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Acute Energy Deprivation Affects Skeletal Muscle Protein Synthesis and Associated Intracellular Signaling Proteins in Physically Active Adults[1,2]

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Abstract

To date, few studies have characterized the influence of energy deprivation on direct measures of skeletal muscle protein turnover. In this investigation, we characterized the effect of an acute, moderate energy deficit (10 d) on mixed muscle fractional synthetic rate (FSR) and associated intracellular signaling proteins in physically active adults. Eight men and 4 women participated in a 20-d, 2-phase diet intervention study: weight maintenance (WM) and energy deficient (ED; ~80% of estimated energy requirements). Dietary protein (1.5 $g \cdot kg^{-1} \cdot d^{-1}$) and fat (~30% of total energy) were constant for WM and ED. FSR and intracellular signaling proteins were measured on d 10 of both interventions using a primed, constant infusion of [$^2H_5$]-phenylalanine and Western blotting techniques, respectively. Participants lost ~1 kg body weight during ED ($P < 0.0001$). FSR was reduced ~19% ($P < 0.05$) for ED (0.06 ± 0.01%/h) compared with WM (0.074 ± 0.01%/h). Protein kinase B and eukaryotic initiation factor 4E binding protein 1 phosphorylation were lower ($P < 0.05$) during ED compared with WM. AMP activated protein kinase phosphorylation decreased ($P < 0.05$) over time regardless of energy status. These findings show that FSR and associated synthetic intracellular signaling proteins are downregulated in response to an acute, moderate energy deficit in physically active adults and provide a basis for future studies assessing the impact of prolonged, and perhaps more severe, energy restriction on skeletal muscle protein turnover. J. Nutr. 140: 745–751, 2010.

Introduction

Skeletal muscle protein turnover, i.e. the balance between protein synthetic and catabolic processes, is affected by energy balance (1,2). Studies have shown that energy deprivation, a common approach to weight management, often results in loss of skeletal muscle, altered whole-body protein metabolism, and a reduction in energy expenditure that can compromise body weight regulation (2,3). The extent to which these metabolic processes are affected depends largely upon the degree to which energy is restricted. In general, acute energy deprivation associated with fasting, as well as more severe energy restriction, results in an increase in whole-body proteolysis, amino acid oxidation, and nitrogen excretion, which becomes less pronounced and plateaus over an extended period of time as the body adapts to conserve energy and protein reserves (i.e. muscle protein) (4–7). Much of the work regarding protein metabolism in the context of energy deprivation has focused on whole-body protein turnover rather than skeletal muscle. To date, limited studies have characterized the skeletal muscle protein metabolic response to an acute energy deficit using muscle biopsies, stable isotope methodology, and strictly controlled diet interventions.

Protein is emerging as a crucial component of energy-restricted diets as a macronutrient that may potentially offset the increased demand for metabolic substrates, maintain energy expenditure, and optimize protein utilization under energy-deprived conditions (8). The literature is replete with studies showing that protein intakes in excess of the current recommended dietary allowance (RDA)[6] of 0.8 $g \cdot kg^{-1} \cdot d^{-1}$, but within

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[6] Abbreviations used: Akt, protein kinase B; AMPK, AMP activated protein kinase; BCAA, branched-chain amino acid; 4E-BP1, eukaryotic initiation factor 4E binding protein 1; ED, energy deficient; FSR, mixed muscle fractional synthetic rate; NBAL, nitrogen balance; NEAA, nonessential amino acids; RDA, recommended dietary allowance; REST, rest isotope infusion protocol; WBPTO, whole body protein turnover; WM, weight maintenance.

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the acceptable macronutrient distribution range, offer metabolic advantages during energy-restricted states (9–11). In these studies, characterization of protein utilization relied on techniques such as body composition, nitrogen balance, and stable isotope methodology to assess whole body protein turnover (WBPTO) (6,7,12). Collectively, this work provides insight into the impact of the level of protein intake on protein metabolism during energy restriction. However, because skeletal muscle comprises ~40% of total body protein stores and contributes to only 30–50% of whole body protein metabolism, findings from such studies do not reflect the specific metabolic response of skeletal muscle to well-controlled energy restriction (13).

This study was designed to characterize skeletal muscle protein synthesis and associated intracellular signaling events in response to an acute, moderate energy deficit in physically active adults. We hypothesized that skeletal muscle protein synthesis, in conjunction with protein anabolic signaling events, would be downregulated following short-term energy deprivation. This study is unique in that stable isotope methodology and muscle biopsies were employed in well-controlled diet intervention studies to simultaneously assess molecular regulatory proteins and skeletal muscle protein kinetics specific to protein synthesis during a state of negative energy balance.

Methods

Participants. Power analysis, based on previously reported skeletal muscle protein turnover measurements, indicated that 5 volunteers would be needed to detect a within-participant effect of energy deprivation with power (β = 0.8) and $P < 0.05$ (14). Therefore, following approval by the Institutional Review Board at the University of Connecticut, 12 physically active men (n = 8) and women (n = 4) were recruited from spring 2006 to summer 2007 to participate in this controlled, 2-phase [weight maintenance (WM) and energy deficient (ED)] diet intervention study. This particular population was of interest given the propensity for either intentional or inadvertent periods of energy restriction by these individuals. Prior to entry into the study, all participants provided a detailed medical history, 7-d physical activity log, and a 3-d diet record. All participants were considered physically active and determined to be fit as indicated by baseline study screening (VO_{2peak} >40 mL · kg^{-1} · min^{-1} for women and >45 mL · kg^{-1} · min^{-1} for men). In addition, all women were eumenorrheic as determined by self-report and were nonpregnant. Any individual reporting metabolic or cardiovascular abnormalities; food allergies; gastrointestinal disorders (i.e. lactose intolerance); vegetarian practices; or use of nutritional/sports supplements, including anabolic steroids, oral contraceptives, and tobacco products were excluded from the study. Consumption of alcohol and caffeinated products was strictly prohibited throughout the study. All study participants provided an informed, written consent prior to the start of the study.

Experimental design. The study consisted of 2 sequential, 10-d diet interventions: WM and ED (~80% of estimated energy requirements). Following 9 d of adaptation to the WM diet, participants underwent a fasted, resting assessment (REST) of mixed muscle fractional synthetic rate (FSR) and intracellular signaling proteins. Upon completion of the REST protocol, participants crossed over and consumed the ED diet for an additional 10 d. Following 9 d of adaptation to the ED diet, participants underwent a second REST protocol in the fasted state.

Diet interventions. Baseline 3-d diet records for each participant were analyzed and menus were created using Nutritionist Pro Softwar (Axxya Systems, version 3.1.0). Individual energy requirements were established relative to resting energy expenditure determined via an open circuit respiratory system (MedGraphics CPX/D, Medical Graphics) and level of physical activity reported in baseline 7-d physical activity logs to maintain body weight during WM. Energy expenditure during physical activity was estimated based upon similar activities and their corresponding metabolic equivalents (15). Participants were provided with copies of their baseline activity log and instructed to repeat similar levels of exercise throughout the study.

Each diet intervention provided 1.5 g · kg^{-1} · d^{-1} protein, 30% of the total energy from fat, and the remaining energy was provided by carbohydrates. Following the initial WM REST protocol, male participants immediately crossed over and consumed the ED diet for 10 d. Both of the experimental phases were carried out during the early follicular phase (i.e. d 1–6 of the menstrual cycle) for female participants to control for any potential confounding variables (16). Although the women were not fed by study researchers between diet interventions, all female participants received dietary instruction between study phases to ensure weight maintenance and consistency of level of protein intake. Random 24-h dietary recalls and weekly body weight assessments were performed and evaluated. Body weight and protein intake did not change during the time period between WM and ED for any female participants. Energy intake was reduced ~2.1 MJ/d for ED to elicit an ~0.5–1.0 kg weight loss over 10 d.

Research assistants developed individualized meal plans based on energy status (WM vs. ED). All meals were prepared, provided, and supervised by research assistants in coordination with University of Connecticut Dining Services. Meals were packed upon request and snacks were provided to each participant for consumption between scheduled meals. Participants were instructed to consume all food that was provided. Intakes were recorded and analyzed to account for any deletions, substitutions, and/or additions that may have occurred during the feedings. Energy, macronutrient composition, and amino acid intake were determined throughout the 20-d intervention.

Additional measurements. Body weight and height measurements were performed using standardized techniques and equipment. Height was measured to the nearest 0.1 cm at baseline using a stationary stadiometer (Health-o-meter Professional). At baseline and daily during both the WM and ED interventions, body weight was measured to the nearest 0.1 kg following an overnight fast and morning void on a portable digital scale (349KLX; Health-o-meter Professional) to ensure weight maintenance and/or loss. Body composition was assessed at baseline by a certified technician using dual energy X-ray absorptiometry (DPX-MD densitometer, LUNAR). All body composition assessments were performed at similar times in the morning following a 12-h overnight fast.

Maximal graded exercise tests were conducted prior to study commencement to determine participant eligibility. VO_{2peak} was determined via an indirect open circuit respiratory system (MedGraphics CPX/D, Medical Graphics) on a treadmill (Quinton MedTrack ST55) (17).

Nitrogen balance. Urinary nitrogen was measured from a single, pooled, 24-h sample for each participant at baseline and the day prior to each REST assessment. Urine was collected in amber bottles provided to the participants and stored (in 15 mL of 0.1 mol/L HCl) in refrigerated containers to preserve urinary ammonia. Urine volume was measured and aliquots stored at −20°C until analyzed. The total nitrogen content of the urine was determined using a micro-Kjeldahl apparatus (Tecator Kjeltec System). Apparent nitrogen balance was calculated as the difference of nitrogen intake minus urinary nitrogen excretion plus estimated integumental losses (18). To verify complete 24-h urine collections, urinary creatinine was measured using a standard colorimetric assay (Creatinine kit 277–10501, Wako Chemicals USA).

Plasma amino acids. Amino acid concentrations were determined using cation exchange, HPLC, with post-column derivatization (Pickering Laboratories PCX5200; Pickering Laboratories). Concentrations of individual amino acids were measured colorimetrically with ninhydrin, which reacts with both primary and secondary amines, detectable at 570 and 440 nm, respectively.

Plasma hormone analysis. Insulin and cortisol concentrations were determined in duplicate from lithium heparin-processed plasma samples using an ELISA (DSL-10–1600 and DSL-10–2000) for insulin and

TABLE 1 Characteristics and energy and macronutrient intakes of healthy men and women prior to the 20-d diet intervention[1]

Characteristic	Total participants	Men	Women
Age, *y*	21 ± 0.5	21 ± 0.6	21 ± 0.5
Height, *cm*	171 ± 3	174 ± 3	164 ± 7
Weight, *kg*	70 ± 4	74 ± 4	64 ± 6
BMI, kg/m^2	24 ± 1	24 ± 1	23 ± 1
Body fat, *%*	19 ± 3	15 ± 2	29 ± 3*
VO_{2peak}, $mL \cdot kg^{-1} \cdot min^{-1}$	54 ± 2	57 ± 1	47 ± 3*
Energy, *MJ/d*	10.1 ± 0.7	10.8 ± 0.5	8.5 ± 1.6
Carbohydrate, *g/d*	308 ± 28	310 ± 27	302 ± 68
Fat, *g/d*	84 ± 10	97 ± 11	60 ± 10**
Protein, *g/d*	102 ± 10	121 ± 6	70 ± 12**

[1] Values are mean ± SEM, $n = 12$ (8 men and 4 women). Asterisks indicate different from men: *$P < 0.001$, **$P < 0.05$.

cortisol, respectively; Diagnostic Systems Laboratories). To ensure that females were studied during the early follicular phase of the menstrual cycle, estradiol concentrations were determined in duplicate from lithium heparin-processed samples using a solid phase, estradiol ultrasensitive EIA (20-DR-4399; ALPCO Diagnostics).

Determination of FSR. Following 9 d of adaptation to their respective diet (i.e. WM vs. ED), participants reported to the Metabolic Assessment Laboratory between 0500 and 0630 h following a 12-h overnight fast to assess FSR. The protocols occurred on d 10 and 20, respectively. Participants refrained from aerobic and resistance exercise for 24 and 72 h, respectively, prior to each REST protocol to minimize the effect of a prior exercise bout on protein kinetic measurements.

Initially, a 20-gauge, 4.4-cm Teflon catheter (Jelco; Medex) was inserted into an antecubital vein for baseline blood sampling followed by a primed, constant infusion of ring-[$^2H_5$]-phenylalanine (2 $\mu mol \cdot kg^{-1}$, 0.05 $\mu mol \cdot kg^{-1} \cdot min^{-1}$; Cambridge Isotope Laboratories) at 0 min that was maintained over the course of the protocol (300 min). At 100 min, a second catheter (3.25 cm) was inserted into a contralateral dorsal hand vein wrapped in a heating pad for "arterialized" blood sampling to ensure that isotopic steady state had been achieved (19). At 120 min, participants were prepped under sterile conditions for a muscle biopsy that was taken from the lateral portion of the vastus lateralis (~20 cm above the knee) as described previously (14). A second muscle biopsy was taken from the same incision at 300 min of the study. A sample of ~100–150 mg of mixed muscle tissue was obtained with each biopsy. Each sample was rinsed with normal saline and blood, visible fat, and connective tissue were removed. Subsequently, the tissue was frozen in liquid nitrogen and stored at −80°C until further analysis.

FSR calculations. FSR was calculated using the direct incorporation method (14,20) based on the rate of ring-[$^2H_5$]-phenylalanine tracer incorporation from muscle intracellular fluid into bound skeletal muscle protein between muscle samples at 120 and 300 min.

Western blot analysis. The phosphorylation and total expression of protein synthetic intracellular signaling proteins were assessed using SDS-PAGE and Western blotting according to methods previously employed by Dryer et al. (21). Briefly, tissue samples were homogenized and total protein concentrations were determined using the Bradford assay (Bio-Rad Laboratories). Equal amounts of protein (65 μg) from the tissue homogenates were subjected to SDS-PAGE using precast Tris-HCl gels (Bio-Rad Laboratories). Proteins were then transferred to polyvinylidene fluoride membranes followed by exposure to the appropriate phospho-specific primary antibodies. Corresponding total proteins were also immunoblotted to confirm equal loading of gels. Phospho-specific and total protein-specific primary antibodies for protein kinase B (Akt; Ser 473), AMP-activated protein kinase α (AMPKα; Thr 172), and eukaryotic initiation factor 4E binding protein 1 (4E-BP1; Thr 37/46) were produced in rabbits and purchased commercially (Cell Signaling Technology). Labeling was performed using anti-rabbit IgG conjugates with horseradish peroxidase (GE Healthcare Life Sciences). Signals were detected using chemiluminescence (GE Healthcare Life Sciences) followed by exposure on imaging film (Kodak). Bands were quantified using ImageJ scanning densitometry software (22). Data are expressed relative to a pooled muscle sample, which was loaded onto each gel to control for between gel differences. The phosphorylation of the intracellular signaling proteins is expressed as relative quantity of phosphorylated protein to relative quantity of total protein.

Statistical analysis. Baseline participant characteristics, energy, and macronutrient data are described using common descriptive statistics. An ANOVA was used to determine baseline differences between sexes. A Levene's test for equality of variance confirmed homogeneity between sexes. With the exception of baseline body composition, fitness, and dietary intake, no criterion measures differed between men and women. Therefore, data were pooled and analyzed using a repeated-measure design with 2 within-group factors (energy: WM vs. ED; time: time over REST protocols) to evaluate the effects of an acute energy deficit on FSR, intracellular signaling activity, and all remaining criterion measures. If significant differences were found, post hoc analysis was performed using paired *t* tests. The α level for significance was set at $P < 0.05$ and adjusted accordingly for multiple comparisons using a Bonferroni correction. All data were analyzed using SPSS 11.0.

Results

Baseline characteristics. Men had a lower percent body fat ($P < 0.001$), greater VO_{2peak} ($P < 0.001$), and consumed more protein and fat ($P < 0.05$) at baseline compared with women (**Table 1**). Other characteristics did not differ between the sexes at baseline.

Diet interventions. Consistent with the study design, total energy intake was reduced ~19% (~2.1 MJ/d; $P < 0.0001$) from WM to ED diet interventions, eliciting a mean 1-kg weight loss ($P < 0.0001$) for all volunteers (**Table 2**). Macronutrient composition of the diet, expressed as a percent of total energy intake, differed ($P < 0.001$) between energy states (WM vs. ED) for carbohydrates (54 ± 1% vs. 49 ± 1%), fat (29 ± 0.3% vs. 30 ± 0.3%), and protein (17 ± 1% vs. 21 ± 1%). Absolute carbohydrate and fat intake decreased ($P < 0.0001$) from WM to ED diet interventions, with no change in total protein intake (**Table 3**). Relative protein intake remained constant at 1.5 $g \cdot kg^{-1} \cdot d^{-1}$ for the WM and ED diet interventions. Essential amino acids (44 ± 2 vs. 45 ± 2 g/d), branched-chain amino acids (BCAA; 20 ± 1 vs. 20 ± 1 g/d), and leucine (9 ± 0.4 vs. 9.1 ± 0.4 g/d) intake did not differ between WM and ED, respectively.

Nitrogen balance. Baseline nitrogen intake was 16.3 ± 1 g/d and did not differ between the WM (17 ± 1 g/d) and ED (17 ± 1 g/d) diet interventions. Baseline nitrogen balance (NBAL) was

TABLE 2 Total energy intakes of healthy men and women during the WM and ED diet interventions[1]

Diet intervention	Total participants	Men	Women
		MJ/d	
WM	10.9 ± 1	11.9 ± 1.3	9.1 ± 1
ED	8.9 ± 1*	9.9 ± 1.2*	6.9 ± 0.5*

[1] Values are mean ± SEM, $n = 12$ (8 men and 4 women). *Different from WM, $P < 0.0001$.

TABLE 3 Absolute macronutrient intakes of healthy men and women during the WM and ED diet interventions[1]

Diet intervention	Carbohydrates	Fat	Protein
		g/d	
WM	367 ± 38	86 ± 8	106 ± 5
ED	272 ± 35*	72 ± 8*	106 ± 5

[1] Values are mean ± SEM, n = 12 (8 men and 4 women). *Different from WM, $P < 0.0001$.

positive (0.6 ± 2 g/24 h) and remained stable during the WM (1.9 ± 1 g/24 h) and ED (0.2 ± 1 g/24 h) diet interventions. Urinary creatinine did not differ between the WM and ED diet interventions, indicative of complete 24-h urine collections.

Plasma amino acids. The 10-d energy deficit did not affect plasma total amino acids, nonessential amino acids, essential amino acids, BCAA, or individual BCAA (i.e. isoleucine, leucine, and valine). Regardless of energy state, BCAA levels were 15% lower ($P < 0.05$) at 300 min (363 ± 21 μmol/L) than at baseline (423 ± 15 μmol/L). Individually, leucine and valine concentrations decreased ($P < 0.05$) over time, as levels were lower at 120, 280, and 300 min compared with baseline (**Table 4**). Similarly, isoleucine concentrations were lower at all time points relative to baseline ($P < 0.05$). There was an energy × time interaction ($P < 0.05$) for plasma leucine and valine concentrations.

Plasma hormones. Energy intake affected plasma insulin with concentrations ~18% lower during the ED period (40.3 ± 2 pmol/L) compared with the WM period (49.3 ± 5 pmol/L) ($P < 0.05$). Insulin concentrations remained stable over time. Cortisol concentrations did not change in response to ED; however, cortisol at 300 min (1203 ± 76 nmol/L) was ~27% lower ($P < 0.001$) compared with 120 min (878 ± 64 nmol/L).

Plasma estradiol was measured to determine phase of the menstrual cycle during which FSR and intracellular signaling were assessed in women. Plasma estradiol concentrations measured at baseline of the 2 interventions did not differ. Furthermore, plasma estradiol during the WM (242 ± 40 pmol/L) and ED (268 ± 48 pmol/L) periods indicated that the women studied during the early follicular phase of their menstrual cycle.

Muscle and plasma phenylalanine enrichments. Individual fluctuations in plasma (**Fig. 1***A*) and muscle (Fig. 1*B*) intracellular ring-[$^2H_5$]-phenylalanine enrichments did not occur, suggesting that plateau isotopic enrichments were achieved during the WM and ED REST protocols.

FSR. In response to an acute energy deficit, FSR was reduced ~19% ($P < 0.05$) during ED (0.06 ± 0.01%/h) compared with the WM (0.074 ± 0.01%/h) diet intervention.

TABLE 4 BCAA concentrations of healthy men and women during the WM and ED REST protocols[1]

BCAA	Baseline	120 min	280 min	300 min
		μmol/L		
Isoleucine	73 ± 3	65 ± 2*	63 ± 2*	65 ± 2*
Leucine	130 ± 5	118 ± 3*	117 ± 4*	120 ± 4
Valine	226 ± 13	210 ± 10*	202 ± 8*	207 ± 8*

[1] Values are mean ± SEM, n = 12 (8 men and 4 women). *Different from baseline, $P < 0.05$.

Western blot analysis. Relative Akt phosphorylation at 300 min was ~35% lower ($P < 0.05$) during ED compared with WM (**Fig. 2***A*). For ED, Akt activation was nearly 54% lower ($P < 0.05$) at 300 min compared with 120 min. Relative AMPK phosphorylation was not affected by energy status (i.e. WM vs. ED diet interventions) during REST protocols (Fig. 2*B*). However, AMPK phosphorylation was lower ($P < 0.05$) at 300 min compared with 120 min for both WM and ED REST protocols. 4E-BP1 phosphorylation at 120 min was ~30% lower ($P < 0.05$) during ED compared with WM. Furthermore, at 300 min, 4E-BP1 activation tended to be lower ($P = 0.07$) compared with WM (Fig. 2*C*). There were no changes over time for relative 4E-BP1 phosphorylation for either REST protocol. Total Akt, AMPK, and 4E-BP1 protein expression, indicative of equal protein loading within gels, did not change over time or differ between energy states.

Discussion

The novel finding from this investigation is the 19% decrease in FSR in response to an acute, 10-d, moderate energy deficit coupled with corresponding reductions in intracellular signaling proteins specific to protein synthesis. Until now, the majority of studies investigating the influence of energy restriction on protein utilization relied upon measures of WBPTO, with varying degrees and durations of energy deprivation (4,7,23–25). In nonobese adults, WBPTO is initially upregulated under fasting conditions (i.e. ≤4 d), as evidenced by increased whole body proteolysis, amino acid oxidation, and plasma BCAA (5,26,27). Studies have consistently shown that WBPTO is diminished substantially in obese adults in response to prolonged periods (i.e. 3–8 wk) of energy deprivation (4,7,24). It is generally accepted that energy-induced downregulations in

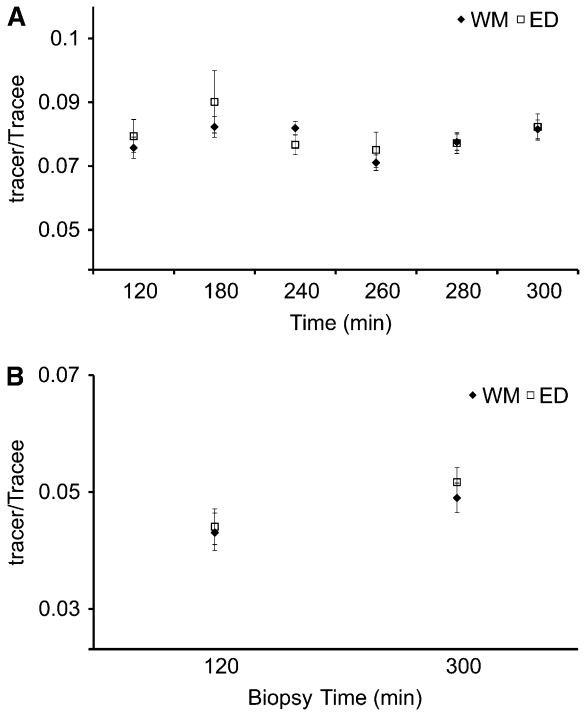

FIGURE 1 Plasma (*A*) and muscle intracellular (*B*) ring-[$^2H_5$]-phenylalanine enrichment data for healthy men and women during WM and ED REST protocols. Values are mean ± SEM, n = 12 (8 men and 4 women).

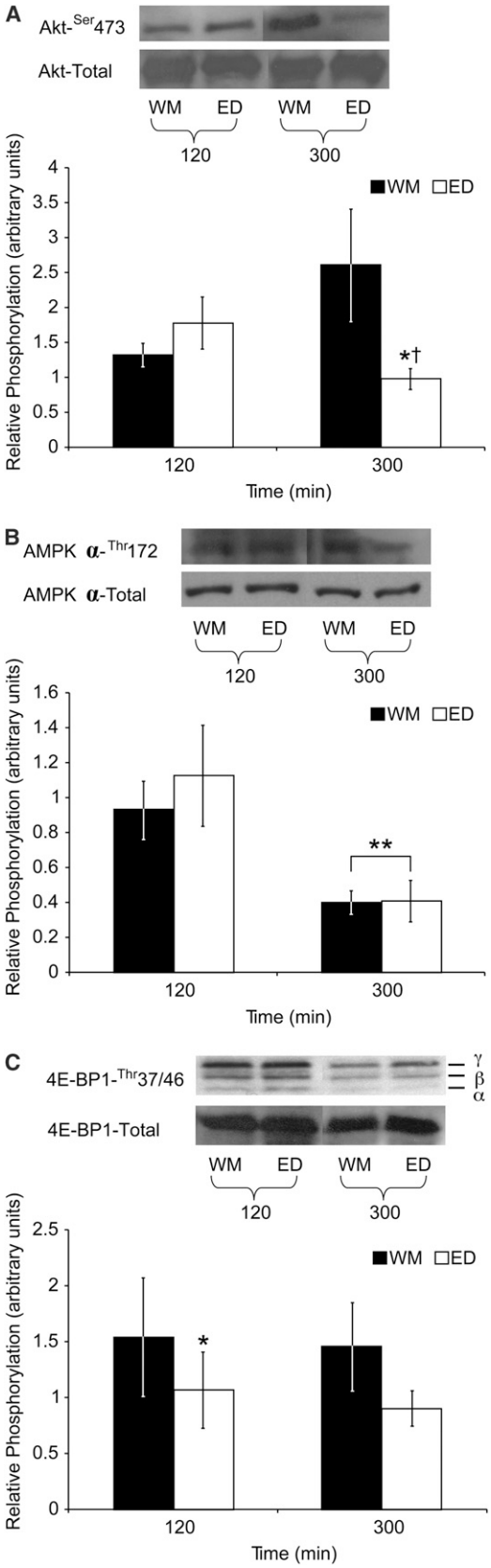

FIGURE 2 Relative phosphorylation of Akt Ser 473 (*A*), AMPKα Thr172 (*B*), and 4E- BP1 Thr37/46 (*C*) in men and women at 120 and 300 min of the WM and ED REST protocols. Values (relative phosphorylation to relative total protein expression) are means ± SEM, $n = 11$, 8 men and 3 women (*A*); $n = 7$, 5 men and 2 women (*B*); and $n = 7$, 5 men and 2 women (*C*). *Different from WM, $P < 0.05$. **Time main effect; different from 120 min, $P < 0.05$. †Different from ED 120, $P < 0.05$.

WBPTO represent a metabolic adaptation for conservation of endogenous protein stores. For the most part, reductions in protein turnover are proportional to the loss of lean body mass and related to degree of energy restriction (4,7,24,28). In the present study, the noted reduction in mixed muscle FSR in response to an acute, modest energy restriction is consistent with the aforementioned downregulation of WBPTO. Because there have been no studies to date, to our knowledge, that have examined the impact of acute energy restriction on skeletal muscle protein synthesis, discussion is limited to investigations that have evaluated WBPTO responses to short- and long-term energy restriction. Potential explanations for the central finding of this investigation, a decrease in mixed muscle FSR during an acute period of negative energy balance, are considered.

Protein intakes in excess of the current RDA have been shown to be beneficial for maintenance of protein utilization under various conditions, including energy restriction (9,29,30). In the present study, the noted reduction in FSR in response to an acute energy deficit was observed at rest in individuals in a fasted state after habitual consumption of a reduced-energy diet that provided 1.5 g protein · kg^{-1} · d^{-1}. Similarly, Yang et al. (6) observed no change in WBPTO in nonobese adult men undergoing a 2-wk, 25% energy reduction for which the diet provided 1.5 g protein · kg^{-1} · d^{-1} and Friedlander et al. (12) reported no change in resting WBPTO in healthy men consuming 1.2 g protein · kg^{-1} · d^{-1} during 21 d of negative energy balance. Recently, Pikosky et al. (31) reported the maintenance of WBPTO in endurance-trained men in response to an exercise-induced energy deficit, with no differences between protein intakes of 0.9 g and 1.8 g protein · kg^{-1} · d^{-1}. Although measures of skeletal muscle protein synthesis were not evaluated in these studies, one might speculate that a reduction in WBPTO in response to energy restriction might correspond to a decrease in mixed muscle FSR. Without question, the unique finding of a reduced FSR in response to a short-term energy deficit is intriguing and warrants further discussion in the context of other criterion measures.

The maintenance of NBAL following a 10-d energy deficit could be due to the provision of dietary protein in excess of the RDA (11). Data from numerous studies attribute enhanced nitrogen retention to greater protein intake during periods of energy deficit (25,31–33). In response to an acute, 7-d, 50% energy restriction, Agus et al. (32) found that NBAL was negative in obese men when protein intakes were slightly below the RDA and positive for those who consumed protein in excess of the recommended amount. These findings coincide with those of Pikosky et al. (31), where NBAL was conserved following a 7-d, 1000-kcal/d (4184-kJ/d) energy deficit, in endurance-trained men who consumed dietary protein at 1.8 g · kg^{-1} · d^{-1}. Similarly, we observed a preservation of NBAL in overweight premenopausal women following a 10-wk weight loss intervention for which protein intake was similar to that of the present study (33). Collectively, these studies, along with the present investigation, show that nitrogen retention is better maintained in response to energy deficits with protein intakes approximately twice the RDA. In the present study, we hypothesize that the noted downregulation in skeletal muscle protein synthesis, coupled with increased protein intake, likely contributed to the maintenance of NBAL. In the present investigation differences in skeletal muscle FSR did not appear to be affected by plasma amino acid levels. Plasma amino acid concentrations were maintained during ED, suggesting that provision of protein at a level in excess of the RDA but well within the current acceptable macronutrient distribution range is sufficient to sustain circu-

lating amino acid concentrations. Earlier investigations have suggested that extracellular (i.e. plasma) amino acid availability, BCAA in particular, contributes to the regulation of skeletal muscle protein synthesis (34–36). More specifically, enhanced muscle protein synthesis has been reported as a result of increased plasma amino acid concentrations subsequent to acute amino acid infusions (34,37,38) or ingestion (39). In contrast, more recent investigations have clearly shown that the stimulation of protein synthesis relies on the sensing of muscle intracellular amino acid concentrations (40,41). This stimulatory effect is likely mediated through leucine-induced alterations of protein synthetic intracellular signaling (42,43). However, it is important to note that muscle intracellular amino acid concentrations were not determined in the present study. Therefore, a limitation of the present study was our inability to address the role of intramuscular amino acid availability with respect to the changes noted in FSR in response to an acute period of moderate energy deprivation.

The phosphorylation of key regulatory intracellular signaling proteins was influenced in the muscle of participants consuming an ED diet for 10 d. Akt and 4E-BP1 phosphorylation were lower in response to an acute energy deficit. Akt can influence mRNA translation initiation through mammalian target of rapamycin-dependent phosphorylation of the translational inhibitory protein, 4E-BP1 (44). Reduced 4E-BP1 phosphorylation will reduce eukaryotic initiation factor 4F formation and protein translation initiation will be diminished, thus limiting protein synthesis (44). These findings are consistent with our original hypothesis that an acute energy deficit would attenuate the activity of key regulatory molecular signaling proteins associated with skeletal muscle protein synthesis and provide insight concerning the intricate molecular regulation of skeletal muscle protein synthesis in response to acute energy restriction.

The endocrine environment is central to many of these changes. In the present study, the possibility exists that lower insulin concentrations may have contributed to reduced phosphatidylinostitol 3-kinse-dependent activation of Akt, ultimately leading to reduced 4E-BP1 phosphorylation, thereby affecting mRNA translation initiation and protein synthesis. Data from Fujita et al. (45) provide some insight into current findings given their observation that increased plasma insulin concentrations in response to feeding stimulated skeletal muscle FSR along with marked increases in Akt and 4E-BP1 phosphorylation. Our findings suggest that the opposite occurs in response to energy deprivation, such that reductions in insulin concentrations downregulate key intracellular signaling intermediates, such as Akt and 4E-BP1.

AMPK functions as a fuel sensor in many tissues, including skeletal muscle. It inhibits many anabolic signaling pathways when cellular ATP levels are reduced and AMP levels rise in response to limited energy availability (46). In the current investigation, AMPK phosphorylation was not influenced by energy status. Although our findings are in disagreement with some animal studies (47,48), it is likely naïve to expect all of these intracellular signaling proteins to respond in concert during a finite time period in human skeletal muscle subsequent to an imposed, in this case, dietary, intervention (49).

In summary, this is the first investigation, to our knowledge, to demonstrate reduced skeletal muscle protein synthesis in response to an acute energy deficit in healthy adults. A novel aspect of this work is the corresponding characterization of skeletal muscle intracellular signaling proteins specific to protein synthesis, all of which were assessed during well-controlled diet interventions. This downregulation of muscle protein synthesis occurred in the presence of reduced insulin concentrations and diminished intracellular signaling essential to skeletal muscle protein synthesis. In total, these data show that a modest reduction in energy intake for as little as 10 d can alter skeletal muscle protein utilization in healthy men and women. Given the current prevalence of obesity and the heightened need for the development of effective diet intervention strategies for weight management that spare lean body mass, our findings demonstrate a need for investigations designed to examine the impact of prolonged, and perhaps more severe, energy restriction on skeletal muscle protein turnover.

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