Effects of Supplemental Energy on Protein Balance during 4-d Arctic Military Training

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ABSTRACT

MARGOLIS, L. M., N. E. MURPHY, S. MARTINI, Y. GUNDERSEN, J. W. CASTELLANI, J. P. KARL, C. T. CARRIGAN, H.-K. TEIEN, E.-H. MADSLIEN, S. J. MONTAIN, and S. M. PASIAKOS. Effects of Supplemental Energy on Protein Balance during 4-d Arctic Military Training. *Med. Sci. Sports Exerc.*, Vol. 48, No. 8, pp. 1604–1612, 2016. Soldiers often experience negative energy balance during military operations that diminish whole-body protein retention, even when dietary protein is consumed within recommended levels (1.5–2.0 $g \cdot kg^{-1} \cdot d^{-1}$). **Purpose**: The objective of this study is to determine whether providing supplemental nutrition spares whole-body protein by attenuating the level of negative energy balance induced by military training and to assess whether protein balance is differentially influenced by the macronutrient source. **Methods**: Soldiers participating in 4-d arctic military training (AMT) (51-km ski march) were randomized to receive three combat rations (CON) ($n = 18$), three combat rations plus four 250-kcal protein-based bars (PRO, 20 g protein) ($n = 28$), or three combat rations plus four 250-kcal carbohydrate-based bars daily (CHO, 48 g carbohydrate) ($n = 27$). Energy expenditure ($D_2{}^{18}O$) and energy intake were measured daily. Nitrogen balance (NBAL) and protein turnover were determined at baseline (BL) and day 3 of AMT using 24-h urine and [^{15}N]-glycine. **Results**: Protein and carbohydrate intakes were highest ($P < 0.05$) for PRO (mean ± SD, 2.0 ± 0.3 $g \cdot kg^{-1} \cdot d^{-1}$) and CHO (5.8 ± 1.3 $g \cdot kg^{-1} \cdot d^{-1}$), but only CHO increased ($P < 0.05$) energy intake above CON. Energy expenditure (6155 ± 515 $kcal \cdot d^{-1}$), energy balance (−3313 ± 776 $kcal \cdot d^{-1}$), net protein balance (NET) (−0.24 ± 0.60 $g \cdot d^{-1}$), and NBAL (−68.5 ± 94.6 $mg \cdot kg^{-1} \cdot d^{-1}$) during AMT were similar between groups. In the combined cohort, energy intake was associated ($P < 0.05$) with NET ($r = 0.56$) and NBAL ($r = 0.69$), and soldiers with the highest energy intake (3723 ± 359 $kcal \cdot d^{-1}$, 2.11 ± 0.45 g protein·$kg^{-1} \cdot d^{-1}$, 6.654 ± 1.16 g carbohydrate·$kg^{-1} \cdot d^{-1}$) achieved net protein balance and NBAL during AMT. **Conclusion**: These data reinforce the importance of consuming sufficient energy during periods of high energy expenditure to mitigate the consequences of negative energy balance and attenuate whole-body protein loss. **Key Words**: ENERGY EXPENDITURE, MACRONUTRIENTS, ENERGY BALANCE, MILITARY, WHOLE-BODY PROTEIN TURNOVER

Energy balance, the nutritional state when energy intake matches energy expenditure, is a primary determinant of whole-body protein balance (5). Periods when energy expenditure exceed energy intake produce a state of negative energy balance. The level of negative energy balance may be detrimental to skeletal muscle mass because proteins are degraded to provide amino acids that can be used as substrates for oxidation and gluconeogenesis, resulting in increased nitrogen excretion and negative protein balance (3,4,35). Achieving energy balance during periods of high energy expenditures can be challenging, particularly in situations where meals are served at discrete times and/or eating is secondary to the activity (30). Increasing protein intake during periods of modest negative energy balance is an effective nutritional countermeasure to attenuate protein loss and protect muscle mass (1,17). The benefits of high-protein diets are largely independent of body size, because consuming twice and three times the recommended dietary allowance for protein (0.8 $g \cdot kg^{-1} \cdot d^{-1}$) equally preserves protein balance and muscle mass in normal weight individuals exposed to sustained, moderate negative energy balance (21 d, 40% energy deficit; percentage of energy required to achieve energy balance based on the difference between energy expenditure and energy intake) (21).

Military personnel commonly experience negative energy balance during training and combat operations. Negative energy balance is largely driven by sustained periods of low-to-moderate physical activity that result in daily energy expenditures that are difficult to match with energy intake,

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because food supply and time availability to eat or prepare a meal are often limited (16,19). As such, protein recommendations for these scenarios range from 1.5 to 2.0 $g \cdot kg^{-1} \cdot d^{-1}$ (23). Whether current military operational protein recommendations are actually sufficient, particularly if degree of negative energy balance is severe (>40% energy deficit), is not clear. Evidence justifying the recommendations of higher protein diets during military operations was generated from controlled feeding studies that used energy deficits that were generally ≤40% of the energy needed to match expenditure (21,26). Energy deficits during "real-world" military operations may exceed 40% of the energy needed to achieve energy balance. For example, energy expenditures of approximately 6800 $kcal \cdot d^{-1}$ were recently observed in Norwegian Soldiers participating in a 3-d arctic military training (AMT) exercise. Despite being provided nearly 5100 $kcal \cdot d^{-1}$, the soldiers only consumed 50% (3400 $kcal \cdot d^{-1}$) of the energy expended (15). Interestingly, dietary protein intake was within current recommendations (1.7 $g \cdot kg^{-1} \cdot d^{-1}$), but whole-body protein balance decreased during the training operation, suggesting that protein intake under these conditions should have been higher. The possibility exists that attenuating negative energy balance to more manageable energy deficits (<40%) by consuming more carbohydrate, a critical fuel source during sustained operations, could reduce the reliance on endogenous protein and spare whole-body protein balance to a similar extent as increasing protein intake.

The objective of this study was to determine whether supplementing standard combat rations with protein- or carbohydrate-based snack bars would sufficiently increase total energy intake and attenuate negative energy balance during short-term challenging military training, such that whole-body protein balance is maintained when compared with consuming combat rations alone. We hypothesized that calorically equivalent protein- and carbohydrate-based supplemental nutrition would similarly diminish the level of negative energy balance by increasing total energy intake, but that whole-body protein balance would be better maintained when protein is the primary form of supplemental nutrition.

MATERIALS AND METHODS

Participants/experimental design. Norwegian army soldiers stationed in Skjold, Norway, participating in a 4-d AMT operation, were recruited to participate in this randomized controlled trial. After providing informed, written consent, participants ($n = 71$ males, $n = 2$ females) were block randomized by body mass to one of three dietary treatment groups, each receiving three Norwegian arctic combat rations, either alone (control, CON) or supplemented with four whey protein-based (PRO) or four carbohydrate-based (CHO) snack products. Baseline (BL) (study days −1 and 0) whole-body protein turnover, nitrogen balance (NBAL), and body mass and composition were measured before starting the 4-d AMT (see Figure, Supplemental Digital Content 1, study design,

http://links.lww.com/MSS/A677). Beginning on day 1, participants performed cross-country skiing in 50/10-min work-to-rest ratios while carrying an approximately 45-kg pack for a total distance of approximately 13 $km \cdot d^{-1}$. The total distance covered was about 51 km. Energy expenditure and energy and macronutrient intake were assessed daily. Whole-body protein turnover and NBAL were reassessed on day 3 of AMT, whereas change in body mass was determined at the conclusion of training (POST) (day 5).

This study was approved by the Institutional Review Board of the US Army Research Institute of Environmental Medicine (Natick, MA) and the Regional Committees for Medical and Health Research Ethics (REK sør-øst, Oslo, Norway; www.clinicaltrials.gov NCT02327208). It is also important to note that the 4-d AMT was not developed for this study; rather, the exercise is an annual mandatory training operation for the second battalion stationed at the garrison in Skjold. Our study did not alter the training exercise, which was conducted in January 2015.

Anthropometrics and body composition. Vertical height was measured at BL to the nearest 0.1 cm using a stadiometer (Seca; Creative Health Products, Plymouth, MI). Seminude (underwear only) body mass was measured using a calibrated digital scale (Befour model PS6600; Befour Inc., Saukville, WI) at BL and POST to the nearest 0.1 kg. Body composition was determined at baseline from skinfold thickness measurements of the chest, triceps, and subscapular for men, and the triceps, suprailiac, and abdomen for women, by a trained technician using Lange calipers (Beta Technology, Santa Cruz, CA) (12,13). Body composition data were used to characterize participants at BL as well as used for estimates of resting metabolic rate (RMR) and in regression modeling, described below, to correct for influence of fat-free mass (FFM) on energy expenditure. This predictive model was then used to estimate daily energy expenditure for the entire study cohort.

Energy intake, expenditure, and components of energy balance. Beginning on day −1 and through the duration of the study, participants were provided three Norwegian arctic combat rations to consume daily as per local command policy. No outside food was permitted for the study participants. Three combat rations provided approximately 3487 $kcal \cdot d^{-1}$, 141 $g \cdot d^{-1}$ protein, 435 $g \cdot d^{-1}$ carbohydrate, and 126 $g \cdot d^{-1}$ fat if entirely consumed. Beginning on day 1 of the AMT, participants assigned to PRO and CHO were provided with four snack bars per day to be consumed in addition to the provided rations. The four whey-based PRO bars provided 1062 $kcal \cdot d^{-1}$, 85 $g \cdot d^{-1}$ protein, 102 $g \cdot d^{-1}$ carbohydrate, and 35 $g \cdot d^{-1}$ fat, whereas four CHO bars provided 1058 $kcal \cdot d^{-1}$, 11 $g \cdot d^{-1}$ protein, 189 $g \cdot d^{-1}$ carbohydrate, and 29 $g \cdot d^{-1}$ fat. The snack bars were manufactured by the Combat Feeding Directorate at the Natick Soldier Systems Center (Natick, MA) and designed to be isocaloric and similar in serving size, taste, and textural qualities.

The primary objective and practical application of this investigation was to determine whether the incorporation of

supplement nutrition in the form of protein- or carbohydrate-based snack bars would augment field feeding practices during real-world military training operations by increasing total energy intake, thereby attenuating the negative energy balance caused by high energy expenditures. There were no specific requirements for participants to consume all ration items and snack bars provided to them; rather, participants were instructed to consume the three combat rations as they normally would during training and to consume snack bars between meals. Participants were instructed to only eat the bars they received and not trade or share bars with other soldiers participating in AMT.

To determine the amount of ration components and snack bars consumed daily, participants were provided with food logs that contained a list of all the items for each provided ration. Before AMT, participants were trained to record the percent of each item consumed using the provided logs. This technique has been used effectively by our laboratory in previous investigations to characterize combat ration intake during military operations (2,15,16). Food logs also included a 10-cm visual analog scale asking participants to rate their level of hunger, with 0 cm being "not hungry at all" and 10 cm being "very hungry." Food logs were collected daily, with trained registered dietitians verifying items consumed with each participant. The amount of each ration item consumed was subtracted from the known initial amount provided to calculate energy, protein, carbohydrate, and fat intake. Nutritional composition of all combat ration items included in this study was confirmed by chemical analysis to verify the accuracy of our dietary analyses (Covance Laboratories, Inc., Madison, WI).

Energy expenditure was assessed in a subset of participants using the doubly labeled water (DLW) technique. After an overnight fast and before dosing of DLW, a subset of participants ($n = 14$ PRO, $n = 14$ CHO, $n = 14$ CON) provided a baseline urine sample to correct for background abundance of ^{18}O and ^{2}H. After ingestion of DLW (0.23 g of $H_2^{18}O$ per total body water (TBW) (kg) and 0.15 g of $^{2}H_2O$ per TBW (kg); Sigma-Aldrich, St. Louis, MO), participants fasted for 4 h to minimize disturbance of isotopic enrichment. At 4 and 6 h postdose, participants provided urine samples to determine peak enrichment. On subsequent study days, daily morning voids were collected for determination of isotopic elimination rates, calculated by linear regression from the rate of disappearance in the urine over AMT. Enrichments of ^{2}H and ^{18}O were assessed using isotope ratio mass spectrometry (Finnigan Mat 252; Thermo Fisher Scientific, Waltham, MA). Determination of CO_2 production to calculate energy expenditure was determined according to Schoeller et al. (29):

$$r CO_2 \left(mol \cdot d^{-1}\right) = (N/2.078)(1.01K_O - 1.04K_H) - 0.0246 r H_2O_f$$

where N is TBW; K_O and K_H are ^{18}O and ^{2}H isotope disappearance rates, respectively; and $r H_2O_f$ is the rate of fractionated evaporated water loss, estimated to be 10.5N (1.01K_O − 1.04K_H). Energy expenditure was calculated using the energy equivalent of CO_2 for a respiratory quotient of 0.86.

To account for ^{2}H and ^{18}O abundance in local drinking water and background shift during the ski march, three participants were dosed with a placebo (tap water) rather than DLW to serve as the control. Drinking water from the garrison and on the ski march was collected and analyzed to correct for background abundance.

To account for the influence of FFM on energy expenditure between groups, regression modeling, with FFM as a covariate, was conducted (27). The predictive equation generated was then used to determine estimated energy expenditure of participants not dosed with DLW. The equation was constructed from the constant and β of FFM determined by regression model.

$$\text{energy expenditure model} \left(kcal \cdot d^{-1}\right) = 1291 + (69.1 FFM)$$

Energy balance was calculated by subtracting mean energy intake from mean total daily energy expenditure during AMT. Furthermore, to determine the contribution of physical activity to total daily energy expenditure, RMR was estimated using baseline measures of FFM (6):

$$\text{RMR} \left(kcal \cdot d^{-1}\right) = 370 + (21.6 FFM)$$

Diet-induced thermogenesis was estimated as 10% total daily energy expenditure (38), with activity-induced energy expenditure calculated as total daily energy expenditure minus RMR and diet-induced thermogenesis (39). Physical activity level (PAL) was defined as the ratio of total daily energy expenditure to RMR (37).

Whole-body protein turnover. The end-product method was used to assess 24-h whole-body protein turnover at BL and during AMT to determine the influence of energy balance, as well as energy and macronutrient intake on whole-body protein kinetics between groups (PRO, CHO, and CON) and over time (36). This method has previously been shown to be effective in estimating whole-body protein turnover in free living participants (8). In addition, assessing total urinary nitrogen enrichment accounts for 80% to 85% of total nitrogen excretion, thereby providing a reasonable estimate of whole-body protein balance (11,32). Before ingestion of a single bolus (4 mg·kg^{-1}) of [^{15}N]-glycine (Cambridge Isotope Laboratories, Andover, MA), participants provided a spot urine to correct for background isotope enrichment and to serve as the starting point of the 24-h urine collection time. The 24-h collection ended with the first void of the following morning. Urine containers from 12 participants were damaged or lost during AMT; as such, 61 total BL and AMT measures were included in the final analyses.

Total nitrogen enrichment was used to determine whole-body protein turnover to maintain consistency with our previous work (15) and to minimize any bias of enrichment

partitioning between the ammonia and the urea nitrogen pools (8). Enrichment of tracer to tracee (tr:T) for [^{15}N]-nitrogen was determined using isotope ratio mass spectrometry (Metabolic Solutions Inc., Nashua, NH). Whole-body protein flux (Q), protein synthesis (PS), protein breakdown (PN), and net protein balance (NET) were calculated as follows:

$$Q \ (gN{\cdot}kg^{-1}{\cdot}d^{-1}) = d/\text{corrected tr:T}/24 \ \times \ \text{body mass}$$

$$PS \ (g{\cdot}kg^{-1}{\cdot}d^{-1}) = Q - (E/24 \ \times \ \text{body mass}) \ \times \ 6.25$$

$$PB \ (g{\cdot}kg^{-1}{\cdot}d^{-1}) = Q - (I/24 \ \times \ \text{body mass}) \ \times \ 6.25$$

$$NET \ (g{\cdot}kg^{-1}{\cdot}d^{-1}) = PS - PB$$

where d is the [^{15}N] oral dose (g glycine $\times$ 0.1972); E is the 24-h urinary nitrogen excretion; I is the 24-h nitrogen intake; and 6.25 is the conversion of nitrogen to protein. All samples were run in duplicate. Quality control samples (ammonia sulfate) at three different enrichments were run at the beginning and end of the batch run. During the batch run, a quality control sample was run after every 10 samples to check performance of the instrument. ^{15}N measurements were reported against atmospheric nitrogen (^{15}N/^{14}N ratio of 0.0036765), with a delta range of 7 to 10 delta per mil at BL and 70 to 750 delta per mil for enriched urines at AMT.

NBAL. Nitrogen excretion and NBAL were determined from the 24-h urine collection used to assess whole-body protein turnover. Nitrogen content was determined from a single pooled 24-h urine sample using pyrochemiluminescence (Antek 9000; Antek Instruments, Houston, TX). NBAL was calculated as the difference of nitrogen intake minus urinary nitrogen excretion plus miscellaneous (estimated as 5 mg·kg^{-1}) and fecal (estimated as 2 mg·kg^{-1}) nitrogen losses (9). To confirm complete 24-h urine collections, urinary creatinine was measured using the Jaffe reaction (UniCel DXC 600 Pro; Beckman Coulter, Brea, CA). For incomplete 24-h urine collections during AMT ($n = 20$, due to weather and logistical constraints), baseline 24-h urinary creatinine was used to correct nitrogen excretion (31). To verify whether it was appropriate to include these data in our report, statistical analyses were performed and measures of NBAL and NET during AMT were not different between data sets (i.e., all urine samples vs urine samples excluding incomplete 24-h collections). Therefore, all 24-h urine collections and their resulting outcomes were included in the final analyses.

Statistical analysis. Participants were randomized 3/1 (intervention/control) to capture differences between PRO and CHO groups during AMT, which were likely to have smaller between-group differences compared with CON. Normality was confirmed using Shapiro–Wilk tests for dependent variables. One-way ANOVA was used to assess between-group (PRO, CHO, and CON) differences for baseline characteristics, energy expenditure, energy and macronutrient intake, and energy balance. Differences between energy expenditure and energy intake were determined using an independent t-test. A χ^2 test was conducted to assess between-group differences for categorical dietary intake data. Repeated-measures ANOVA was used to determine the main effects of time (BL and AMT or POST), group, and time–group interactions for body mass, hunger, and whole-body protein flux, synthesis, and breakdown. An ANCOVA was used to assess AMT NBAL and NET by group adjusting for BL NBAL and NET.

To further examine associations between dietary intake and NBAL and whole-body protein turnover, the full cohort was combined and exploratory analyses conducted irrespective of the diet group. Linear regression was used to examine associations between energy, protein, carbohydrate, and fat intakes (expressed as percentage energy intake), and NBAL, flux, PS, PB, and NET during AMT. Regression analysis showed that energy intake was a positive predictor of NBAL and NET during AMT. Associations between energy intake and NBAL and NET were further examined by separating energy intake into quartiles and expressed as a categorical variable. In this model, differences in energy expenditure, energy and macronutrient intake, energy balance, and change in body mass between quartiles were assessed using one-way ANOVA. An ANCOVA was used to assess AMT NBAL and NET by energy intake quartile, adjusting for BL NBAL and NET. Bonferroni adjustment was used for *post hoc* analysis for significant main effects. Data were analyzed using IBM SPSS Statistics for Windows (version 22.0; IBM Corp., Armonk, NY). Significance was set at $P < 0.05$, and data are presented as mean ± SD.

RESULTS

Participant characteristics. Although sample size differed between the PRO ($n = 28$) and the CHO ($n = 27$) compared with the CON ($n = 18$), there were no group differences for any baseline characteristic, particularly age (PRO, 20 ± 1; CHO, 20 ± 1; CON, 19 ± 2), weight (PRO, 77.8 ± 9.1; CHO, 78.2 ± 8.8; CON, 77.7 ± 6.6), and FFM (PRO, 71.4 ± 8.6; CHO, 69.7 ± 7.3; CON, 69.7 ± 7.9) (see Table, Supplemental Digital Content 2, participant descriptions, http://links.lww.com/MSS/A678).

Energy and macronutrient intake. During AMT, participants in PRO, CHO, and CON consumed 62% ± 13%, 69% ± 14%, and 72% ± 12%, respectively, of the total energy provided. The percentage of energy intake attributed to the energy provided in the combat rations was lower ($P < 0.05$) for PRO (56% ± 15%) than CON (72% ± 12%, Table 1). There was no difference in the percentage energy intake from the rations provided for CHO (64% ± 14%) compared with PRO and CON. Participants in the PRO group consumed less ($P < 0.05$) of all ration items compared with CON, expect for condiments, with the greatest difference observed for consumption of snacks, dried fruit, and bread (Table 2). Participants in the PRO and CHO groups consumed 82% ± 19% and 85% ± 21%, respectively, of the provided energy

TABLE 1. Energy and macronutrient intake during AMT.

	PRO	CHO	CON
Ration			
Energy (kcal·d^{-1})	1953 ± 518^a (1001–3099)	2227 ± 493^{a,b} (1387–2918)	2506 ± 409^{b,c} (1557–3111)
Protein (g·d^{-1})	78 ± 19^a (38–117)	88 ± 20^{a,b} (50–113)	100 ± 15^{b,c} (65–124)
Carbohydrate (g·d^{-1})	238 ± 70^a (87–401)	274 ± 62^{a,b} (163–368)	312 ± 47^{b,c} (193–385)
Fat (g·d^{-1})	73 ± 20^a (37–110)	82 ± 21^{a,b} (45–116)	91 ± 20^{b,c} (57–117)
Supplements			
Energy (kcal·d^{-1})	872 ± 202^a (259–1128)	904 ± 224^a (282–1140)	—
Protein (g·d^{-1})	70 ± 15^a (24–90)	10 ± 3^b (3–20)	—
Carbohydrate (g·d^{-1})	83 ± 20^a (21–108)	160 ± 40^b (48–204)	—
Fat (g·d^{-1})	29 ± 7^a (9–37)	25 ± 6^b (9–32)	—
Total (absolute)			
Energy (kcal·d^{-1})	2825 ± 599^{a,b} (1691–4029)	3131 ± 633^a (1843–3940)	2506 ± 410^{b,c} (1557–3111)
Protein (g·d^{-1})	148 ± 25^a (96–191)	98 ± 22^b (58–130)	100 ± 15^b (65–124)
Carbohydrate (g·d^{-1})	321 ± 77^a (171–490)	434 ± 86^b (253–543)	312 ± 47^a (193–385)
Fat (g·d^{-1})	102 ± 23^a (59–141)	107 ± 24^a (56–146)	91 ± 20^a (57–117)
Total (relative)			
Energy (kcal·kg^{-1}·d^{-1})	37.3 ± 7.8^{a,b} (22.2–50.2)	42.2 ± 9.9^a (23.9–57.6)	33.6 ± 6.2^{b,c} (19.1–43.2)
Protein (g·kg^{-1}·d^{-1})	2.0 ± 0.3^a (1.3–2.6)	1.3 ± 0.3^b (0.7–1.9)	1.3 ± 0.2^b (0.8–1.7)
Carbohydrate (g·kg^{-1}·d^{-1})	4.2 ± 1.0^a 3(2.2–6.1)	5.8 ± 1.3^b (3.3–8.0)	4.2 ± 0.7^a (2.4–5.3)
Fat (g·kg^{-1}·d^{-1})	1.3 ± 0.3^a (0.8–1.8)	1.4 ± 0.4^a (0.7–2.0)	1.2 ± 0.3^a (0.7–1.7)

The values are presented as mean ± SD and range (min–max).
Values not sharing the same superscript are different, $P < 0.05$.

from treatment snack bars, with no difference between groups (Table 1). Provision of protein and carbohydrate snack bars during AMT was successful in altering macronutrient intake between groups, but despite PRO and CHO receiving an additional approximately 1000 kcal·d^{-1}, only CHO achieved a significantly higher ($P < 0.05$) total daily energy intake compared with CON, with no difference between PRO and CHO, or PRO and CON. Mean hunger increased ($P < 0.05$) from 5.4 ± 1.7 at BL to 6.4 ± 1.3 during AMT, with no difference between groups.

Energy balance. Total daily energy expenditure of all participants during AMT was 6155 ± 515 kcal·d^{-1}, with no

TABLE 2. Percent ration items consumed (%).

	Energy (kcal·d^{-1})*	PRO	CHO	CON
Entreés	517 (450–543)	88^a	88^a	94^b
Snacks	152 (72–314)	51^a	62^b	74^c
Dried fruit	156 (93–157)	47^a	51^{a,b}	64^b
Candy	77 (5–261)	40^a	40^a	52^b
Bread	205 (N/A)‡	38^a	48^a	68^b
Spreads	47 (46–49)	28^a	29^a	33^b
Sides	132 (N/A)	18^a	18^a	29^b
Condiments	20 (N/A)	10^a	34^b	12^a
Powdered beverages	36 (4–130)	18^a	19^a	17^b

Values not sharing the same superscript are different, $P < 0.05$.
*Mean (range) energy content of rations component.
‡No range.

TABLE 3. Energy balance and expenditure.

	PRO	CHO	CON
Energy balance (kcal·d^{-1})	−3402 ± 687	−3050 ± 888	−3595 ± 606
Energy expenditure (kcal·d^{-1})^a	6167 ± 592	6181 ± 505	6096 ± 412
RMR (kcal·d^{-1})	1894 ± 185	1898 ± 158	1871 ± 129
Thermic effect of feeding (kcal·d^{-1})	617 ± 59	618 ± 50	610 ± 41
Activity-induced energy expenditure (kcal·d^{-1})	3657 ± 348	3665 ± 297	3615 ± 242
PAL	3.29 ± 0.03	3.29 ± 0.03	3.29 ± 0.02

The values are presented as mean ± SD.
Means were not different between groups, $P > 0.05$.
^{a}Adjusted energy expenditure for FFM using regression modeling.

differences between groups (Table 3). Estimated RMR accounted for 31% (1890 ± 161 kcal·d^{-1}) of total daily energy expenditure, with thermic effect of feeding estimated to contribute 10% (615 ± 51 kcal·d^{-1}) of total daily energy expenditure; activity-induced energy expenditure accounted for the remaining 59% (3649 ± 302 kcal·d^{-1}) of total energy expenditure. High daily activity-induced energy expenditures resulted in a PAL of 3.3 ± 0.1 for all groups. There were no differences between dietary treatment groups for any energy expenditure-related variable.

Energy expenditure exceeded ($P < 0.05$) energy intake similarly across dietary treatment groups, because the negative energy balance for CON was not different from the deficits observed for PRO and CHO, averaging 54% (3313 ± 776 kcal·d^{-1}) of the total energy requirements for all participants (Table 3). As a result, loss of body mass was similar for PRO (2.6 ± 1.2 kg), CHO (2.9 ± 1.3 kg), and CON (2.7 ± 1.1 kg).

Whole-body protein turnover and NBAL. For calculations of whole-body protein turnover and NBAL on day 3 of AMT, dietary protein and nitrogen intake were highest ($P < 0.05$) for PRO (155 ± 40 g·d^{-1}, 25 ± 6 g·d^{-1}) compared with CHO (101 ± 29 g·d^{-1}, 16 ± 5 g·d^{-1}) and CON (114 ± 22 g·d^{-1}, 18 ± 3 g·d^{-1}). Within groups, daily protein and nitrogen intakes were similar ($P > 0.05$). The AMT resulted in a downregulation ($P < 0.05$) of whole-body protein flux (−0.19 ± 0.35 gN·kg^{-1}·d^{-1}), PS (−1.62 ± 2.41 g·kg^{-1}·d^{-1}), and PB (−1.47 ± 2.49 g·kg^{-1}·d^{-1}) compared with baseline, regardless of the dietary treatment group (Fig. 1A–C). NET was negative during AMT for PRO (−0.15 ± 0.66 g·kg^{-1}·d^{-1}), CHO (−0.24 ± 0.64 g·kg^{-1}·d^{-1}), and CON (−0.38 ± 0.46 g·kg^{-1}·d^{-1}) with no differences between groups. Similarly, NBAL was negative (PRO, −55.4 ± 106.8; CHO, −70.0 ± 101.4; CON, −85.1 ± 66.5 mg·kg^{-1}·d^{-1}) during AMT, but not affected by the dietary treatment group.

Given that no treatment effects were observed and that energy and macronutrient intake varied considerably across groups, linear regression analysis was used to evaluate associations between energy, protein, carbohydrate, and fat intake on NBAL and NET. Energy intake was positively ($P < 0.05$) associated with NBAL ($r = 0.59$, $r^2 = 0.34$) and NET ($r = 0.56$, $r^2 = 0.32$), whereas energy balance was ($P < 0.05$) associated with NBAL ($r = 0.51$, $r^2 = 0.26$) and NET ($r = 0.50$, $r^2 = 0.25$). Because of the stronger association for energy intake, data were used to separate participants into quartiles (quartile 1,
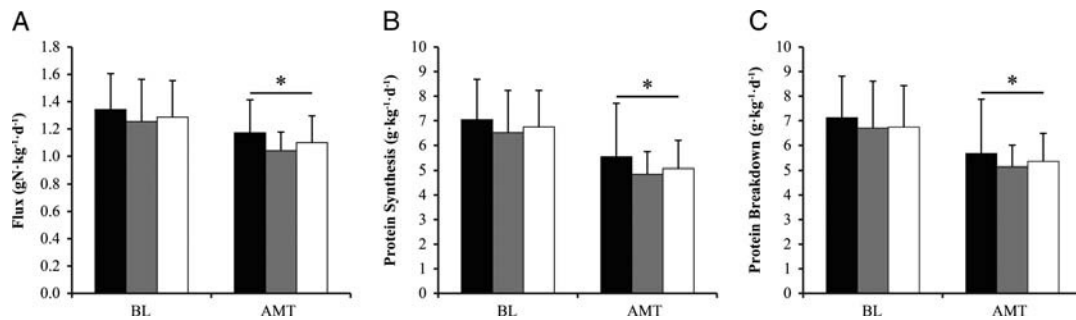
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FIGURE 1—Determination of whole-body protein flux (A), synthesis (B), and breakdown (C) from BL to AMT between PRO (■), CHO (■), CON (□). Values presented as mean ± SD. *Main effect of time AMT different from BL, $P < 0.05$.

<2356 kcal·d^{-1}; quartile 2, 2356–2862 kcal·d^{-1}; quartile 3, 2863–3303 kcal·d^{-1}; quartile 4, >3303 kcal·d^{-1}). Participants in quartile 4 consumed more energy, resulting in a lower negative energy balance compared with quartiles 3, 2, and 1 ($P < 0.05$, Table 4). Participants in quartile 4 also tended ($P = 0.06$) to lose less body mass, particularly when compared with quartile 1. NBAL and NET were also higher ($P < 0.05$) in quartile 4 during AMT than quartile 1 (Fig. 2). No other differences were observed across quartiles.

DISCUSSION

The primary finding of this study was that during a challenging AMT, which produced energy expenditures as high as 6000 kcal·d^{-1}, increasing energy intake, regardless of the macronutrient source, attenuated negative energy balance and spared whole-body protein. High daily energy expenditures were the product of activity-induced energy expenditures (3650 kcal·d^{-1}). Whole-body protein losses were only reduced in those that consumed enough energy to reduce negative energy balance to levels comparable with those typically used to elicit moderate weight loss (approximately 40% energy deficit). Although supplementing combat rations with eat-on-the-move protein- or carbohydrate-based snack bars was an effective approach in altering macronutrient intake, eating the bars did not independently attenuate negative energy balance or decrements in protein balance. These findings highlight the difficulty of optimizing military field feeding, as participants in

PRO and CHO compensated for the extra energy consumed in the snack products by consuming less of their combat rations.

The dietary intervention largely failed to attenuate the severity of negative energy balance, despite higher energy intake for those assigned to CHO compared with CON. As such, there was no apparent benefit of providing supplemental protein or carbohydrate on NBAL and NET during the 4-d AMT. Energy balance, although highly variable (-1700 to -5200 kcal·d^{-1}), was associated with NBAL and NET. In fact, whole-body protein loss was negated for the participants who consumed the most energy, an observation consistent with previous works establishing the relation between energy balance and protein use (18,33,41). This finding emphasizes that during periods of high-level physical activity, attention to food discipline so as to better match energy intake and energy expenditure, to reduce the level of negative energy balance, is warranted.

Although participants with the highest energy intake achieved protein balance, they remained in a severe state of negative energy balance (-2400 kcal·d^{-1}, 39% energy deficit), suggesting that at this level of negative energy balance, macronutrient intake may have had influence in mitigating protein losses. In agreement with our previous study (15), consuming 1.7 g·kg^{-1}·d^{-1} or more of dietary protein failed to independently modulate NBAL and NET, when negative energy balance exceeded 3000 kcal·d^{-1} ($>50\%$ energy deficit). However, consistent with our hypothesis, attenuating negative energy balance limited whole-body protein loss with elevated protein and carbohydrate intakes. In fact, 14 of the 15 participants in

TABLE 4. Energy balance and macronutrient intake by energy intake quartile.

	Quartile 1 ($n = 15$)	Quartile 2 ($n = 15$)	Quartile 3 ($n = 15$)	Quartile 4 ($n = 15$)
Energy intake (kcal·d^{-1})	1783 ± 437[a]	2658 ± 149[b]	3076 ± 136[c]	3723 ± 360[d]
Energy expenditure (kcal·d^{-1})	5920 ± 483[a]	6375 ± 637[a]	6276 ± 427[a]	6133 ± 359[a]
Energy balance (kcal·d^{-1})	−4138 ± 696[a]	−3740 ± 700[a,b]	−3229 ± 489[b]	−2410 ± 582[c]
△Body mass*	−3.17 ± 1.20[a]	−3.11 ± 1.38[a]	−2.59 ± 1.17[a]	−1.95 ± 1.33[a],†
Protein (g·kg^{-1}·d^{-1})	1.16 ± 0.36[a]	1.68 ± 0.47[b]	1.74 ± 0.27[b]	2.11 ± 0.45[c]
Carbohydrate (g·kg^{-1}·d^{-1})	3.26 ± 0.97[a]	4.13 ± 0.59[a,b]	4.97 ± 0.94[b]	6.54 ± 0.1.16[c]
Fat (g·kg^{-1}·d^{-1})	0.77 ± 0.25[a]	1.13 ± 0.21[b]	1.34 ± 0.19[c]	1.66 ± 0.19[d]

The values are presented as mean ± SD.
Values not sharing the same superscript are different, $P < 0.05$.
*Delta body mass calculated as POST − BL.
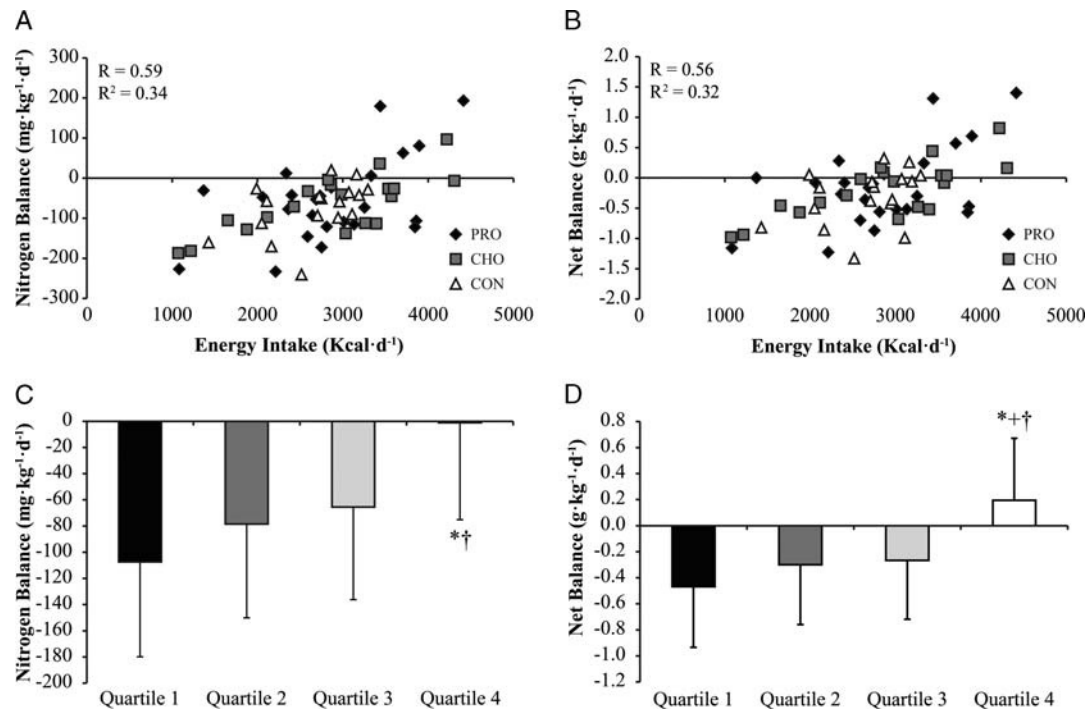†Tendency to being different from quartile 1, $P = 0.06$.

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FIGURE 2—Correlation of energy intake for PRO (♦), CHO (■), and CON (△) to NBAL (A) and NET (B). Comparison for NBAL (C) and NET (D) between energy quartiles. Values presented as mean ± SD. *Different from quartile 1, $P < 0.05$. †Different from quartile 2, $P < 0.05$. +Different from quartile 3, $P < 0.05$.

quartile 4, where protein balance was maintained, were assigned to PRO ($n = 7$) and CHO ($n = 7$). Those soldiers not only consumed more food but also consumed protein at or slightly above the upper limit of current military operational protein recommendations (>2.0 $g·kg^{-1}·d^{-1}$) (20). Likewise, carbohydrate intake for those who consumed the most energy was about 6.5 $g·kg^{-1}·d^{-1}$, a level within current recommendations (6–10 $g·kg^{-1}·d^{-1}$) for endurance-type exercise (28). These data suggest that achieving a more manageable energy deficit during demanding military operations by consuming both protein (2.0 $g·kg^{-1}·d^{-1}$) and carbohydrate (6.5 $g·kg^{-1}·d^{-1}$) within recommended levels could positively affect skeletal muscle mass and performance.

Whole-body protein turnover was downregulated during AMT. Typically, during acute negative energy balance (≤3 day), whole-body protein flux and breakdown are upregulated to liberate amino acids for energy production (5,14,18). Down-regulations in whole-body protein turnover generally occur with sustained (≥14 d) negative energy balance, because adaptation occurs and amino acids are no longer heavily relied on as an energy source (22,40). Measuring whole-body protein turnover over a 24-h period (10) that included high levels of physical activity (14), in both fasted and fed states (14,18), is likely the reason why our turnover data differs from past studies. Our findings show that during periods of severe negative energy balance caused primarily by exercise-induced energy expenditure, there is a downregulation in whole-body protein turnover, representing an adaptive response to spare endogenous protein.

Providing energy-dense supplemental nutrition during sustained (8 wk), metabolically challenging (5000 $kcal·d^{-1}$) military operations has been an effective countermeasure, attenuating negative energy balance and reducing total body mass loss from 5.0 to 1.6 kg (7). In the current investigation, despite strong compliance with the supplemental nutrition intervention, the treatment failed to attenuate negative energy balance, decrements in NBAL and NET, and losses in body mass compared with CON. The lack of an effect can be explained by the fact that snack bars were consumed at the expense of some of the food in the rations. Participants in the PRO and CHO groups consumed about 500 and 300 $kcal·d^{-1}$ less from their rations compared with CON. Diminished ration consumption and wide ranges in energy and macro-nutrient consumption within groups can be attributed to *ad libitum* intake during the training operation.

We recognize that not controlling dietary intake during this field feeding study limits our ability to assess the effects of increased energy intake from protein or carbohydrate, but *ad libitum* intake more accurately depicts how soldiers actually eat during military operations. That the soldiers chose to eat less when provided with our snack items raises questions as how or what approach should be used to increase voluntary energy intake. Drinks rather than bars might minimize reductions in ration intake by reducing feeling of fullness (34), but powdered carbohydrate drinks were the least consumed food product in the rations provided. Providing additional food or augmenting current ration items to provide energy in other forms may allow soldiers to eat as they train,

minimize the effect on overall ration intake, and improve energy intake to support performance and function during sustained operations (24,25).

In conclusion, the current investigation reinforces the importance of eating during challenging military training operations, because protein balance was maintained in those soldiers who ate enough to reduce the level of negative energy balance to an energy deficit of <40% of the total daily energy requirements. Those soldiers who maintained protein balance in the present investigation consumed protein at the upper end of the recommendations (2.0 g·kg^{-1}·d^{-1}), suggesting a benefit of higher protein intake, as long the deficits, as a percentage of energy requirements, are less than 40%. In addition, consumption of 6.5 g·kg^{-1}·d^{-1} carbohydrate likely also contributed to the maintenance of whole-body protein balance by providing a readily available substrate for exercise metabolism. Although benefits were observed with increased energy intake, attempting to increase energy intake with supplemental snack bars was ineffective. Future studies are warranted to identify effective military field feeding strategies that maintain recommended intakes of protein and carbohydrate, attenuate negative energy balance, sustain exercise metabolism, and spare whole-body protein.

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