

RESEARCH ARTICLE

Supplementing an energy adequate, higher protein diet with protein does not enhance fat-free mass restoration after short-term severe negative energy balance

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¹Military Nutrition Division, US Army Research Institute of Environmental Medicine, Natick, Massachusetts; ²Oak Ridge Institute for Science and Education, Belcamp, Maryland; and ³Department of Geriatrics, Center for Translational Research in Aging and Longevity, Donald W. Reynolds Institute on Aging, University of Arkansas for Medical Sciences, Little Rock, Arkansas

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Berryman CE, Sepowitz JJ, McClung HL, Lieberman HR, Farina EK, McClung JP, Ferrando AA, Pasiakos SM. Supplementing an energy adequate, higher protein diet with protein does not enhance fat-free mass restoration after short-term severe negative energy balance. *J Appl Physiol* 122: 1485–1493, 2017. First published April 6, 2017; doi:10.1152/jappphysiol.01039.2016.—Negative energy balance during military operations can be severe and result in significant reductions in fat-free mass (FFM). Consuming supplemental high-quality protein following such military operations may accelerate restoration of FFM. Body composition (dual-energy X-ray absorptiometry) and whole body protein turnover (single-pool [¹⁵N]alanine method) were determined before (PRE) and after 7 days (POST) of severe negative energy balance during military training in 63 male US Marines (means ± SD, 25 ± 3 yr, 84 ± 9 kg). After POST measures were collected, volunteers were randomized to receive higher protein (HIGH: 1,103 kcal/day, 133 g protein/day), moderate protein (MOD: 974 kcal/day, 84 g protein/day), or carbohydrate-based low protein control (CON: 1,042 kcal/day, 7 g protein/day) supplements, in addition to a self-selected, ad libitum diet, for the 27-day intervention (REFED). Measurements were repeated POST-REFED. POST total body mass (TBM; −5.8 ± 1.0 kg, −7.0%), FFM (−3.1 ± 1.6 kg, −4.7%), and net protein balance (−1.7 ± 1.1 g protein·kg^{−1}·day^{−1}) were lower and proteolysis (1.1 ± 1.9 g protein·kg^{−1}·day^{−1}) was higher compared with PRE (*P* < 0.05). Self-selected, ad libitum dietary intake during REFED was similar between groups (3,507 ± 730 kcal/day, 2.0 ± 0.5 g protein·kg^{−1}·day^{−1}). However, diets differed by protein intake due to supplementation (CON: 2.0 ± 0.4, MOD: 3.2 ± 0.7, and HIGH: 3.5 ± 0.7 g·kg^{−1}·day^{−1}; *P* < 0.05) but not total energy (4,498 ± 725 kcal/day). All volunteers, independent of group assignment, achieved positive net protein balance (0.4 ± 1.0 g protein·kg^{−1}·day^{−1}) and gained TBM (5.9 ± 1.7 kg, 7.8%) and FFM (3.6 ± 1.8 kg, 5.7%) POST-REFED compared with POST (*P* < 0.05). Supplementing ad libitum, energy-adequate, higher protein diets with additional protein may not be necessary to restore FFM after short-term severe negative energy balance.

NEW & NOTEWORTHY This article demonstrates 1) the majority of physiological decrements incurred during military training (e.g., total and fat-free mass loss), with the exception of net protein balance, resolve and return to pretraining values after 27 days and 2) protein supplementation, in addition to an ad libitum, higher protein (~2.0

g·kg^{−1}·day^{−1}), energy adequate diet, is not necessary to restore fat-free mass following short-term severe negative energy balance.

negative energy balance; whey; casein; protein balance; recovery; muscle

NEGATIVE ENERGY BALANCE OCCURS when total energy expenditure exceeds the dietary energy intake necessary to maintain normal physiological processes and match energy required for physical activity, resulting in an energy deficit (21). Scientific investigations of negative energy balance and its physiological implications have demonstrated decrements in total body mass (TBM), including both fat-free mass (FFM) and fat mass (FM) (18). Some instances of negative energy balance are deliberate, such as during strenuous military training, leading to FFM loss that may be associated with decrements in physical performance typically observed during strenuous military operations (10, 13, 22, 28, 29, 40). During military training and combat operations, energy deficits are often more severe than those imposed to elicit weight loss, and the physiological consequences, including the loss of FFM, are likely more pronounced (15, 16, 25, 39). In these circumstances, energy deficits can approach 100% of total daily energy requirements needed to establish energy balance due to constant physical activity, limited access to food, and inadequate time or desire to eat (16, 21, 22).

Dietary interventions that increase protein intake above the recommended dietary allowance (RDA; 0.8 g·kg^{−1}·day^{−1}) but within the acceptable macronutrient distribution range for protein (10–35% of total calories) (17) have been shown to prevent significant FFM loss during negative energy balance. In a clinical weight loss study that combined 40% energy restriction with controlled exercise, overweight and obese adults consuming 2.4 g protein·kg^{−1}·day^{−1} gained more FFM and lost more FM compared with the group consuming 1.2 g protein·kg^{−1}·day^{−1} (20). In another clinical study investigating a 40% energy deficit in healthy, normal weight adults, dietary protein intakes above the RDA (1.6–2.4 g protein·kg^{−1}·day^{−1}) also protected against FFM loss (31). The mechanism by which higher protein diets spare FFM during moderate weight loss is likely due to the anabolic stimulus of essential amino acids (EAA) on muscle protein synthesis (4, 12, 27, 31, 33). How-

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ever, interventions to increase protein intake during military operation-induced energy deficits have not been successful (21). In such instances, effective interventions that restore FFM may be more feasible than interventions aiming to prevent FFM loss during the period of negative energy balance. Interventions leveraging the anabolic stimulus of EAA by increasing quantity and quality of protein intake to restore FFM have not been studied in the context of severe negative energy balance induced by strenuous military training.

Therefore, the current study characterized the consequences of short-term severe negative energy balance in combination with strenuous military training and determined whether high-quality protein supplementation, in addition to an ad libitum diet, promotes FFM recovery. We hypothesized protein supplementation would promote greater FFM restoration in a dose-dependent manner.

METHODS AND MATERIALS

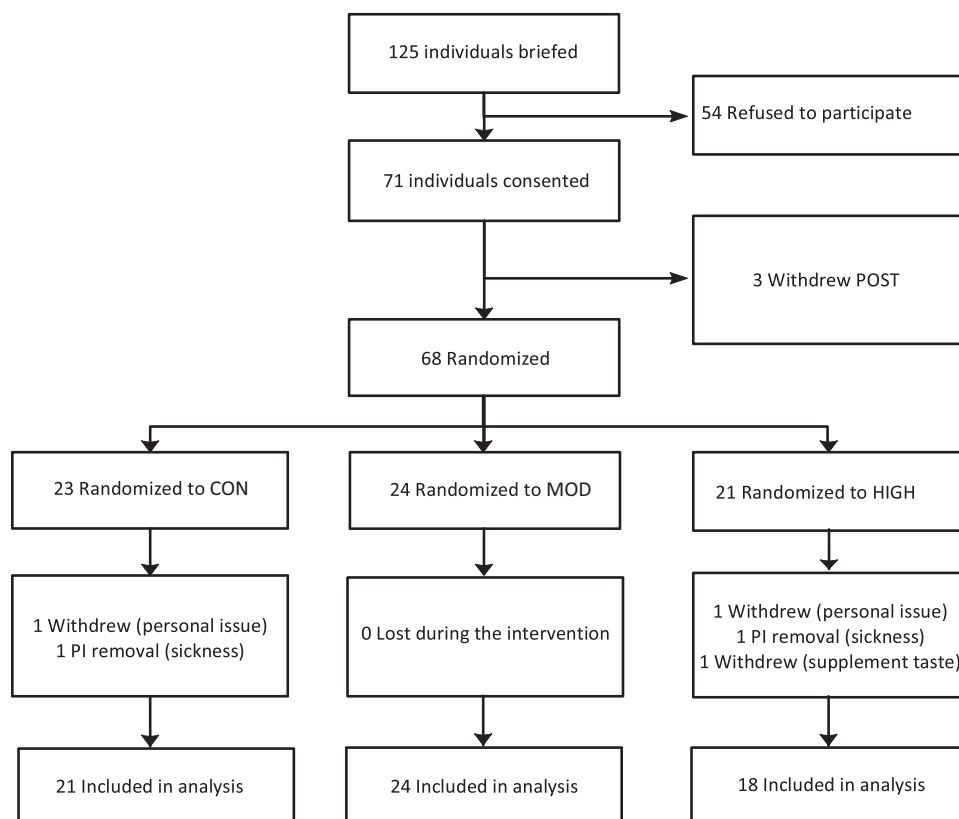
Participants. US Marines stationed at Stone Bay, Camp Lejeune, NC, who were at least 18 yr and had no known allergies to dairy, were eligible for the study. The military training exercise, Survival, Evasion, Resistance, Escape (SERE) school, lasted ~18 days. The study occurred from January 2014 through March 2015 and did not interfere or alter the execution of training. Details regarding participant recruitment, randomization, and attrition are presented in Fig. 1. In total, 68 participants were randomized and 63 volunteers completed the study and were included in the final analyses. All volunteers provided written informed consent before participation. This study was approved by the Institutional Review Board at the US Army Research Institute of Environmental

Medicine (Natick, MA) and registered at <https://clinicaltrials.gov/> as NCT02057094.

Experimental design. This study was designed to assess whether supplemental protein at various levels restores FFM after a short-term period of negative energy balance. The intent of the study was not to evaluate the effects of SERE per se but to use the training as a model of severe metabolic stress, uniformly applied to each volunteer. Previous studies have shown SERE is an excellent model of physiological stress (19, 26). With the use of a randomized, double-blind, placebo-controlled design, participants were studied before SERE (PRE), immediately after SERE (POST), and following a 27-day supplement refeeding intervention (POST-REFED; Fig. 2). Anthropometrics, demographics, whole body protein turnover, body composition, and circulating biomarkers of physiological status were measured PRE (*days 2 and 3*). These measures, with the exception of height and demographics, were repeated immediately POST (*days 18 and 19*). After completing all POST measures, participants were randomized to one of three [carbohydrate-based, low protein control (CON); protein-based (MOD); or higher protein-based (HIGH)] supplement groups and received three beverages daily to consume in addition to their ad libitum diet. Participants were randomized by FFM loss during SERE using Tave's minimization method of treatment assignment (38). Whole body protein turnover, body composition, and circulating biomarkers of physiological status were reassessed POST-REFED (*days 45 and 46*).

Military training exercise. The current study used SERE as a model of short-term severe negative energy balance. Military personnel at high risk of capture from the enemy (i.e., captivity, isolation, starvation, physical and mental abuse, and ex-

Fig. 1. Schematic of volunteer recruitment and retention.



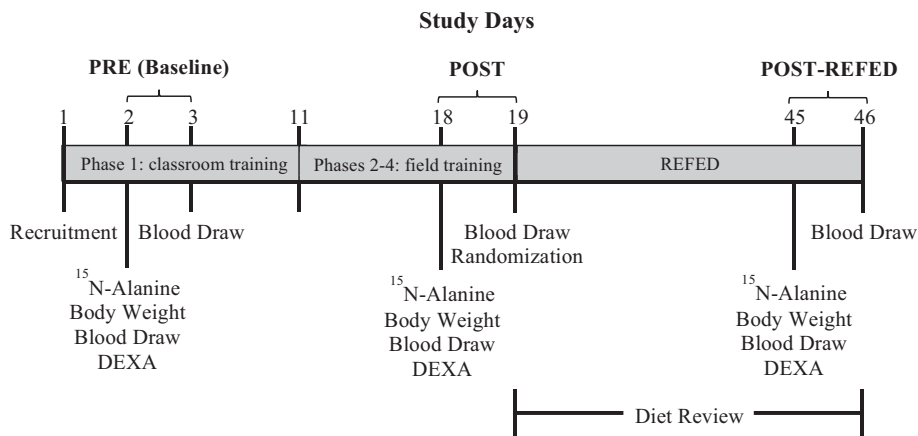


Fig. 2. Experimental design.

plottation) are required to complete SERE training. The training lasted ~18 days and consisted of four sequential phases: 1) 10-day academic classroom training; 2) 2.5-day survival skills training, conducted in a natural environment; 3) 2.5-day evasion training; and 4) 2.5-day captivity training. During *phase 1*, physical activity consisted of 1.5–2 h of organized physical training (PT) approximately every other day and dietary intake remained ad libitum. At the start of *phase 2*, participants were split into 4 separate, 12-person teams and provided minimal food [i.e., only 1 combat ration (Meal Ready-to-Eat, ~1,300 kcal) per participant and a limited amount of vegetables and meat to portion between team members] for the duration of *phases 2* and 3 (5 days). During *phase 2*, participants were active ≥ 14 h/day while completing survival skills training, which included food and water procurement, signaling, evasion shelter, fire-craft, survival medicine, and navigation principles. During *phase 3*, participants were physically active ≥ 16 h/day in varied environmental conditions and carrying a pack weighing approximately 20–30 kg. The intentional limited availability of food (approximately 300 kcal·day⁻¹ or 5 kcal·kg FFM⁻¹·day⁻¹ throughout *phases 2* and 3) coupled with high levels of physical activity resulted in severe negative energy balance. During *phase 4*, participants were “captured” and placed in a stressful simulated captivity environment. They were provided water on a regular basis but only given two meals during the entirety of *phase 4*; each meal consisted of a piece of bread and approximately one cup of rice. Participants were not physically active during the captivity phase.

Twenty-seven-day supplement refeeding intervention (REFED). The objective of REFED was to determine whether an ad libitum diet with supplemental protein would enhance FFM restoration compared with energy intake via a carbohydrate-based supplement. As such, participants were allowed ad libitum access to foods and beverages and were not instructed to follow any particular diet pattern during REFED. Based on previous studies (23), we anticipated that participants would self-select a diet composed of approximately 50–65% carbohydrate, <30% fat, and protein at 1.0 g·kg⁻¹·day⁻¹. Our objective was to increase dietary protein to levels two to three times the current RDA (0.8 g·kg⁻¹·day⁻¹) immediately following POST.

At the start of REFED, and throughout the remainder of the study, participants were provided three CON, MOD, or HIGH beverages per day, to be consumed immediately after morning

PT, between lunch and dinner meals, and before bed (Table 1). MOD and HIGH supplements consumed after PT and between lunch and dinner meals contained whey protein (7, 32, 41), whereas the MOD and HIGH supplements consumed before bed contained casein protein (11, 34). Volunteers assigned to the CON group consumed a flavor-matched, carbohydrate-based beverage at similar times throughout the day. All supplement drinks were designed to be isocaloric and similar in serving size, taste, and textural qualities. Study staff members were blinded to treatment assignment and distributed coded supplements to participants every 3 days during REFED.

Energy and macronutrient intake were determined every 3 days during REFED by registered dietitians using 24-h dietary recall and analysis software (Food Processor, version 11.0; ESHA, Salem, OR) to ensure supplement compliance and to characterize ad libitum dietary intake. Since participants consumed the majority (~80%) of their total daily dietary intake from the local military dining facility, a comprehensive nutritional database was developed before study implementation to more accurately estimate dietary intake by obtaining information on all foods/meals, menus, menu cycles, serving size, and recipes provided by the dining facility from the dining facility manager. Volunteers were able to report consumption of spe-

Table 1. Supplement composition

	CON		MOD		HIGH	
	Day	Night	Day	Night	Day	Night
Energy, kcal	260	522	226	522	289	525
Carbohydrate, g	64	113	36	68	32	56
Fat, g	0	6	1	8	1	9
Protein, g	1	5	20	44	39	55
EAA	0.0	0.9	11.8	18.9	23.5	23.6
BCAA	0.0	0.4	5.6	8.7	11.3	10.9
Leucine	0.0	0.2	3.1	4.1	6.2	5.1

Volunteers were instructed to consume 3 supplements per day (2 “Day” supplements and 1 “Night” supplement): 1 “Day” supplement immediately following morning physical training, the other “Day” supplement between lunch and dinner meals, and the “Night” supplement before bed. BCAA, branch-chain amino acids; CON, control; EAA, essential amino acids; HIGH, higher protein; MOD, moderate protein. Supplements were manufactured by the Combat Feeding Directorate, Natick Soldier Research, Development, and Engineering Center. CON supplements contained powdered beverage mix (Gatorade). MOD and HIGH “Day” supplements contained whey protein isolate (895, Fonterra); “Night” supplements contained instant micellar casein (American Casein).

cific meals (or individual foods) and serving number. No vitamins, minerals, or other nutritional supplements were permitted during the study. Physical activity during REFED was at the discretion of course instructors and included daily PT geared toward subsequent course objectives. Study volunteers all participated in the same organized PT to ensure consistency between intervention groups.

Anthropometrics and body composition. Vertical height was measured to the nearest 0.1 cm using a stadiometer (Seca; Creative Health Products, Plymouth, MI) at PRE. Body mass was measured to the nearest 0.1 kg using a calibrated digital scale (Befour Model PS6600; Befour, Saukville, WI) at PRE, POST, and POST-REFED. TBM, FM, and FFM were determined using dual energy X-ray absorptiometry (DEXA; Lunar IDXA; GE Lunar, Madison, WI). DEXA scans were performed under fasted conditions by trained personnel. Changes in body energy stores (Δ ES) were used to estimate energy balance (16):

$$\Delta\text{ES} = (\Delta\text{FM} \times 9.51 \text{ kcal/g}) + [\Delta\text{FFM} \times (1 - \text{FFM hydration}) \times 4.40 \text{ kcal/g}]$$

with FFM hydration representing the aqueous fraction of FFM, estimated as 0.73 (35).

Whole body protein turnover. Whole body protein turnover was measured by a single-pool whole body method at PRE, POST (during phase 4, i.e., captivity), and POST-REFED and as described by Ferrando et al. (9). After providing a urine sample to correct for background isotope enrichments, volunteers ingested a single dose of [^{15}N]alanine (99% enriched; Cambridge Isotope Laboratories, Andover, MA) at 4 mg ^{15}N /kg body mass after consuming their evening meal. Volunteers were instructed to fast and collect their urine for the next 10–12 h, ending with the first void the following morning. Nitrogen flux (Q ; g N/24 h) was determined using urinary urea enrichment according to Fern et al. (8). Protein synthesis (PS) and breakdown (PB) were calculated according to Stein et al. (36).

$$Q = \text{PS} + N_{\text{EX}} \text{ and } Q = \text{PB} + N_{\text{IN}}$$

$$\text{PB} = Q - N_{\text{IN}} \text{ and } \text{PS} = Q - N_{\text{EX}}$$

$$\text{NET} = \text{PS} - \text{PB}$$

where N_{EX} is urinary urea nitrogen excretion and N_{IN} represents nitrogen intake calculated from a diet recall of the evening meal before ingesting the isotope. Enrichment of tracer to tracee for [^{15}N]urea was determined using isotope ratio mass spectrometry (Metabolic Solutions, Nashua, NH).

Circulating biomarkers. Blood samples were collected after an overnight fast by antecubital venipuncture. Serum and plasma were isolated, frozen, and shipped on dry ice to the Pennington Biomedical Research Center (Baton Rouge, LA) for analysis of amino acids [i.e., total (TAA), essential (EAA), nonessential (NEAA), branched-chain (BCAA), and leucine alone; Agilent 1100 Series HPLC, Agilent Technologies, Foster City, CA] and insulin (Immulite 2000; Siemens Healthcare Diagnostic, Deerfield, IL).

Statistical analysis. Normality was assessed for each variable using the univariate procedure to quantitatively evaluate skewness and visually inspect box and probability plots. Log transformation was used to correct for nonnormally distributed data when necessary. One-way ANOVA was used to assess

differences between treatment groups at baseline. Repeated-measures ANOVA was used to determine main effects of time (PRE, POST, and POST-REFED) for body mass and composition, whole body protein synthesis, breakdown, and net and circulating biomarkers. One-way ANOVA was used to assess differences among groups (CON, MOD, and HIGH) for body mass and composition, whole body protein synthesis, breakdown, and net and circulating biomarkers POST-REFED and energy and macronutrient intake during REFED. An ANCOVA, adjusted for POST measures, was used to further assess differences among intervention groups POST-REFED. Bonferroni adjustment was used for post hoc analysis for significant main effects. Data were analyzed using SAS (Version 9.3; SAS Institute, Cary, NC). Significance was set at $P < 0.05$, and data are presented as means \pm SD. Power analyses indicated that 19 volunteers/group would provide 90% power to detect between-group differences in FFM restoration at POST-REFED with an effect size of 0.54 and an α of 0.05 (31).

RESULTS

Baseline characteristics. Participants ($n = 63$) were young (25 ± 3 yr) enlisted male Marines with a body fat percentage of $17 \pm 5\%$. On average, participants engaged in aerobic exercise 5 days/wk and upper body, lower body, and core exercises 3 day/wk (Table 2).

Longitudinal responses to military training. Significant reductions in TBM, FFM, FM, leg mass, and trunk mass were observed at POST compared with PRE ($P < 0.05$; Table 3). At POST-REFED, TBM, FFM, FM, leg mass, and trunk mass increased compared with POST ($P < 0.05$), resulting in no differences between PRE and POST-REFED measures ($P > 0.05$). Body energy stores were decreased at POST (FFM: $-3,694 \pm 1,956$ and FM: $-25,729 \pm 13,303$ kcal) and increased POST-REFED (FFM: $4,239 \pm 2,088$ and FM: $22,141 \pm 12,386$ kcal). Based on changes in body energy stores over the 7 days of severe negative energy balance (POST), participants were in an energy deficit of $-4203 \pm 1,686$ kcal/day; based on changes in body energy stores over the 27-day REFED period, participants were in a positive energy balance of 977 ± 435 kcal/day.

Protein synthesis and net protein balance were lower and protein breakdown was higher at POST vs. PRE ($P < 0.05$; Table 3). Both protein synthesis and proteolysis were higher POST-REFED compared with PRE and POST ($P < 0.05$). Net

Table 2. Participant characteristics and demographics at study enrollment

	ALL	CON	MOD	HIGH
Age, yr	25.2 \pm 2.5	25.9 \pm 3.0	24.7 \pm 2.5	25.0 \pm 1.8
Height, cm	178 \pm 6	179 \pm 6	176 \pm 5	178 \pm 8
Body mass, kg	83.8 \pm 9.5	85.3 \pm 8.4	83.1 \pm 11.0	83.0 \pm 8.8
BMI, kg/m ²	26.4 \pm 2.1	26.5 \pm 1.9	26.6 \pm 2.5	26.0 \pm 1.6
Exercise, days/wk				
Aerobic	4.7 \pm 1.3	4.7 \pm 1.1	4.6 \pm 1.4	4.7 \pm 1.4
Upper body pushing	3.2 \pm 1.6	3.3 \pm 1.7	3.2 \pm 1.6	3.2 \pm 1.6
Upper body pulling	2.7 \pm 1.5	2.8 \pm 1.4	2.5 \pm 1.6	2.9 \pm 1.6
Lower body	2.5 \pm 1.5	2.7 \pm 1.7	2.3 \pm 1.3	2.7 \pm 1.6
Core	3.3 \pm 1.5	3.2 \pm 1.2	3.1 \pm 1.7	3.6 \pm 1.6

Data are means \pm SD. CON, control ($n = 21$); MOD, moderate protein ($n = 24$); HIGH, higher protein ($n = 18$). There were no differences between groups.

Table 3. Longitudinal responses to SERE

	PRE	POST	POST-REFED	Time, <i>P</i> -Value
Body mass, kg				
Total	83.8 ± 9.5 ^a	78.0 ± 9.1 ^b	83.9 ± 8.7 ^a	<0.0001
Fat free	66.3 ± 6.7 ^a	63.2 ± 6.6 ^b	66.8 ± 6.6 ^a	<0.0001
Fat	14.0 ± 4.7 ^a	11.3 ± 4.5 ^b	13.6 ± 3.8 ^a	<0.0001
Leg mass, kg				
Total	28.8 ± 3.8 ^a	27.5 ± 3.9 ^b	29.0 ± 3.6 ^a	<0.0001
Fat free	22.4 ± 2.7 ^a	21.8 ± 2.8 ^b	22.6 ± 2.7 ^a	<0.0001
Fat	5.0 ± 1.7 ^a	4.3 ± 1.7 ^b	5.0 ± 1.5 ^a	<0.0001
Trunk mass, kg				
Total	38.9 ± 4.4 ^a	35.3 ± 4.0 ^b	38.8 ± 4.0 ^a	<0.0001
Fat free	30.7 ± 3.0 ^a	28.9 ± 2.9 ^b	31.0 ± 2.8 ^a	<0.0001
Fat	7.1 ± 2.6 ^a	5.3 ± 2.3 ^b	6.7 ± 2.1 ^c	<0.0001
Protein turnover, g protein·kg ⁻¹ ·day ⁻¹				
Synthesis	6.84 ± 1.24 ^a	6.28 ± 1.69 ^b	8.40 ± 2.35 ^c	<0.0001
Breakdown	5.79 ± 1.58 ^a	6.91 ± 1.96 ^b	8.02 ± 2.59 ^c	<0.0001
Net	1.05 ± 1.18 ^a	-0.64 ± 0.43 ^b	0.38 ± 0.98 ^c	<0.0001
Amino acids, μmol/l				
Total	2,671 ± 540 ^a	3,374 ± 525 ^b	2,989 ± 259 ^c	<0.0001
Essential	947 ± 191 ^a	1,122 ± 241 ^b	1,122 ± 104 ^b	<0.0001
Branched chain	507 ± 112 ^a	584 ± 149 ^b	615 ± 71 ^b	<0.0001
Leucine	143 ± 30 ^a	157 ± 47 ^a	174 ± 20 ^b	0.0003
Nonessential	1,724 ± 381 ^a	2,252 ± 329 ^b	1,867 ± 189 ^a	<0.0001
Insulin, μU/ml	4.46 ± 1.80 ^a	8.47 ± 8.31 ^b	5.77 ± 3.28 ^{a,b}	<0.0001

Data are mean ± SD, *n* = 63. Repeated-measures ANOVA was used to determine the main effects of time. Protein turnover synthesis and insulin data were log transformed. SERE, Survival, Evasion, Resistance, Escape. ^{a,b,c}*P* < 0.05, different lowercase letters within a row indicate significant differences; Bonferroni adjustments were made for multiple comparisons.

protein balance was also higher POST-REFED compared with POST; however, net protein balance POST-REFED remained lower than PRE (*P* < 0.05).

Increased TAA, EAA, BCAA, and NEAA were observed when POST was compared with PRE (*P* < 0.05), whereas leucine was unchanged at POST (*P* > 0.05). Lower TAA were observed POST-REFED compared with POST; however, POST-REFED measures remained significantly higher compared with PRE (*P* < 0.05). POST-REFED measures of EAA and BCAA were no different than POST (*P* > 0.05) but significantly elevated compared with PRE (*P* < 0.05). Decreased NEAA were observed POST-REFED compared with POST (*P* < 0.05), resulting in no differences between PRE and POST-REFED (*P* > 0.05). Leucine concentrations were higher POST-REFED compared with both PRE and POST (*P* < 0.05). Insulin concentrations were greater at POST compared with PRE (*P* < 0.05), but neither PRE nor POST differed from POST-REFED (*P* > 0.05).

Twenty-seven day supplement intervention (REFED). Supplements provided significantly different amounts of protein (HIGH > MOD > CON; *P* < 0.01) and carbohydrate (CON > MOD > HIGH; *P* < 0.01; Table 4). Protein and carbohydrate intake from the diet did not differ across groups (*P* > 0.05). Thus MOD and HIGH did not differ in total protein intake (*P* = 0.06), while both groups did consume significantly more protein than CON (*P* < 0.01). CON consumed more total carbohydrate than HIGH (*P* < 0.01) but neither group differed from MOD (*P* = 0.08 and *P* = 0.11, respectively). Total energy and fat intake were similar between treatment groups. Compliance with supplement consumption was above 95% across all groups.

There were no significant effects of the CON, MOD, or HIGH supplements on any outcome measure POST-REFED (Table 5 and Fig. 3).

DISCUSSION

The current study investigated the effects of high-quality protein supplementation, in addition to an ad libitum diet, on FFM restoration after short-term (7 days) severe negative energy balance caused by SERE training. The current findings indicate 1) SERE training induced significant weight loss, including loss of FFM, and a negative net protein balance; 2) the majority of physiological decrements incurred during SERE (POST), with the exception of net protein balance, resolved and returned to PRE values after 27 days; and 3) protein supplementation, in addition to consuming an ad libitum, higher protein, energy adequate diet, is not necessary to restore FFM following periods of severe negative energy balance.

It is generally not feasible to achieve the same level of study control and apply the same measurement techniques in military field studies as those used in laboratory settings. Therefore, there are a number of logistical constraints and inherent limitations in the current military field study that need to be acknowledged before interpreting the study outcomes. For example, changes in FFM may have been overestimated due to the inability of DEXA to capture changes in muscle glycogen and total body water content, which decrease during periods of severe negative energy balance and rapid weight loss (5). Ideally, and in a highly controlled laboratory setting, a four-compartment model for body composition, controlled feeding, muscle kinetic studies, and resting metabolic rate and/or doubly labeled water methods would replace the three-compartment model for body composition, 24-h recalls, whole body protein turnover, and estimated energy balance by changes in body energy stores, respectively. In addition, due to concerns of interference with course objectives, we were unable to determine whether the decrement and restoration of FFM were

Table 4. Dietary intakes during the 27-day supplement intervention (REFED) following SERE

	CON	MOD	HIGH
Relative, kcal/kg, g/kg			
Energy			
Supplement	12 ± 1 ^{a,b}	12 ± 2 ^a	13 ± 2 ^b
Diet	43 ± 10	47 ± 12	42 ± 11
Total	56 ± 11	59 ± 13	55 ± 12
Protein			
Supplement	0.1 ± 0.0 ^a	1.0 ± 0.1 ^b	1.6 ± 0.2 ^c
Diet	1.9 ± 0.4	2.2 ± 0.6	1.9 ± 0.6
Total	2.0 ± 0.4 ^a	3.2 ± 0.7 ^b	3.5 ± 0.7 ^b
Carbohydrate			
Supplement	2.9 ± 0.3 ^a	1.7 ± 0.2 ^b	1.4 ± 0.2 ^c
Diet	4.9 ± 1.4	5.4 ± 1.4	4.8 ± 1.6
Total	7.8 ± 1.6 ^a	7.1 ± 1.5 ^{a,b}	6.3 ± 1.8 ^b
Fat			
Supplement	0.1 ± 0.0 ^a	0.1 ± 0.0 ^b	0.1 ± 0.0 ^b
Diet	1.8 ± 0.4	1.8 ± 0.6	1.6 ± 0.4
Total	1.8 ± 0.4	2.0 ± 0.6	1.8 ± 0.4
Relative, kcal/kg FFM, g/kg FFM			
Energy			
Supplement	15 ± 1 ^{a,b}	15 ± 2 ^a	16 ± 2 ^b
Diet	54 ± 12	58 ± 13	51 ± 12
Total	69 ± 13	72 ± 14	67 ± 14
Protein			
Supplement	0.1 ± 0.0 ^a	1.3 ± 0.1 ^b	2.0 ± 0.3 ^c
Diet	2.4 ± 0.4	2.7 ± 0.7	2.4 ± 0.7
Total	2.5 ± 0.4 ^a	3.9 ± 0.8 ^b	4.3 ± 0.8 ^b
Carbohydrate			
Supplement	3.5 ± 0.3 ^a	2.1 ± 0.2 ^b	1.8 ± 0.2 ^c
Diet	6.1 ± 1.7	6.6 ± 1.6	5.9 ± 1.9
Total	9.6 ± 1.8 ^a	8.8 ± 1.7 ^{a,b}	7.7 ± 2.0 ^b
Fat			
Supplement	0.1 ± 0.0 ^a	0.1 ± 0.0 ^b	0.1 ± 0.0 ^b
Diet	2.2 ± 0.5	2.3 ± 0.7	2.0 ± 0.5
Total	2.3 ± 0.5	2.4 ± 0.7	2.1 ± 0.5
Absolute, kcal, g			
Energy			
Supplement	1,001 ± 34 ^a	937 ± 29 ^b	1,050 ± 72 ^c
Diet	3,505 ± 689	3,674 ± 747	3,287 ± 736
Total	4,506 ± 674	4,612 ± 747	4,337 ± 764
Protein			
Supplement	6 ± 0 ^a	80 ± 2 ^b	127 ± 9 ^c
Diet	155 ± 27	170 ± 44	152 ± 43
Total	161 ± 27 ^a	250 ± 44 ^b	280 ± 47 ^b
Carbohydrate			
Supplement	232 ± 8 ^a	135 ± 4 ^b	114 ± 8 ^c
Diet	395 ± 97	425 ± 98	379 ± 106
Total	627 ± 94 ^a	559 ± 98 ^{a,b}	493 ± 109 ^b
Fat			
Supplement	5.7 ± 0.2 ^a	8.7 ± 0.3 ^b	9.4 ± 0.6 ^c
Diet	144 ± 29	144 ± 42	130 ± 33
Total	149 ± 29	153 ± 42	139 ± 33
Supplement compliance, %	96.7 ± 3.3	96.9 ± 3.0	95.8 ± 6.6

Data are means ± SD. CON, control ($n = 21$); MOD, moderate protein ($n = 24$); HIGH, higher protein ($n = 18$). One-way ANOVA was used to determine the main effects of treatment. ^{a,b,c} $P < 0.05$, different lowercase letters within a row indicate significant differences; Bonferroni adjustments were made for multiple comparisons.

functionally relevant using validated physical performance tests. The results of this study should be interpreted in the context of these limitations.

Evidence consistently demonstrates military training and operations cause severe physiological and psychological stress (13, 19). In the current study, while acknowledging the limitations of DEXA-measured body composition, participants lost 7.0 and 4.7% of initial TBM and FFM, respectively, while

consuming ~300 kcal/day (5 kcal·kg FFM⁻¹·day⁻¹) during strenuous military training (POST), resulting in negative net protein balance (-0.64 ± 0.43 g protein·kg⁻¹·day⁻¹). Previous reports indicate 7-day US Army SERE school induces similar decrements in TBM (-6.7 kg, -7.8%) (19), and 3- and 4-day Norwegian Army arctic military training operations, which also result in severe energy deficits ($-3,390 \pm 669$ and $-3,313 \pm 776$ kcal/day, respectively), produce comparable decrements in net protein balance (-1.41 ± 1.11 g protein·kg⁻¹·10 h⁻¹ and -0.24 ± 0.60 g protein·kg⁻¹·day⁻¹, respectively) (21, 22). During various combat missions, soldiers can expend 3,500–4,600 kcal/day (39) and, at the extreme, total energy expenditures may exceed 7,000 kcal/day, as documented in US Marines engaged in mountain warfare training (14, 15).

Body composition measures returned to PRE after the intervention period (POST-REFED), suggesting ad libitum intake of ~4,500 kcal/day (70 kcal·kg FFM⁻¹·day⁻¹) for 27 days is sufficient to fully restore FFM after short-term severe negative energy balance. Protein synthesis decreased POST and increased POST-REFED, whereas protein breakdown was greater at POST and greatest POST-REFED. Muscle protein synthesis (4, 33) and mTORC1 signaling (24) are downregulated during periods of energy deficit, while protein breakdown is increased to mobilize substrate stores (i.e., amino acids) for oxidation and acute-phase protein synthesis (6), resulting in an overall decrease in net protein balance that contributes, in part, to FFM loss. In the current study, this was reflected by an increase in circulating amino acid concentrations POST. Insulin is known to mitigate proteolysis and, in the presence of adequate amino acid substrate, promote protein synthesis (1). Despite elevated insulin concentrations POST, protein breakdown was higher than PRE and protein synthesis decreased, which may indicate that a severe energy deficit is a stronger regulator of protein metabolism than insulin when individuals are in a depleted state. At POST-REFED, a positive net protein balance was observed, likely due to restored energy intake ($+977 \pm 435$ kcal/day). The difference between PRE and POST-REFED net protein balance, taken in context with the increase in protein turnover and concomitant increases in the volume and intensity of physical activity during REFED, may indicate a persistent stress response. This suggests that at the whole body protein level, 27 days may not be adequate time for Warfighters engaged in successive training or operations to fully recover before subsequent assignments. Future studies incorporating performance measures may help elucidate the physiological significance of the whole body protein measures.

Overall, protein kinetics indicated a net catabolic state after SERE. It is important to note that this measure is an integrated representation of a fixed period of time (i.e., 10–12 h). However, it represents a “snapshot” of whole body protein kinetics in response to a consolidation of physiological stressors. On the contrary, FFM measures represent the cumulative changes/responses to the 7-day field exercise of SERE. Furthermore, this measure of FFM is most likely an overestimation due to the concomitant glycogen depletion and total body water loss (5). While one must exercise caution in correlating one measure to the other, each demonstrates an important physiological response to a combination of severe metabolic stressors.

Our primary hypothesis stated high-quality protein supplementation, in addition to an ad libitum diet, would maximize

Table 5. Treatment responses to the 27-day supplement intervention (REFED)

	CON	MOD	HIGH	Treatment, <i>P</i> Value*	Treatment, <i>P</i> Value†
Body mass, kg					
Total	85.2 ± 7.5	83.3 ± 10.1	83.1 ± 8.0	0.70	0.94
Fat free	67.4 ± 5.4	66.2 ± 7.7	66.9 ± 6.4	0.85	0.79
Fat	14.2 ± 3.5	13.7 ± 4.5	12.8 ± 3.2	0.50	0.29
Leg mass, kg					
Total	29.4 ± 3.2	28.7 ± 4.1	28.8 ± 3.6	0.79	0.88
Fat free	22.8 ± 2.2	22.4 ± 3.1	22.7 ± 2.8	0.85	0.78
Fat	5.2 ± 1.4	5.0 ± 1.6	4.7 ± 1.4	0.66	0.36
Trunk mass, kg					
Total	39.9 ± 4.0	38.2 ± 4.2	38.2 ± 3.4	0.28	0.55
Fat free	31.5 ± 2.6	30.7 ± 3.2	30.8 ± 2.7	0.62	0.65
Fat	7.3 ± 2.1	6.5 ± 2.4	6.3 ± 1.4	0.26	0.24
Protein turnover, g protein·kg ⁻¹ ·day ⁻¹					
Synthesis	7.71 ± 2.35	8.53 ± 2.02	9.05 ± 2.67	0.14	0.11
Breakdown	7.13 ± 2.61	8.16 ± 2.30	8.87 ± 2.73	0.10	0.11
Net	0.58 ± 0.70	0.37 ± 1.19	0.18 ± 0.95	0.46	0.42
Amino acids, μmol/l					
Total	3,000 ± 281	3,017 ± 249	2,939 ± 253	0.62	0.59
Essential	1,102 ± 128	1,154 ± 91	1,103 ± 84	0.17	0.31
Branch-chain	603 ± 87	640 ± 60	597 ± 56	0.09	0.24
Leucine	173 ± 25	180 ± 17	167 ± 17	0.16	0.23
Nonessential	1,898 ± 193	1,863 ± 186	1,835 ± 195	0.60	0.56
Insulin, μU/ml	5.20 ± 3.05	6.85 ± 4.16	5.07 ± 1.62	0.28	0.27

Data are means ± SD. CON, control (*n* = 21); MOD, moderate protein (*n* = 24); HIGH, higher protein (*n* = 18). Protein turnover synthesis and insulin data were log transformed. *One-way ANOVA was used to determine the main effects of treatment. †Model adjusted for POST as a covariate.

FFM restoration. Instead, all volunteers regained FFM and TBM to similar PRE levels regardless of treatment assignment. The inability to detect a treatment difference may be attributable to a combination of factors: 1) FFM loss POST may have been overestimated, as muscle glycogen and its associated water content can influence the DEXA derived FFM estimates (5); 2) total daily energy intake, to which supplements contributed a relatively small amount, may have been under or overestimated given limitations of self-reported dietary data (37); and 3) ad libitum protein intake during REFED was already at the upper end of recommendations, without supplementation. Supplementation only contributed ~22% of total energy intake during REFED. The remainder of caloric intake came from the self-selected ad libitum diet. The supplements were designed to complement the diet, with the assumption that ad libitum protein intake would be similar to our past studies (23) and consistent with

Army regulation 40–25 recommendations of 0.8 to 1.6 g·kg⁻¹·day⁻¹ (2). In the current study, military special forces consumed ~2 g protein·kg⁻¹·day⁻¹ from their ad libitum diet alone. This amount meets current recommendations (1.2–2.0 g protein·kg⁻¹·day⁻¹) during periods of elevated physical activity (39a). Although the study is limited by the absence of a true control group (i.e., group that receives no supplement), and we are unable to determine if providing supplemental calories, regardless of macronutrient source, was better than providing no supplement at all, the data suggest that in the context of military training, it is unlikely that protein consumption in excess of 2.0 g protein·kg⁻¹·day⁻¹ provides additional FFM benefits (30).

While we acknowledge limitations in the current study, there are also strengths that should be highlighted. One strength was the use of SERE school as the model of substantial metabolic stress and negative energy balance, which produced uniform losses in TBM and FFM. In addition, during REFED, participants engaged in the same organized PT, which ensured consistency between intervention groups. Many studies intervene before and/or during military training operations, but few intervene during a follow-up period to determine physiological status for subsequent training and operations. The current study was strengthened by investigating both the physiological stress of military training and the 27 days following that training.

In conclusion, US Marines met or exceeded current recommendations for protein (1, 2) when eating primarily at a military dining facility following strenuous military training that produced severe negative energy balance, which allowed them to adequately restore FFM. Supplementing ad libitum, energy-adequate, higher protein diets with additional protein did not augment the restoration of FFM.

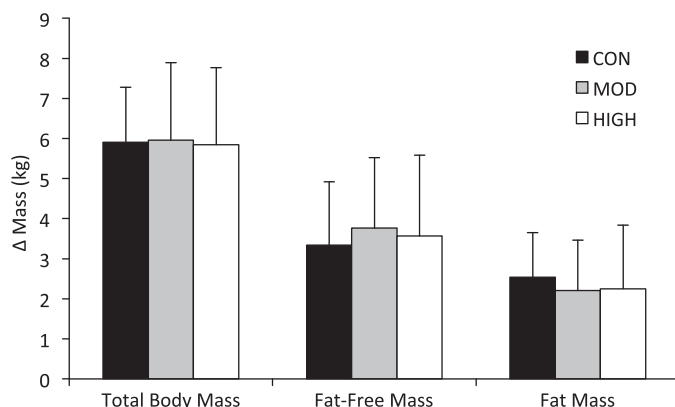


Fig. 3. Body composition changes at POST-REFED by supplement group. CON, control (*n* = 21); MOD, moderate protein (*n* = 24); HIGH, higher protein (*n* = 18). Values are presented as means ± SD. *P* < 0.05.

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DISCLAIMERS

The investigators adhered to the policies for protection of human subjects as prescribed in Army Regulation 70-25, and the research was conducted in adherence with the provisions of 32 CFR part 219. The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Army or the Department of Defense. Any citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

J.J.S., H.L.M., H.R.L., E.K.F., J.P.M., A.A.F., and S.M.P. conceived and designed research; J.J.S., H.L.M., E.K.F., J.P.M., and S.M.P. performed experiments; C.E.B., J.J.S., A.A.F., and S.M.P. analyzed data; C.E.B., J.J.S., A.A.F., and S.M.P. interpreted results of experiments; C.E.B., J.J.S., A.A.F., and S.M.P. prepared figures; C.E.B. and S.M.P. drafted manuscript; C.E.B., J.J.S., H.L.M., H.R.L., E.K.F., J.P.M., A.A.F., and S.M.P. edited and revised manuscript; C.E.B., J.J.S., H.L.M., H.R.L., E.K.F., J.P.M., A.A.F., and S.M.P. approved final version of manuscript.

REFERENCES

- Abdulla H, Smith K, Atherton PJ, Idris I. Role of insulin in the regulation of human skeletal muscle protein synthesis and breakdown: a systematic review and meta-analysis. *Diabetologia* 59: 44–55, 2016. doi:10.1007/s00125-015-3751-0.
- Anonymous. *Nutrition and Menu Standards for Human Performance Optimization*. Army Regulation 40–25 OPNAVINST 10110.1/MCO 10110.49 AFI 44–141. Washington, DC: Headquarters, Depts. of the Army, Navy, and Air Force, 2017.
- Areta JL, Burke LM, Camera DM, West DW, Crawshay S, Moore DR, Stellingwerff T, Phillips SM, Hawley JA, Coffey VG. Reduced resting skeletal muscle protein synthesis is rescued by resistance exercise and protein ingestion following short-term energy deficit. *Am J Physiol Endocrinol Metab* 306: E989–E997, 2014. doi:10.1152/ajpendo.00590.2013.
- Bone JL, Ross ML, Tomcik KA, Jeacocke NA, Hopkins WG, Burke LM. Manipulation of muscle creatine and glycogen changes dxa estimates of body composition. *Med Sci Sports Exerc* 2016 Nov 28. [Epub ahead of print]. doi:10.1249/MSS.0000000000001174.
- Carbone JW, McClung JP, Pasiakos SM. Skeletal muscle responses to negative energy balance: effects of dietary protein. *Adv Nutr* 3: 119–126, 2012. doi:10.3945/an.111.001792.
- Drummond MJ, Rasmussen BB. Leucine-enriched nutrients and the regulation of mammalian target of rapamycin signalling and human skeletal muscle protein synthesis. *Curr Opin Clin Nutr Metab Care* 11: 222–226, 2008. doi:10.1097/MCO.0b013e3282fa17fb.
- Fern EB, Garlick PJ, Waterlow JC. Apparent compartmentation of body nitrogen in one human subject: its consequences in measuring the rate of whole-body protein synthesis with ¹⁵N. *Clin Sci (Lond)* 68: 271–282, 1985. doi:10.1042/cs0680271.
- Ferrando AA, Lane HW, Stuart CA, Davis-Street J, Wolfe RR. Prolonged bed rest decreases skeletal muscle and whole body protein synthesis. *Am J Physiol Endocrinol Metab* 270: E627–E633, 1996.
- Fortes MB, Diment BC, Greeves JP, Casey A, Izard R, Walsh NP. Effects of a daily mixed nutritional supplement on physical performance, body composition, and circulating anabolic hormones during 8 weeks of arduous military training. *Appl Physiol Nutr Metab* 36: 967–975, 2011. doi:10.1139/h11-124.
- Groen BB, Res PT, Pennings B, Hertle E, Senden JM, Saris WH, van Loon LJ. Intra-gastric protein administration stimulates overnight muscle protein synthesis in elderly men. *Am J Physiol Endocrinol Metab* 302: E52–E60, 2012. doi:10.1152/ajpendo.00321.2011.
- Hector AJ, Marcotte GR, Churchward-Venne TA, Murphy CH, Breen L, von Allmen M, Baker SK, Phillips SM. Whey protein supplementation preserves postprandial myofibrillar protein synthesis during short-term energy restriction in overweight and obese adults. *J Nutr* 145: 246–252, 2015. doi:10.3945/jn.114.200832.
- Henning PC, Park BS, Kim JS. Physiological decrements during sustained military operational stress. *Mil Med* 176: 991–997, 2011. doi:10.7205/MILMED-D-11-00053.
- Hoyt RW, Friedl KE. Field studies of exercise and food deprivation. *Curr Opin Clin Nutr Metab Care* 9: 685–690, 2006. doi:10.1097/01.mco.0000247472.72155.7c.
- Hoyt RW, Jones TE, Stein TP, McAninch GW, Lieberman HR, Askeew EW, Cymerman A. Doubly labeled water measurement of human energy expenditure during strenuous exercise. *J Appl Physiol* (1985) 71: 16–22, 1991.
- Hoyt RW, Opstad PK, Haugen AH, DeLany JP, Cymerman A, Friedl KE. Negative energy balance in male and female rangers: effects of 7 d of sustained exercise and food deprivation. *Am J Clin Nutr* 83: 1068–1075, 2006.
- Institute of Medicine. *Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (Macronutrients)*. Washington, DC: The National Academies Press, 2005.
- Keys A, Brozek J, Henschel A, Mickelsen O. *The Biology of Human Starvation*. Minneapolis, MN: Univ of Minnesota Press, 1950.
- Lieberman HR, Farina EK, Caldwell J, Williams KW, Thompson LA, Niro PJ, Grohmann KA, McClung JP. Cognitive function, stress hormones, heart rate and nutritional status during simulated captivity in military survival training. *Physiol Behav* 165: 86–97, 2016. doi:10.1016/j.physbeh.2016.06.037.
- Longland TM, Oikawa SY, Mitchell CJ, Devries MC, Phillips SM. Higher compared with lower dietary protein during an energy deficit combined with intense exercise promotes greater lean mass gain and fat mass loss: a randomized trial. *Am J Clin Nutr* 103: 738–746, 2016. doi:10.3945/ajcn.115.119339.
- Margolis LM, Murphy NE, Martini S, Gundersen Y, Castellani JW, Karl JP, Carrigan CT, Teien HK, Madslie EH, Montain SJ, Pasiakos SM. Effects of supplemental energy on protein balance during 4-d arctic military training. *Med Sci Sports Exerc* 48: 1604–1612, 2016. doi:10.1249/MSS.0000000000000944.
- Margolis LM, Murphy NE, Martini S, Spitz MG, Thrane I, McGraw SM, Blatny JM, Castellani JW, Rood JC, Young AJ, Montain SJ, Gundersen Y, Pasiakos SM. Effects of winter military training on energy balance, whole-body protein balance, muscle damage, soreness, and physical performance. *Appl Physiol Nutr Metab* 39: 1395–1401, 2014. doi:10.1139/apnm-2014-0212.
- Margolis LM, Pasiakos SM, Karl JP, Rood JC, Cable SJ, Williams KW, Young AJ, McClung JP. Differential effects of military training on fat-free mass and plasma amino acid adaptations in men and women. *Nutrients* 4: 2035–2046, 2012. doi:10.3390/nu4122035.
- Margolis LM, Rivas DA, Berrone M, Ezzayat Y, Young AJ, McClung JP, Fielding RA, Pasiakos SM. Prolonged calorie restriction downregulates skeletal muscle mTORC1 signaling independent of dietary protein intake and associated microRNA expression. *Front Physiol* 7: 445, 2016. doi:10.3389/fphys.2016.00445.
- Margolis LM, Rood J, Champagne C, Young AJ, Castellani JW. Energy balance and body composition during US Army special forces training. *Appl Physiol Nutr Metab* 38: 396–400, 2013. doi:10.1139/apnm-2012-0323.
- Morgan CA III, Hazlett G, Southwick S, Rasmussen A, Lieberman HR. Effect of carbohydrate administration on recovery from stress-induced deficits in cognitive function: a double-blind, placebo-controlled study of soldiers exposed to survival school stress. *Mil Med* 174: 132–138, 2009. doi:10.7205/MILMED-D-58-7808.
- Murphy CH, Churchward-Venne TA, Mitchell CJ, Kolar NM, Kassis A, Karagounis LG, Burke LM, Hawley JA, Phillips SM. Hypoenergetic

- diet-induced reductions in myofibrillar protein synthesis are restored with resistance training and balanced daily protein ingestion in older men. *Am J Physiol Endocrinol Metab* 308: E734–E743, 2015. doi:10.1152/ajpendo.00550.2014.
28. Nindl BC, Barnes BR, Alemany JA, Frykman PN, Shippee RL, Friedl KE. Physiological consequences of U.S. Army Ranger training. *Med Sci Sports Exerc* 39: 1380–1387, 2007. doi:10.1249/MSS.0b013e318067e2f7.
 29. Nindl BC, Leone CD, Tharion WJ, Johnson RF, Castellani JW, Patton JF, Montain SJ. Physical performance responses during 72 h of military operational stress. *Med Sci Sports Exerc* 34: 1814–1822, 2002. doi:10.1097/00005768-200211000-00019.
 30. Pasiakos SM, Austin KG, Lieberman HR, Askew EW. Efficacy and safety of protein supplements for U.S. Armed Forces personnel: consensus statement. *J Nutr* 143: 1811S–1814S, 2013. doi:10.3945/jn.113.176859.
 31. Pasiakos SM, Cao JJ, Margolis LM, Sauter ER, Whigham LD, McClung JP, Rood JC, Carbone JW, Combs GF Jr, Young AJ. Effects of high-protein diets on fat-free mass and muscle protein synthesis following weight loss: a randomized controlled trial. *FASEB J* 27: 3837–3847, 2013. doi:10.1096/fj.13-230227.
 32. Pasiakos SM, McClung HL, McClung JP, Margolis LM, Andersen NE, Cloutier GJ, Pikosky MA, Rood JC, Fielding RA, Young AJ. Leucine-enriched essential amino acid supplementation during moderate steady state exercise enhances postexercise muscle protein synthesis. *Am J Clin Nutr* 94: 809–818, 2011. doi:10.3945/ajcn.111.017061.
 33. Pasiakos SM, Vislocky LM, Carbone JW, Altieri N, Konopelski K, Freake HC, Anderson JM, Ferrando AA, Wolfe RR, Rodriguez NR. Acute energy deprivation affects skeletal muscle protein synthesis and associated intracellular signaling proteins in physically active adults. *J Nutr* 140: 745–751, 2010. doi:10.3945/jn.109.118372.
 34. Res PT, Groen B, Pennings B, Beelen M, Wallis GA, Gijzen AP, Senden JM, VAN Loon LJ. Protein ingestion before sleep improves postexercise overnight recovery. *Med Sci Sports Exerc* 44: 1560–1569, 2012. doi:10.1249/MSS.0b013e31824cc363.
 35. Siri WE. The gross composition of the body. *Adv Biol Med Phys* 4: 239–280, 1956. doi:10.1016/B978-1-4832-3110-5.50011-X.
 36. Stein TP, Rumpler WV, Leskiw MJ, Schluter MD, Staples R, Bodwell CE. Effect of reduced dietary intake on energy expenditure, protein turnover, and glucose cycling in man. *Metabolism* 40: 478–483, 1991. doi:10.1016/0026-0495(91)90228-O.
 37. Subar AF, Freedman LS, Tooze JA, Kirkpatrick SI, Boushey C, Neuhauser ML, Thompson FE, Potischman N, Guenther PM, Tarasuk V, Reedy J, Krebs-Smith SM. Addressing current criticism regarding the value of self-report dietary data. *J Nutr* 145: 2639–2645, 2015. doi:10.3945/jn.115.219634.
 38. Taves DR. Minimization: a new method of assigning patients to treatment and control groups. *Clin Pharmacol Ther* 15: 443–453, 1974. doi:10.1002/cpt.1974155443.
 39. Tharion WJ, Lieberman HR, Montain SJ, Young AJ, Baker-Fulco CJ, Delany JP, Hoyt RW. Energy requirements of military personnel. *Appetite* 44: 47–65, 2005. doi:10.1016/j.appet.2003.11.010.
 - 39a. Thomas DT, Erdman KA, Burke LM. American College of Sports Medicine joint position statement. Nutrition and athletic performance. *Med Sci Sports Exerc* 48: 543–568, 2016. doi:10.1249/MSS.0000000000000852.
 40. Welsh TT, Alemany JA, Montain SJ, Frykman PN, Tuckow AP, Young AJ, Nindl BC. Effects of intensified military field training on jumping performance. *Int J Sports Med* 29: 45–52, 2008. doi:10.1055/s-2007-964970.
 41. West DW, Burd NA, Coffey VG, Baker SK, Burke LM, Hawley JA, Moore DR, Stellingwerff T, Phillips SM. Rapid aminoacidemia enhances myofibrillar protein synthesis and anabolic intramuscular signaling responses after resistance exercise. *Am J Clin Nutr* 94: 795–803, 2011. doi:10.3945/ajcn.111.013722.

