats, there are several knowledge gaps. First, although there appears to be supporting evidence for the efficacy of enriching intact proteins, further work is required to define the exact dosages and amino acid compositions that will deliver the greatest benefit. Similarly, the comparable effects of blended and single-source isolated intact proteins on MPS support blending these protein sources. However, whether this relationship remains equivalent between single-sources and blends of whole-foods (i.e., whole-food co-ingestion) must be tested. Overall, the information surrounding whole-food proteins is limited [28,29]. Further characterization of whole-food proteins is required and may inform the development of meal patterns specifically aimed at achieving optimal protein and/or EAA intake. Lastly, for scenarios of energy

deficit, additional studies are needed to determine whether feeding formats that provide greater quantities of amino acids and/or total energy beyond the current recommended dosages are more advantageous compared to those that do not. Greater quantities of both essential and nonessential amino acids and/or energy may be required during energy deficit to offset or attenuate increased catabolism and support increased whole-body energy and protein requirements.

7. Conclusions

In this review, we summarized how supplemental free-form EAA, supplemental intact protein, and protein-containing mixed meal formats moderate MPS during energy balance. It appears that ingesting formats that provide ~20 to 30 g or 0.25–0.30 g/kg protein or ~10g or 0.10g/kg free-form EAA per dose optimizes MPS in healthy, non-obese individuals consuming habitual protein intakes of 1.6 g/kg/day during optimal conditions, such as following resistance exercise and in the absence of energy deficit. However, knowledge gaps remain, including the optimal dose of EAA during the catabolic stress of energy deficit, as well as how whole-food-containing mixed meals directly compare to supplemental free-form EAA and isolated intact proteins. Lastly, future studies should include simultaneous assessments of MPS, muscle protein breakdown, and whole-body protein balance to fully evaluate the efficacy of feeding formats in supporting body protein balance. Systematically addressing these gaps, while clarifying previous work, will provide the information required to quantify the relationship between feeding format and MPS as well as whole-body protein balance. This will ultimately aid in practical recommendations for when supplemental free-form EAA, supplemental intact protein, and protein-containing mixed meals should be consumed to optimally support total-body protein during energy deficit.

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