

Reduced resting skeletal muscle protein synthesis is rescued by resistance exercise and protein ingestion following short-term energy deficit

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Areta JL, Burke LM, Camera DM, West DW, Crawshaw 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. First published March 4, 2014; doi:10.1152/ajpendo.00590.2013.—The myofibrillar protein synthesis (MPS) response to resistance exercise (REX) and protein ingestion during energy deficit (ED) is unknown. In young men ($n = 8$) and women ($n = 7$), we determined protein signaling and resting postabsorptive MPS during energy balance [EB; 45 kcal·kg fat-free mass (FFM)⁻¹·day⁻¹] and after 5 days of ED (30 kcal·kg FFM⁻¹·day⁻¹) as well as MPS while in ED after acute REX in the fasted state and with the ingestion of whey protein (15 and 30 g). Postabsorptive rates of MPS were 27% lower in ED than EB ($P < 0.001$), but REX stimulated MPS to rates equal to EB. Ingestion of 15 and 30 g of protein after REX in ED increased MPS ~16 and ~34% above resting EB ($P < 0.02$). p70 S6K Thr³⁸⁹ phosphorylation increased above EB only with combined exercise and protein intake (~2–7 fold, $P < 0.05$). In conclusion, short-term ED reduces postabsorptive MPS; however, a bout of REX in ED restores MPS to values observed at rest in EB. The ingestion of protein after REX further increases MPS above resting EB in a dose-dependent manner. We conclude that combining REX with increased protein availability after exercise enhances rates of skeletal muscle protein synthesis during short-term ED and could in the long term preserve muscle mass.

body composition; fat-free mass; myofibrillar protein synthesis; weight loss

ENERGY DEFICIT (ED) can be achieved through reduced energy intake and/or increased energy expenditure and subsequently leads to loss of fat mass (FM). A reduction in FM is a goal for improved health (19, 33); however, when achieved by energy restriction alone, it typically results in the concomitant weight loss comprised of ~25% fat-free mass (FFM) (52), of which skeletal muscle is the main component (37, 39). Given that the quality and quantity of skeletal muscle is a major determinant of whole body metabolic rate and functional capacity throughout the lifespan (25), nutritional and exercise strategies to prevent or minimize loss of FFM while losing fat mass are crucial.

Pasiakos et al. (41) reported a 19% reduction in basal rates of mixed-muscle protein synthesis in young healthy males and females after 10 days of ED (~500 kcal/day). In contrast, a more recent study from Pasiakos et al. (40) found no decrease in rates of resting muscle protein synthesis after 30 days of moderate ED. If a potential decrease in basal rates of muscle protein synthesis was not accompanied by a concomitant reduction in muscle protein breakdown, then ED would presumably result in a marked loss of skeletal muscle protein. Indeed, prolonged ED-induced body weight loss can be comprised of up to 60% FFM (40). In contrast, exercise has been shown to attenuate the loss of lean body tissue that typically occurs with periods of ED alone (50). However, it is currently unknown whether the anabolic effects of resistance exercise (REX) are attenuated during periods of ED.

Provision of dietary amino acids increases skeletal muscle protein synthesis, an effect that is enhanced by prior REX (3, 36). To date, only one study has examined whether skeletal muscle exhibits “anabolic resistance” to exercise and protein ingestion following short-term ED (40). However, in that investigation, rates of mixed-muscle protein synthesis and not myofibrillar protein synthesis (MPS; i.e., the contractile protein fraction of muscle) were measured. Furthermore, there was no examination of the impact of exercise, and the cohort under investigation was comprised mainly of males. Hence, the primary aim of the current study was to determine the effects of ED in combination with REX and two levels of protein intake on skeletal muscle translation initiation signaling, mRNA expression, and rates of MPS. In addition, because women may be more susceptible to dysregulation of normal metabolism during periods of ED (30), a secondary aim was to identify potential sex-based differences in skeletal muscle anabolism in response to ED. Our general hypothesis was that short-term ED would reduce basal rates of muscle protein synthesis, but this impairment would be overcome by a combination of REX and protein feeding. We also examined two levels of postexercise protein intake, as higher protein availability may be more efficacious in ED.

MATERIALS AND METHODS

Ethical Approval

Subjects were informed of any potential risks involved in the study before providing their written, informed consent. The study was approved by the Australian Institute of Sport Ethics Committee and

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Table 1. *Subject characteristics*

	Males (n = 8)	Females (n = 7)
Age, yr	27 ± 5	28 ± 4
Body mass, kg*	82.7 ± 6.6	70.3 ± 7
Fat, %BM*	14.9 ± 3.5	28.7 ± 5.3
Lean mass, kg*	67.2 ± 6.2	48.1 ± 4.3
1-RM, kg*	300 ± 70	200 ± 38
1-RM (kg)/BM (kg)*	3.6 ± 0.8	2.8 ± 0.3
1-RM (kg)/FFM (kg)	4.5 ± 0.9	4.2 ± 0.7

Values are means ± SD. 1-RM, 1-repetition maximum; BM, body mass; FFM, fat-free mass. Data were analyzed by using multiple *t*-tests. *Different between sexes ($P < 0.05$).

conformed to the standards set by the latest revision of the Declaration of Helsinki.

Subjects

Sixteen young, healthy, resistance-trained subjects (8 females, 8 males) commenced the study, but one female participant withdrew from the trial before completion (Table 1). Body composition was measured 1–2 wk before the first experimental trial using a whole body scan-narrowed fan beam dual-energy X-ray absorptiometry (DXA Lunar Prodigy; GE Healthcare, Madison, WI) with GE Encore 13.60 software (GE Healthcare).

Experimental Design

The study employed a within-subject design, with subjects completing four experimental interventions: energy balance (EB) at rest, ED at rest, and then ED with exercise both with and without protein feeding. All trials were performed in a randomized order, with the exception of the EB trial, which was always undertaken first to avoid any potential dysregulation in EB trial induced by previous ED interventions (Fig. 1).

Dietary Intervention

Subjects were each provided with individualized prepackaged meals for 5 days before each experimental trial. Before the resting EB trial, subjects were provided with meals equivalent to an energy availability (EA) of 45 kcal·kg FFM⁻¹·day⁻¹, where EA is defined as energy intake minus the energy cost of exercise. For all ED trials, diets consisted of an energy availability of 30 kcal·kg FFM⁻¹·day⁻¹. Between days 1 and 3 of the dietary control period, subjects were permitted to exercise, and the diet was adjusted to account for the energy expenditure of the exercise sessions and thus restore EA to the set level. However, in the 48 h prior to an experimental trial, subjects refrained from strenuous physical activity/training. The protein, carbohydrate, and fat content of the diets was 1.4–1.6, 4–4.5, and 1.5–2.5 g·kg body mass (BM)⁻¹·day⁻¹ for EB and 1.4–1.6, 3–3.5 and 0.5–1.5 g·kg BM⁻¹·day⁻¹ for ED, respectively. The ranges for protein and carbohydrate depend on the exercise energy requirements for each day, whereas the amount of fat provided was that required to match the target EA. No alcohol was consumed by the subjects during the 5-day dietary control period, and they refrained from caffeine intake 24 h before each trial day. Between experimental trials, there was a 9 day “washout” period, during which subjects continued with their normal exercise and dietary habits.

Rationale for Dietary Interventions and Washout Periods

We chose to employ 5 days of ED prior to each experimental trial based on previous data showing that such time is sufficient for inducing disruption to whole body metabolic homeostasis (23, 31). In addition, a negative nitrogen balance can be generated after as little as 4 days of ED (17, 21, 40, 48). Our 9-day washout period was used based on data showing that nitrogen balance returns to positive values

after only 3 days of refeeding following a 3-wk fasting period (22) and that reductions in resting metabolic rate after 20 days of ED return to basal values after 10 days of EB (53).

Experimental Trials

After 5 days of dietary control, subjects reported to the laboratory between 0700 and 0800 after an ~10-h overnight fast, and a Teflon catheter was inserted in the antecubital vein of each arm for blood sampling and tracer infusion. A first (baseline) blood sample was drawn for the resting EB trial (or muscle biopsy from the vastus lateralis was obtained for the ED trials) immediately before a primed, continuous (0.05 μmol·kg⁻¹·min⁻¹, 2 μmol/kg prime) infusion of L-[ring-¹³C₆]phenylalanine (Cambridge Isotopes Laboratories, Woburn, MA) commenced.

After a 3-h resting period, a muscle biopsy was obtained. For the three nontracer naïve subjects, the first muscle biopsy was taken before the commencement of the infusion in their EB trial.

The ED trials were undertaken after the resting EB trial, with the protein/placebo (PL) ingestion randomized and counterbalanced. Drinks contained 15 or 30 g of protein (86.8 g of protein, 1.5 g of fat, and 3.1 g of carbohydrates/100 g; ISO8 WPI, Musashi) or no protein given in the form of a flavor- and volume-matched placebo drink. Each protein drink was enriched with 5% L-[ring-¹³C₆]phenylalanine and mixed with water to a total volume of 500 ml. The first ED trial for each subject was divided in two periods. The first (resting) period of the trial determined resting ED and was identical to the EB trial, with the exception of an initial muscle biopsy. In the second period (nutrition/exercise), subjects undertook a bout of REX (described subsequently), with further muscle biopsies obtained 1 and 4 h postexercise. Drinks were ingested immediately following cessation of REX. In the remaining ED trials, a primed, constant infusion of tracer commenced prior to exercise to ensure that isotopic equilibrium was reached before/during the measurement periods (between 1 and 4 h).

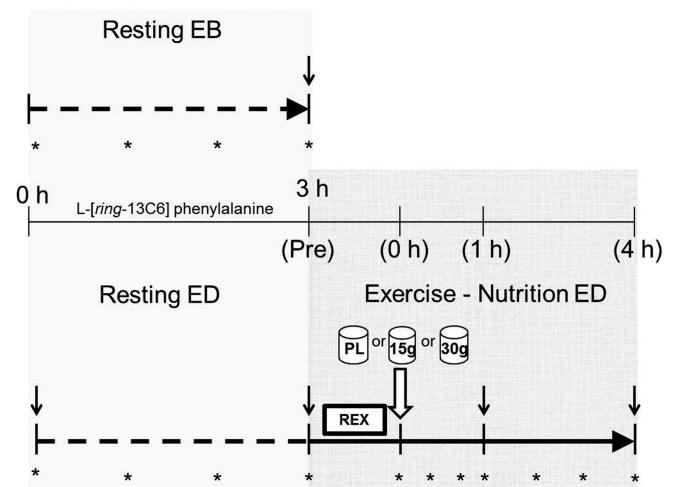


Fig. 1. Schematic of the experimental design. The resting energy balance (EB) trial was preceded by 5 days of controlled diet providing 45 kcal·kg fat-free mass (FFM)⁻¹·day⁻¹. The resting/exercise in energy deficit (ED) trials were preceded by 5 days of controlled diet providing 30 kcal·kg FFM⁻¹·day⁻¹. Subjects returned to normal daily activities in EB for 9 days between the ED periods. ↓ Skeletal muscle biopsy sample; *blood sample. PL, 15 g, and 30 g represent the respective placebo or whey protein drinks (500 ml). Dashed timeline represents trials undertaken a single time by each subject. Times in parentheses are for ED trials involving resistance exercise (REX) and protein intake.

Exercise

A one-repetition maximum (1-RM) inclined (45°) leg press (GLPH1100; Body-Solid, Forest Park, IL) test was completed by each subject for a minimum of 1 wk prior to the experimental trials. After a warmup of two sets of five moderate-intensity repetitions, the 1 RM was determined as the highest successfully lifted weight during a maximum of six attempts. On the day of an ED experimental trial, subjects completed two warmup sets of five repetitions at ~50 and ~60% 1-RM with 2 min of rest between sets. The REX bout incorporated six sets of eight repetitions at ~80% 1-RM with 3 min of rest between sets. Exercise range of motion was ~85° for the knee joint, with leg extension end point set at ~5° from full extension.

Biological Samples

Blood samples (4 ml) were taken at rest, before the exercise bout, and at repeated time points throughout recovery (Fig. 1). Skeletal muscle biopsy samples were taken from different incisions separated by ~1 cm using 5-mm Bergström needles adapted for manual suction. Muscle was cleaned with saline solution to remove excess blood and immediately frozen in liquid N₂. Muscle and plasma samples were stored at -80°C until subsequent analysis.

Analytical Procedures

Insulin and amino acid concentration. Plasma insulin concentration was measured using an automated enzyme-amplified chemiluminescence Immulite 1000 system (Siemens Diagnostics) according to the manufacturer's guidelines. Plasma amino acids were analyzed by gas chromatography-mass spectrometry using the EZ:faast kit (Phenomenex).

Western blot. Intracellular proteins were extracted, isolated, and quantified as described previously (11). The amount of protein loaded in each well was 40 µg. Polyclonal anti-phospho-mammalian target of rapamycin (mTOR) Ser²⁴⁴⁸ (no. 2,971), monoclonal anti-phospho-Akt Ser⁴⁷³ (no. 9,271), ribosomal protein S6 Ser^{235/6} (no. 4,856), eukaryotic initiation factor 4E binding protein 1 Thr^{37/46} (no. 2,855), eukaryotic elongation factor 2 (eEF2) Thr⁵⁶ (no. 2,331), AMP-activated protein kinase (AMPK) Thr¹⁷² (no. 2,535), and anti-α-tubulin control protein (no. 3,873) were purchased from Cell Signaling Technology (Danvers, MA). Polyclonal anti-phospho-p70 S6K Thr³⁸⁹ (no. PK1015) was from Millipore (Temecula, CA). Monoclonal anti-SLC7A5 (ab134121) was obtained from Abcam (Cambridge, UK). All densitometric analysis was carried out by the same researcher using specialized software (Image J 1.47; National Institutes of Health) by quantifying the area under the peak of each plot for each lane of the measured membrane.

Fractional synthetic rate. Preinfusion plasma sample proteins, extracted by acetonitrile, were used for the baseline enrichment values in "tracer-naïve subjects" (7). For non-tracer-naïve subjects ($n = 3$; males) a preinfusion muscle biopsy was used for baseline enrichment. Muscle tissue was processed as described previously (36).

Calculations. The fractional synthetic rate (FSR) of myofibrillar proteins was calculated using the standard precursor-product method $FSR (\%h^{-1}) = [E_{p2} - E_{p1}] / E_{ic} \times 1/t \times 100$, where $E_{p2} - E_{p1}$ represents the change in bound protein enrichment between two biopsy samples, E_{ic} is the average enrichment of intracellular phenylalanine between the two biopsy samples; and t is the time between biopsies. The utilization of tracer-naïve subjects ($n = 12$) allowed us to use the preinfusion blood sample (i.e., mixed plasma protein fraction) as the baseline enrichment (E_{p1}) for the calculation of resting MPS (8).

RNA extraction, reverse transcription, and RT-PCR. Skeletal muscle tissue (~20 mg) was used to isolate RNA using a modification of the acid guanidinium thiocyanate-phenol-chloroform extraction, as described previously (12). Reverse transcription and real-time polymerase chain reaction (RT-PCR) were performed as described previ-

ously (10, 54). Taqman-FAM-labeled primer/probes for atrogin-1 (Hs01041408_m1), muscle RING finger-1 (MuRF-1; Hs00822397_m1), SLC38A2 (Hs00255854_m1), and SLC7A5 (Hs00185826_m1) primers (Applied Biosystems, Carlsbad, CA) were used. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH; HS99999905_m1) was used as the housekeeping gene. The relative amounts of mRNAs were calculated using the relative quantification ($\Delta\Delta C_T$) method (29).

Statistical analysis. Data were analyzed using two-way repeated-measures analysis of variance (ANOVA) with Student-Newman-Keuls post hoc analysis (sex \times time) for cell signaling, RT-PCR, and myofibrillar FSR (SigmaStat for Windows, version 3.10). There were no differences between sexes, and data were subsequently combined for further analysis using one-way repeated-measures ANOVA with Student-Newman-Keuls post hoc test. Data for plasma insulin and amino acids concentration were analyzed using two-way repeated-measures ANOVA with Student-Newman-Keuls post hoc test, where resting EB and ED trials were independently analyzed from the exercise trials. Data for Western blotting were log-transformed prior to analysis. All data are presented as means \pm SD, and the level of statistical significance was set at $P < 0.05$.

RESULTS

Plasma Insulin Concentration

There were no differences in plasma insulin concentration during the resting EB and ED trials. There was a time \times group interaction for plasma insulin concentration ($P < 0.001$) after exercise and protein feeding (Fig. 2A).

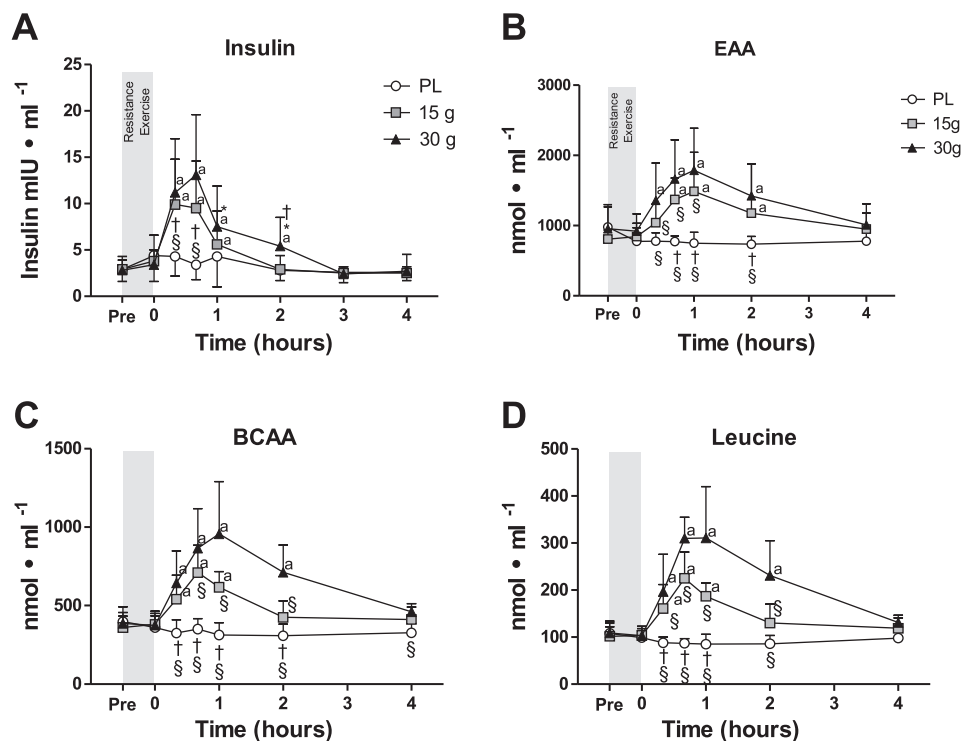
Amino Acid Concentration

There were no differences in resting essential amino acids (EAA), branched-chain amino acids (BCAA), or leucine plasma concentrations during the EB and ED trials. Plasma concentrations of EAA, BCAA, and leucine increased above preexercise values between 20 and 120 min postexercise (Fig. 2, B–D) for both the 15- and 30-g treatments. The 30-g protein feeding protocol resulted in higher aminoacidemia at 20 min postexercise (1.4-fold, $P < 0.004$) compared with 40 min postexercise following 15 g of protein ingestion (1.7-fold, $P < 0.001$). Plasma concentration peaked 40–60 min postexercise (1.8- to 1.9-fold, $P < 0.001$) and remained above preexercise values until 2 h postexercise (1.6- to 1.9-fold, $P < 0.02$) in both the 15- and 30-g treatments. Plasma EAA concentration increased in 30 compared with 15 g between 20 min and 1 h postexercise (1.2- to 1.3-fold, $P < 0.03$; Fig. 2B). Plasma BCAA and leucine concentration followed a similar pattern, but the differences between 15 and 30 g remained until 2 h postexercise (1.2- to 1.8-fold, $P < 0.02$; Fig. 2, C and D).

Muscle myofibrillar FSR

Intracellular free phenylalanine enrichments showed a stable precursor pool throughout infusion in all groups. Resting post-absorptive MPS after ED was lower compared with EB (0.019 vs 0.026%/h⁻¹, $P < 0.001$; Fig. 3). Resistance exercise in ED returned MPS to values comparable with resting EB in the acute postexercise recovery period. Resistance exercise followed by 15- and 30-g protein ingestion increased postexercise MPS ~16 and ~34% above resting EB, respectively (0.030 and 0.038%/h, respectively, $P < 0.02$; effect sizes $d = 0.86$ and 2, respectively). The 30-g protein treatment also increased MPS above 15 g by ~14% ($P < 0.003$; effect size $d = 0.83$). There were no differences between males and

Fig. 2. Plasma insulin (A), essential amino acids (EAA; B), branched-chain amino acids (BCAA; C), and leucine (D) concentration after 5 days of ED (30 kcal·kg FFM⁻¹·day⁻¹) and following a bout of leg press (6 sets × 8 repetitions at 80% 1-repetition maximum) and postexercise ingestion of a PL or 15 or 30 g of whey protein drinks. Data were analyzed by using 2-way repeated-measures ANOVA with Student-Newman-Keuls post hoc analysis. Values are mean and individual values. *Different vs. rest within treatment; †PL; ‡15 g; §30 g at equivalent time point ($P < 0.05$).



females in any of the treatments. Linear regression analysis revealed a positive correlation between the quantity of protein ingested per kilogram of BM or FFM and MPS ($r^2 = 0.43$ and 0.42 respectively, $P < 0.001$; Fig. 4).

Cell Signaling

There were no differences in phosphorylation status between resting EB and ED for any of the proteins quantified. Akt Ser⁴⁷³ is phosphorylated directly by mTOR, and phosphorylation of this site is required for full activation of Akt (45). Akt Ser⁴⁷³ phosphorylation was higher than resting ED in all treatments 1 h postexercise (1.8- to 3.2-fold, $P < 0.05$;

Figs. 5A and 6A). Protein intake increased Akt Ser⁴⁷³ phosphorylation above resting EB to a similar extent 1 h postexercise regardless of protein quantity (15 g: ~2.1-fold; 30 g: ~2.4-fold; $P < 0.02$). There were similar effects on mTOR Ser²⁴⁴⁸ and S6K Thr³⁸⁹ phosphorylation. Protein intake increased mTOR Ser²⁴⁴⁸ phosphorylation above resting EB levels and placebo at the 1-h postexercise time point (~2.5-fold from resting EB, ~2-fold from PL 1 h; $P < 0.006$; Figs. 5B and 6B). However, only the 30-g treatment prolonged the elevation in mTOR phosphorylation to 4 h postexercise (~2.1-fold, $P < 0.05$). The p70 S6K Thr³⁸⁹ phosphorylation increased above resting levels 1 and 4 h following resistance exercise and protein ingestion (2.6- to 7-fold, $P < 0.05$; Figs. 5C and 6C). Peak phosphorylation above rest was observed with 30 g of protein at 1 h postexercise (7-fold, $P < 0.001$) and was higher than 15 g of protein at the equivalent time point (1.8-fold, $P = 0.051$). Phosphorylation of rpS6 Ser^{236/237} above resting EB was highest 1 h after exercise with postexercise protein ingestion (12.5- to 19.2-fold, $P < 0.001$; Figs. 5D and 6D). There were no differences in AMPK Thr¹⁷², 4E-BP1 Thr^{36/47}, or eEF2 Thr⁵⁶ phosphorylation at any time (data not shown).

mRNA Expression

C_T values for GAPDH were stable across all time points. There were only minor changes in MuRF-1 mRNA content from resting EB, but MuRF-1 was different from rest and select postexercise time points after 4 h postexercise recovery in the 15-g protein treatment (1.85-fold, $P < 0.003$; Fig. 7A). Atrogin-1 mRNA content at 4 h postexercise was higher than resting EB, ED, and 1-h recovery (1.98- to 2.27-fold, $P < 0.006$; Fig. 7B). There were no differences in system A amino acid transporter (sodium-coupled neutral amino acid trans-

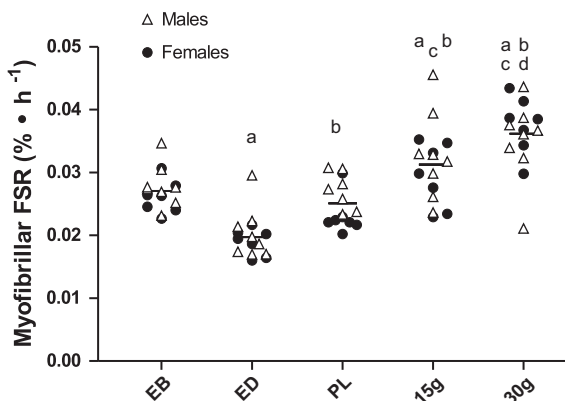


Fig. 3. Myofibrillar fractional synthetic rate (FSR) at rest after 5 days of EB (45 kcal·kg FFM⁻¹·day⁻¹), after 5 days of ED (30 kcal·kg FFM⁻¹·day⁻¹), and following a bout of leg press (6 sets × 8 repetitions at 80% 1-repetition maximum) and postexercise ingestion PL or 15 or 30 g of whey protein drinks. Data were analyzed by using repeated-measures ANOVA with Student-Newman-Keuls post hoc analysis. Values are mean and individual values. Different vs. the following ($P < 0.02$): *EB; †ED; ‡PL; §15 g.

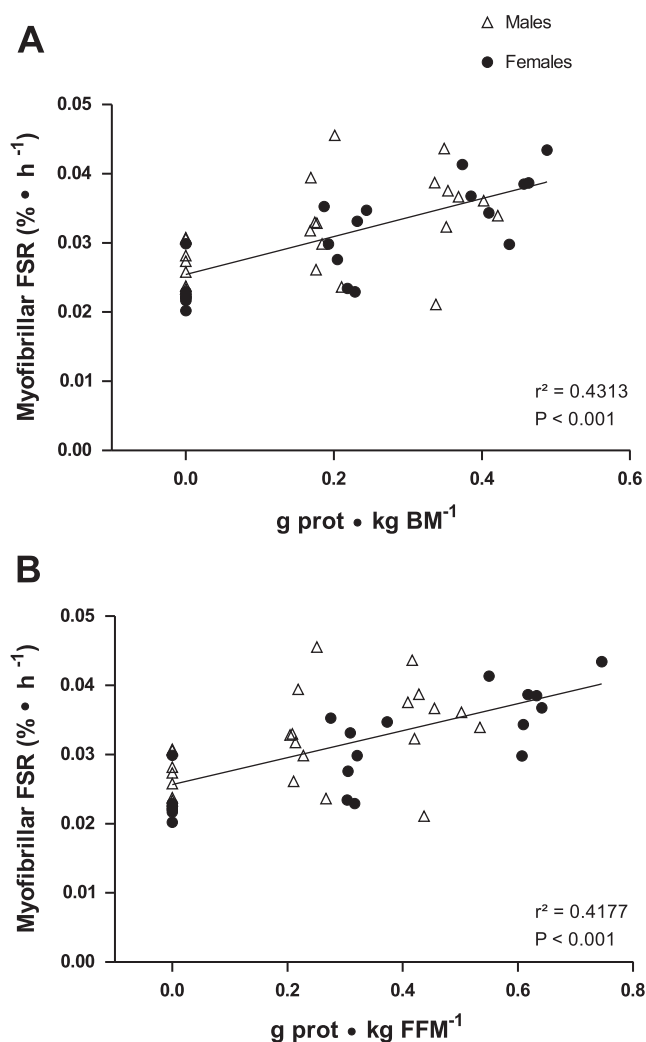


Fig. 4. Myofibrillar FSR after 5 days of ED (30 kcal·kg FFM⁻¹·day⁻¹) following bout of leg press (6 sets × 8 repetitions at 80% 1-repetition maximum) plotted against postexercise protein intake in g of protein/kg of body mass (BM; A) and FFM (B). Data were analyzed using linear regression.

porter 2) mRNA content (Fig. 7C), but in all treatments there was a decrease in system L amino acid transporter (LAT1) mRNA content at 1 and 4 h postexercise compared with resting EB (−0.49 to 0.6, $P < 0.03$). In addition, LAT1 mRNA content following resistance exercise with PL (4 h) and 15 g of protein ingestion (1 h) was lower compared with resting ED (0.55–0.64, $P < 0.04$; Fig. 7D).

Protein Content

We found no differences in the amount of SLC7A5 protein content at any time points between treatments (Fig. 8).

DISCUSSION

The first novel finding of the present study was that 5 days of moderate ED resulted in a 27% reduction in resting rates of myofibrillar protein synthesis in young, healthy men and women. A second finding was that, even when in ED, a single bout of REX was sufficient to restore MPS to values observed at rest in EB. Finally, the ingestion of protein after REX further increased rates of MPS above those observed at rest in EB in

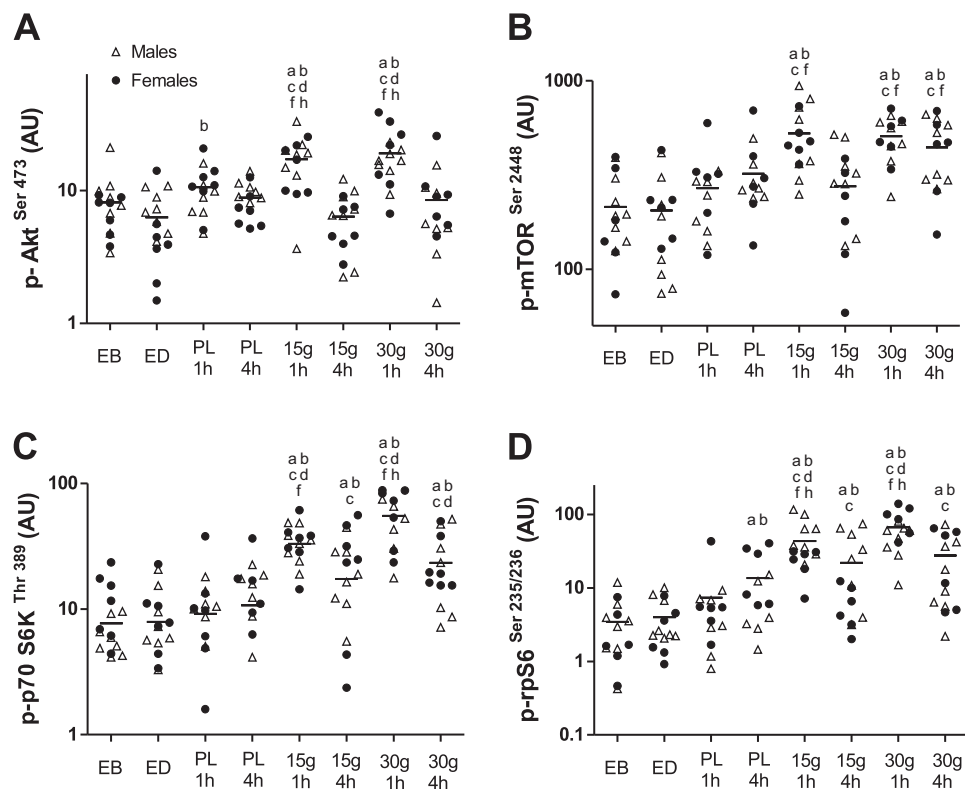
a dose-dependent manner. Taken collectively, our results demonstrate that a combination of REX with increased protein availability postexercise can enhance rates of skeletal muscle protein synthesis during short-term ED, which could in the long term preserve muscle mass.

Pasiakos et al. (41) have reported previously that, compared with EB, 10 days of moderate ED (~500 kcal/day) resulted in a 19% reduction in basal rates of mixed protein synthesis. Here, we extend their findings (41) and show that just 5 days of moderate ED (an energy availability of 30 kcal·kg FFM⁻¹·day⁻¹) resulted in a similar reduction in the rates of MPS in healthy individuals. Previous studies that have measured muscle protein synthesis responses to perturbations in energy status have based their protocols on a model of ED from estimated energy requirements (40, 41, 51). Instead, we chose a model of energy availability to set the energy deficit in our subjects, with a level of 30 kcal·kg FFM⁻¹·day⁻¹ corresponding to a threshold below which there is significant disruption to metabolic and hormonal systems within the body (30). The notional energy deficit of ~15 kcal·kg FFM⁻¹·day⁻¹ in our subjects was typically equivalent to an energy availability of 1,690–2,200 kcal/day for males and 1,210–1,640 kcal/day for female subjects. Despite the extensive resistance training history of our subjects, coupled with the high relative dietary protein intake (1.4 g·kg BM⁻¹·day⁻¹) during ED, postabsorptive MPS rates were not preserved compared with EB. The reduction in MPS in ED that we and others (41) have observed may be an adaptive response since MPS is an energetically expensive process.

Consistent with observations when individuals are in EB (36, 43), the anabolic stimulus generated by REX during energy restriction in the fasted state elevated rates of MPS above resting levels in the early postexercise period. However, despite this elevation, exercise merely restored MPS to a level that was similar to but not exceeding rates measured in EB. Accordingly, it appears that the metabolic status of the muscle during short-term (5 days) ED plus an ~10-h fast may dictate that contractile overload in isolation is not enough to increase MPS to values that otherwise would be observed when subjects are in EB.

The anabolic effect of protein ingestion on cell signaling and rates of protein synthesis is well accepted (6). A recent study on young healthy subjects involving 21 days of moderate ED (750 kcal/day) found that high protein intake (1.6 and 2.4 g·kg BM⁻¹·day⁻¹) rescued the FFM loss seen with protein intake at RDA levels (0.8 g/kg BM) (40). Our study is the first to determine the acute muscle anabolic response to resistance exercise with two different doses of protein ingested after exercise during short-term ED. Our results highlight the importance of combining REX with increased protein availability to maximize rates of protein synthesis. Furthermore, we report a dose-dependent response of MPS to protein ingestion in individuals in short-term ED; we observed a hierarchical increase above resting EB for rates of muscle protein synthesis with ingestion of 15 and 30 g of whey protein (Fig. 3). This effect was evident when protein ingestion was expressed in both absolute and relative terms to BM and FFM (Fig. 4). Our results suggest that the optimal amount of protein to maximize the response to a single bout of resistance training while in ED may be above the level (20 g) found to maximize MPS postexercise for individuals who are in EB (35). Given that

Fig. 5. Phosphorylation of skeletal muscle Akt Ser⁴⁷³ (A), mammalian target of rapamycin (mTOR) Ser²⁴⁴⁸ (B), p70 S6K Thr³⁸⁹ (C), and rpS6 Ser^{235/236} (D) at rest after 5 days of EB (45 kcal·kg FFM⁻¹·day⁻¹), after 5 days of ED (30 kcal·kg FFM⁻¹·day⁻¹), and following a bout of leg press (6 sets × 8 repetitions at 80% 1-repetition maximum) and postexercise ingestion of a PL or 15 or 30 g of whey protein drinks. Data were analyzed by repeated-measures ANOVA with Student-Newman-Keuls post hoc analysis. Values are mean and individual values. Different vs. the following (*P* < 0.05): ^aEB; ^bED; ^cPL, 1.5 h; ^dPL, 4.5 h; ^e15 g, 4.5 h; ^f30 g, 4.5 h. AU, arbitrary units.



previous studies have shown chronic resistance training and protein supplementation can promote gains in muscle mass when individuals are in EB or ED (20), our findings add support to the view that there are favorable interactions between REX and increased protein availability during periods of low energy availability that lead to improvements in body composition. However, the precise dose of protein necessary to preserve (or increase) rates of protein synthesis while simultaneously reducing fat mass in the face of different levels of

energy restriction has not been determined systematically (24, 28). Regardless, the provision of exogenous amino acid during ED appears to be a prerequisite for supporting muscle protein synthesis and allowing an increase in net muscle protein synthesis above that achieved at rest while in EB (2, 43).

The current data set indicates that the physiological response in skeletal muscle following the short-term ED protocol employed in the current study was similar in male and female subjects, and they appear to be equally responsive to an acute bout of REX and postexercise protein intake in ED. Indeed, we failed to observe any sex-based differences for the cellular markers of “muscle anabolism” under investigation, providing further support for the notion that both acute and chronic responses to resistance exercise and/or protein ingestion are similar between younger men and women (42, 46, 47, 54). Our muscle anabolic responses in ED persisted despite a wide range of differences in BM and body composition (Table 1). Given that the subjects in the current study had a history of REX, we cannot discount this as a possible factor that may have reduced our capacity to detect sex-based differences in MPS. Moreover, since there was a moderate relationship between the relative quantity of protein ingested and MPS, we cannot completely rule out the possibility that (smaller) females may have benefited at least in part from a greater relative protein dose (Fig. 4) and that this may have attenuated any potential sex-based differences. Indeed, Phillips et al. (42) have recently reported that the capacity of skeletal muscle to hypertrophy during 20 wk of the REX program is to a large extent determined genetically rather than sex dependent. Therefore, our current findings support the hypothesis that sexual dimorphism in absolute muscle mass of healthy adult males and females is likely determined by factors other than the magnitude of the

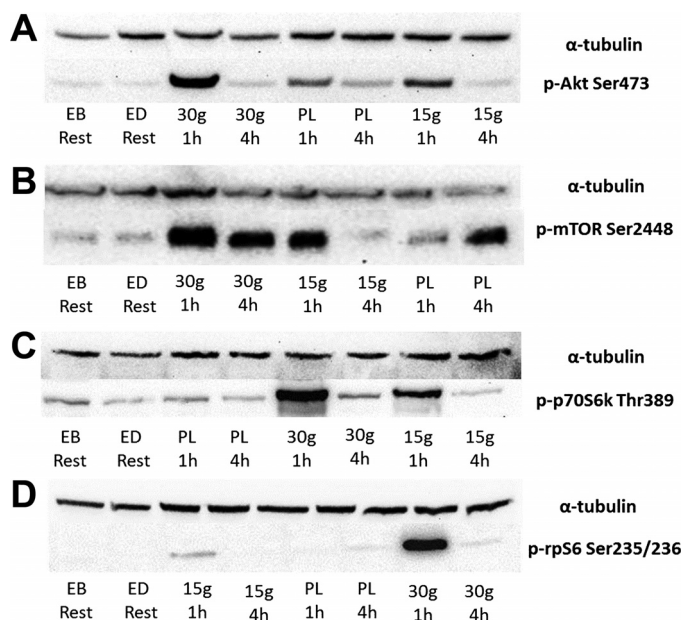


Fig. 6. Representative blots for signaling proteins.

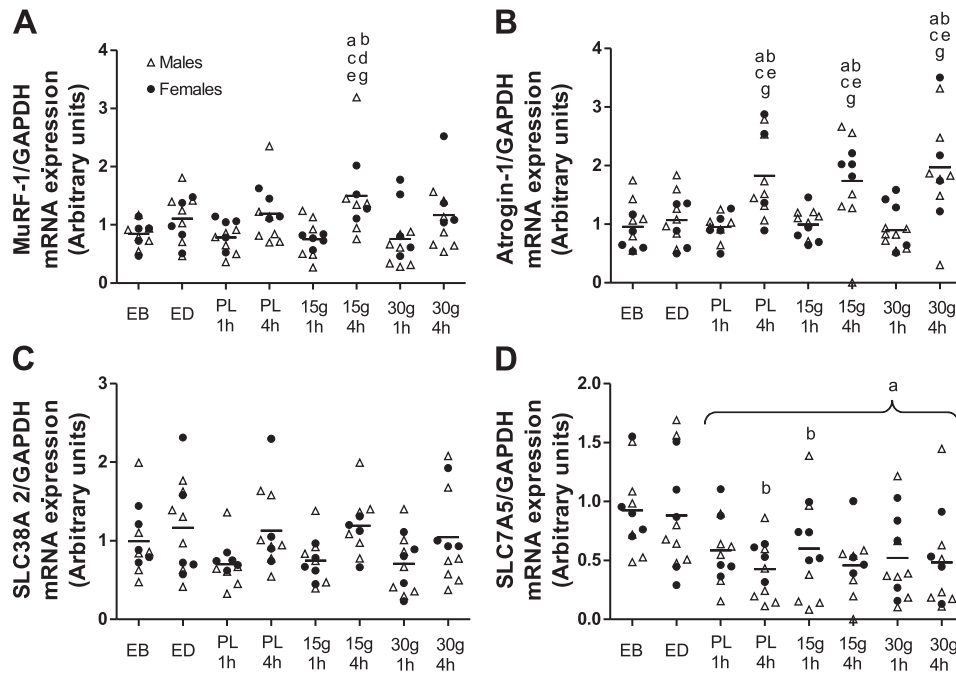


Fig. 7. Muscle RING finger-1 (MuRF-1; A), atrogin-1 (B), SLC38A2/SNAT 2 (C), and SLC7A5/LAT1 (D) mRNA abundance at rest after 5 days of EB (45 kcal·kg FFM⁻¹·day⁻¹), after 5 days of ED (30 kcal·kg FFM⁻¹·day⁻¹), and following a bout of leg press (6 sets × 8 repetitions at 80% 1-repetition maximum) and postexercise ingestion of a PL or 15 or 30 g of whey protein drinks. Data were analyzed by repeated-measures ANOVA with Student-Newman-Keuls post hoc analysis. Values are mean and individual values. Different vs. the following ($P < 0.05$): ^aEB; ^bED; ^cPL, 1.5 h; ^dPL, 4.5 h; ^e15 g, 1.5 h; ^f30 g, 1.5 h.

hypertrophic response to REX and protein intake (38). Although sex-based differences in skeletal muscle fiber composition have been reported previously (49), these differences are unlikely to have influenced our findings, as previous studies have failed to show any meaningful fiber type-specific effects on rates of MPS (26, 34).

In agreement with recent observations by others (40), our mTOR-associated translational signaling responses were similar at rest whether subjects were in EB or ED. Indeed, REX performed under conditions of ED and an overnight fast had little effect on promoting the phosphorylation of any of the proteins measured in the current study. This finding was in

contrast to our previous results (9) and those from several other groups (13, 15) when REX was undertaken in the fasted state in EB. However, we did observe a marked increase in translational signaling following the postexercise ingestion of protein, with subtle differences between the response to 15 and 30 g of whey protein. Moreover, we have previously reported a hierarchical signaling response to increasing quantities of whey protein ingestion (i.e., greater protein availability resulted in greater phosphorylation of p70 S6K) (1). The results of the current study indicate that ED may alter the magnitude of signal for translation initiation in response to acute exercise and protein intake (Fig. 5). Importantly, the similar phosphorylation responses were not mirrored by MPS rates and support previous work showing that translation initiation signaling can be indicative of increases in MPS compared with rest (8, 18, 27) but do not accurately reflect the magnitude or duration of the MPS response (1).

The ubiquitin ligases MuRF-1 and atrogin are key regulatory steps of the ubiquitin-proteasomal protein degradation. Linked originally to muscle atrophy (4), they seem to be important in the myofibril remodeling process after a bout of resistance exercise (55). ED did not generate any differences in muscle transcriptional activity of any gene of interest at rest compared with resting EB (Fig. 7). Protein intake in sufficient quantities has been shown previously to blunt the exercise induced increase in MuRF-1 mRNA abundance (1, 5, 32). Interestingly, increases in Atrogin mRNA abundance following high-intensity exercise are not consistently observed in human skeletal muscle (1, 32, 44), and our results showing elevated atrogin mRNA following resistance exercise in all treatments suggests that ED may promote the catabolic activity of this specific atrogene. Importantly, protein ingestion did not alter the elevated transcriptional activity of atrogin during recovery from resistance exercise in ED, but direct measurements of protein breakdown are required to determine the physiological relevance of the increase in atrogin mRNA expression when

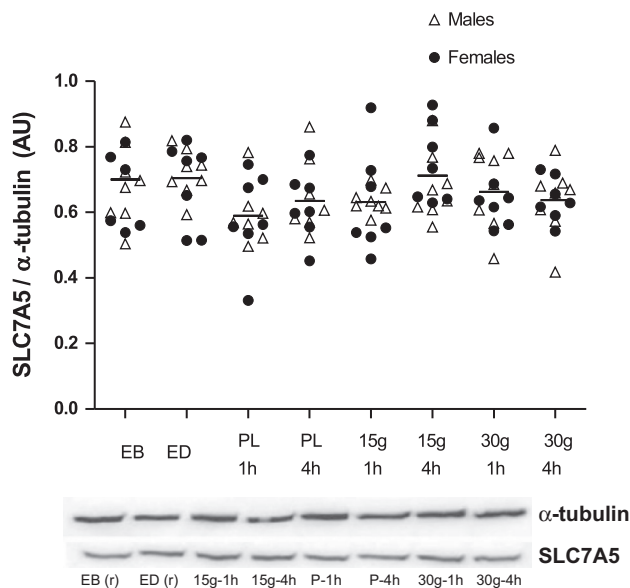


Fig. 8. LAT 1/SLC7A5 protein content relative to α -tubulin protein content. Data were analyzed by repeated-measures ANOVA with Student-Newman-Keuls post hoc analysis. Values are mean and individual values.

exercising in ED. We observed a downregulation of the system L amino acid transporter gene SLC7A5. Although SLC7A5 mRNA has been shown to be elevated after REX alone (16) and also following REX and protein ingestion when subjects are in EB (14), our results indicate that REX undertaken in ED acts to suppress the typical exercise/nutrient-mediated upregulation of SLC7A5 mRNA abundance (Fig. 7D). Notwithstanding any perturbations to SLC7A5 mRNA that may be induced by energy status, we failed to detect changes in LAT1 protein content during the early recovery period for any intervention (Fig. 8).

In conclusion, our results are the first to determine the effect of short-term ED on rates of myofibrillar protein synthesis. We show that as little as 5 days of ED (energy availability 30 kcal·kg FFM⁻¹·day⁻¹) was sufficient to reduce rates of post-absorptive MPS. However, this impairment was “rescued” to values observed at rest in EB by a single bout of resistance exercise. The ingestion of protein after REX further increased MPS above resting EB in a dose-dependent manner. Accordingly, we suggest that chronic resistance training combined with increased postexercise protein availability would enhance rates of skeletal muscle protein synthesis during prolonged periods of moderate ED, which would ultimately preserve lean (muscle) mass and reduce fat mass. Finally, we suggest that the amount of protein required to maximally stimulate muscle protein synthesis under conditions of mild ED is likely to be higher than for individuals in EB.

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DISCLOSURES

The authors report no conflicts of interest, financial or otherwise.

AUTHOR CONTRIBUTIONS

J.L.A., L.M.B., D.R.M., T.S., S.M.P., J.A.H., and V.G.C. conception and design of research; J.L.A., D.M.C., D.W.D.W., and S.C. performed experiments; J.L.A. and V.G.C. analyzed data; J.L.A., L.M.B., D.R.M., T.S., S.M.P., J.A.H., and V.G.C. interpreted results of experiments; J.L.A. prepared figures; J.L.A. drafted manuscript; J.L.A., L.M.B., D.R.M., T.S., S.M.P., J.A.H., and V.G.C. edited and revised manuscript; J.L.A., L.M.B., D.M.C., D.W.D.W., S.C., D.R.M., T.S., S.M.P., J.A.H., and V.G.C. approved final version of manuscript.

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