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

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Authors' contributions to manuscript

JLA, LMB, SMP, DRM, TS, JAH and VGC designed research. JLA, DWDW, SC and DMC conducted research. JLA, JAH and VGC analyzed data and/or performed statistical analysis. JLA, LB, SMP, JAH and VGC wrote paper. JLA, JAH and VGC had primary responsibility for final content.

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Abstract
The myofibrillar protein synthesis (MPS) response to resistance exercise (REX) and protein ingestion during energy deficit (ED) is unknown. We determined, in young men (n=8) and women (n=7), protein signaling, resting post-absorptive MPS during energy balance [EB: 45 kcal·(kg FFM·d)^{-1}] and after 5d of ED [30 kcal·(kg FFM·d)^{-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). Post-absorptive 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 post-absorptive 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.

Key words: Body composition; Fat Free Mass; Myofibrillar Protein Synthesis; Weight loss
Introduction

Energy deficit (ED) can be achieved through reduced energy intake and/or increase 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, 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 life span (25), nutritional and exercise strategies to prevent or minimize loss of FFM while losing fat mass are crucial.

Pasiakos and colleagues (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\(^{-1}\)). In contrast, a recent study from the same group (40) found no decrease in rates of resting muscle protein synthesis after 30 day of moderate ED. If a potential decrease in basal rates of muscle protein synthesis were 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 to ED, 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 if 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) was measured. Furthermore, there was no examination of the impact of exercise, and the cohort
under investigation comprised mainly 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, as 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 energy deficit. 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 post-exercise protein intake as higher protein availability may 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 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 weeks 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, Madison, WI).

Experimental design. The study employed a within subject design, with subjects completing
four experimental interventions: energy balance (EB) at rest; energy deficit (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
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avoid any potential dysregulation in EB trial induced by previous ED interventions (Figure 1).

**Dietary intervention.** Subjects were each provided with individualized pre-packaged meals for five 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⁻¹ FFM · 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⁻¹ FFM · day⁻¹. Between days 1-3 of the dietary control period subjects were permitted to exercise and the diet 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 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 day 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, as little as 4 days of ED is capable of generating a negative nitrogen balance (17, 21, 40, 48). Our 9 day washout period was used based on data showing nitrogen balance returns to positive values after only 3 days of re-feeding following a 3 week 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 five days of dietary control, subjects reported to the laboratory between 0700 and 0800 h after a ~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⁻¹ prime) infusion of L-[ring-¹³C₆]phenylalanine (Cambridge Isotopes Laboratories, Woburn, MA, USA) commenced. After a 3 h resting period, a muscle biopsy was obtained. For the three non-tracer 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 ingestion randomized and counterbalanced. Drinks contained 15 g or 30 g of protein (ISO8 WPI; 86.8 g protein, 1.5 g fat, 3.1 g carbohydrates per 100 g; Musashi, Australia) 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 h and 4 h post-exercise. 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 isotopic equilibrium was reached before/during the measurement periods (between 1 and 4 h).

Exercise. A one repetition maximum (1 RM) inclined (45º) leg press (GLPH1100, Body-Solid, Forest Park, IL, USA) test was completed by each subject a minimum of one week prior to the experimental trials. After a warm up of 2 sets of 5 moderate intensity repetitions
the 1 RM was determined as the highest successfully lifted weight during a maximum of 6 attempts. On the day of an ED experimental trial subjects completed two warm up sets of 5 repetitions at ~50 and ~60% 1 RM with 2 min rest between sets. The REX bout incorporated 6 sets of 8 repetitions at ~80% 1 RM with 3 min rest between sets. Exercise range of motion was ~85° for the knee joint, with leg extension endpoint 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 (Figure 1). 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, Australia) according to manufacturer’s guidelines. Plasma amino acids (AA) were analyzed by gas chromatography-mass spectrometry using EZ:faast kit (Phenomenex, USA)

Western Blot. Intracellular proteins were extracted, isolated and quantified as previously described (11). Amount of protein loaded in each well was 40 μg. Polyclonal anti-phospho mammalian target of rapamycin (mTOR) Ser2448 (#2971), monoclonal anti-phospho-Akt Ser473 (#9271), ribosomal protein S6 Ser 235/6 (#4856), 4E-BP1 Thr37/46 (# 2855), eEF2 Thr56 (# 2331), AMPK Thr172 (#2535) and anti-α-tubulin control protein (#3873) were purchased from Cell Signaling Technology (Danvers, MA, USA). Polyclonal anti-phospho-p70S6K Thr 389 (#PK1015) was from Millipore (Temecula, CA, USA). Monoclonal anti-
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SLC7A5 (ab134121) was obtained from Abcam (Cambridge, England). All densitometric analysis was carried out by the same researcher using specialized software (ImageJ 1.47, National Institutes of Health, USA) by quantifying the area under the peak of each plot for each lane of the measured membrane.

Fractional Synthetic Rate. Pre-infusion 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 pre-infusion muscle biopsy was used for baseline enrichment. Muscle tissue was processed as previously described (36).

Calculations. The fractional synthetic rate of myofibrillar proteins was calculated using the standard precursor–product method:

\[
FSR \, (\% \cdot h^{-1}) = \frac{E_{p2} - E_{p1}}{E_{ic}} \times \frac{1}{t} \times 100
\]

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-naive’ subjects (n=12) allowed us to use the pre-infusion blood sample (i.e. mixed plasma protein fraction) as the baseline enrichment (Ep1) 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 previously described (12). Reverse transcription and real-time Polymerase Chain Reaction (RT-PCR) was performed as previously described (10, 54). Taqman-FAM-labeled primer/probes for Atrogin-1 (Hs01041408_m1*), MuRF-1 (Hs00822397_m1*), SLC38A2 (Hs00255854_m1*), and SLC7A5 (Hs00185826_m1) primers (Applied Biosystems, Carlsbad, CA, USA) 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 (ΔΔCT) method (29).

Statistical analysis. Data were analyzed using two way repeated measures analysis of variance (ANOVA) with Student-Newman-Keuls post hoc analysis (gender × 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 energy balance and energy deficit trials were independently analyzed from the exercise trials. Data for Western blotting were log-transformed prior to analysis. All data are presented as mean ± standard deviation (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 × group interaction for plasma insulin concentration (P<0.001) after exercise and protein feeding (Figure 2).

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

Muscle myofibrillar fractional synthetic rate. Intracellular free phenylalanine enrichments showed a stable precursor pool throughout infusion in all groups. Resting post-absorptive MPS after ED was lower compared to EB (0.019 vs 0.026 %·h⁻¹, P < 0.001; Figure 3). Resistance exercise in ED returned MPS to values comparable to resting EB in the acute post exercise recovery period. Resistance exercise followed by 15 g and 30 g protein ingestion increased post-exercise 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 females in any of the treatments. Linear regression analysis revealed a positive correlation between the quantity of protein ingested per kg of BM or FFM and MPS (r² =0.43 and 0.42 respectively, P< 0.001, Figure 4).

Cell signaling. There were no differences in phosphorylation status between resting EB and ED for any of the proteins quantified. Akt^{Ser473} is directly phosphorylated 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 post exercise (1.8 -3.2 fold; P < 0.05; Figure 5A). Protein intake increased Akt phosphorylation above resting EB to a similar extent 1 h post exercise 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 post exercise time point (~2.5 fold from resting EB, ~2 fold from PL1 h; P < 0.006; Figure 5B). However, only the 30 g treatment prolonged the elevation in mTOR phosphorylation to
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4 h post-exercise (~2.1 fold; P < 0.05). The p70 S6K$^{\text{Thr389}}$ phosphorylation increased above resting levels 1 h and 4 h following resistance exercise and protein ingestion (2.6-7 fold; P < 0.05; Figure 5C). Peak phosphorylation above rest was observed with 30 g protein at 1 h post-exercise (7 fold; P < 0.001) and was higher than 15 g protein at the equivalent time point (1.8 fold, P=0.051). Phosphorylation of rpS6$^{\text{Ser236/237}}$ above resting EB was highest 1 h after exercise with post exercise protein ingestion (12.5-19.2 fold; P < 0.001 Figure 5D). There were no differences in AMPK$^{\text{Thr172}}$, 4EBP1$^{\text{Thr36/47}}$ or eEF2$^{\text{Thr56}}$ phosphorylation at any time (data not shown).

mRNA Expression. Ct 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 post exercise time points after 4 h post-exercise recovery in the 15 g protein treatment (1.85 fold; P < 0.003; Figure 7A). Atrogin-1 mRNA content at 4 h post exercise was higher than resting EB and ED, 1 h recovery (1.98-2.27 fold; P < 0.006; Figure 7B). There were no differences in system A amino acid transporter (SNAT2) mRNA content but in all treatments there was a decrease in system L amino acid transporter (LAT1) mRNA content at 1 and 4 h post-exercise compared to resting EB (-0.49-0.6; P < 0.03). In addition, LAT1 mRNA content following resistance exercise with PL (4 h) and 15 g protein ingestion (1 h) was lower compared with resting ED (0.55-0.64; P < 0.04; Figure 7D).

Protein content. We found no differences in the amount of SLC7A5 protein content at any time-points between treatments (Figure 8).
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**Discussion**

The first novel finding of the present study was that five 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 post exercise 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 previously reported that compared with EB, 10 days of moderate ED (~500 kcal · day⁻¹) resulted in a 20% reduction in basal rates of mixed protein synthesis. Here we extend their findings (41) and show that just five days of moderate ED (an energy availability of 30 kcal·kg⁻¹ FFM·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). We chose, instead, a model of energy availability to set the energy deficit in our subjects, with a level of 30 kcal·kg⁻¹ FFM·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⁻¹ FFM·day⁻¹ in our subjects was typically equivalent to an energy availability of 1690-2200 kcal · day⁻¹ for males and 1210-1640 kcal·day⁻¹ for female subjects. Despite the extensive resistance training history of our subjects, coupled with the high relative dietary protein intake (1.4 g·kg⁻¹ BM·day⁻¹) during ED, post-absorptive MPS rates were not preserved compared to EB. The reduction in MPS in
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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 post-exercise 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 the metabolic status of the muscle during short-term (5 days) ED plus a ~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⁻¹) found that high (1.6 and 2.4 g·kg⁻¹ BM·day⁻¹) protein intake rescued the FFM loss seen with protein intake at RDA levels (0.8 g·kg⁻¹ 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 energy balance for rates of muscle protein synthesis with ingestion of 15 and 30 g whey protein (Figure 3). This effect was evident when protein ingestion was expressed in both absolute and relative terms to body mass and fat free mass (Figure 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 post-exercise for
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individuals who are in EB (35). Given that 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 systematically determined (24, 28). Regardless, the provision of exogenous amino acid during ED appears to be a pre-requisite for supporting muscle protein synthesis and allowing an increase in net muscle protein synthesis above that achieved at rest while in energy balance (2, 43).

The current data set indicate 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 equally responsive to an acute bout of REX and post exercise 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 body mass 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, as 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 (Figure 4) and that this may have attenuated any potential sex-based differences. Indeed Phillips and co-workers (42)
have recently reported that the capacity of skeletal muscle to hypertrophy during 20 wk REX program is to a large extent, genetically determined, rather than being 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 hypertrophic response to REX and protein intake (38). While sex-based differences in skeletal muscle fiber-composition have previously been reported (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 in promoting the phosphorylation of any of the proteins measured in the current study. This finding is 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 post exercise ingestion of protein, with subtle differences between the response to 15 g 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 (Figure 5). Importantly, the similar phosphorylation responses were not mirrored by MPS rates and support previous work showing translation initiation signaling can be indicative of increases in MPS compared to rest (8, 18, 27), but does 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. Originally linked to muscle atrophy (4) they seem to be important in the myofibril remodeling process after a bout of resistance exercise (55). Energy deficit did not generate any differences in muscle transcriptional activity of any gene of interest at rest compared with resting energy balance (Figure 7). Protein intake in sufficient quantities has been previously shown to blunt the exercise induced increase in MuRF-1 mRNA abundance (1, 5, 32). Interestingly, increases in Atrogin mRNA abundance following high intensity exercise is 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 energy deficit 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 energy deficit but direct measures of protein breakdown are required to determine the physiological relevance of the increase in Atrogin mRNA expression when exercising in ED. We observed a down-regulation of the system L amino acid transporter gene SLC7A5. While 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 up-regulation of SLC7A5 mRNA abundance (Figure 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 (Figure 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 five days of ED (energy availability
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30 kcal·kg\(^{-1}\)·FFM·day\(^{-1}\)) was sufficient to reduce rates of post-absorptive MPS. However, this impairment was ‘rescued’ to values observed at rest in energy balance 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 post-exercise 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 energy deficit is likely to be higher than for individuals in energy balance.

Acknowledgements

The authors would like to thank Evelyn Parr, Alisa Nana, Louise Cato, Iona Halliday, Greg Shaw, Felicity Galvez, Graeme Allbon, Ryan Kohler, Tom Hilton, Dr. Peter Velloza, Dr. Andrew Garnham, Todd Prior and Tracy Rerecich for technical assistance during clinical trials and laboratory analysis.

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Grants

This study was funded by an Australian Research Council Linkage Project Grant LP100100010.

Disclosures

All authors report no conflict of interest.
References


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**Table 1.** Subjects characteristics. 1RM, 1 Repetition Maximum; BM, Body Mass; FFM, Fat Free Mass. Data were analyzed by using multiple T-tests. Values are mean ± SD.* different between sexes (P < 0.05).

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**Figure 1.** Schematic of the experimental design. The resting energy balance (EB) trial was preceded by 5 days of controlled diet providing 45 kcal • kg⁻¹ FFM • day . The
Energy deficit and skeletal muscle protein synthesis

resting/exercise in energy deficit (ED) trials were preceded by 5 days of controlled diet providing 30 kcal • kg⁻¹ FFM • day. Subjects returned to normal daily activities in energy balance for 9 days between the periods energy deficit. Solid arrow, muscle biopsy sample; *, blood sample; REX, resistance exercise; 0 g, 15 g and 30 g represent the respective placebo or whey protein drinks (500 mL). Dashed time-line represent trials undertaken a single time by each subject. Times in parentheses are for ED trials involving exercise and protein intake.

Figure 2. Plasma Insulin concentration after 5 days of energy deficit (30 kcal • kg⁻¹ FFM • day) following a bout of leg press (6 sets × 8 repetitions at 80% one repetition maximum) and post-exercise ingestion of a placebo (PL) or 15 or 30g of whey protein drinks. Data were analyzed by two way repeated measures ANOVA with Student-Newman-Keuls post hoc analysis. Values are mean ± SD. Different vs a, rest within treatment; *, PL , †, 15g; §, 30 g at equivalent time point (P < 0.05).

Figure 3. Plasma essential amino acids (EAA; A), branched-chain amino acids (BCAA; B), and leucine (C) concentration after 5 days of energy deficit (30 kcal • kg⁻¹ FFM • day) and following a bout of leg press (6 sets × 8 repetitions at 80% one repetition maximum) and post-exercise ingestion of a placebo (PL), 15 or 30 g of whey protein drinks. Data were analyzed by using two way repeated measures ANOVA with Student-Newman-Keuls post hoc analysis. Values are mean and individual values. Different vs. a, rest within treatment; †, 15 g; §, 30 g at equivalent time point (P < 0.05).

Figure 3. Myofibrillar fractional synthetic rate (FSR) at rest after 5 days of energy balance (45 kcal • kg⁻¹ FFM • day; EB), after 5 days of energy deficit (30 kcal • kg⁻¹ FFM • day; ED) and following a bout of leg press (6 sets × 8 repetitions at 80% one repetition maximum) and post-exercise ingestion of a placebo (PL), 15 or 30 g of whey protein drinks. Data were
Energy deficit and skeletal muscle protein synthesis analyzed by using repeated measures ANOVA with Student-Newman-Keuls post hoc analysis. Values are mean and individual values. Different vs. a, EB; b, ED; c, PL; d, 15 g (P < 0.02).

**Figure 4.** Myofibrillar fractional synthetic rate (FSR) after 5 days of energy deficit (30 kcal · kg⁻¹ FFM · day) following bout of leg press (6 sets × 8 repetitions at 80% one repetition maximum) plotted against post exercise protein intake in grams of protein per kg of body mass (BM; A) and fat free mass (FFM; B). Data were analyzed using linear regression.

**Figure 5.** Phosphorylation of skeletal muscle Akt Ser⁴⁷³ (A), mTOR Ser²⁴⁴⁸ (B), p70 S6K Thr³⁸⁹ (C), and rpS6 Ser²³⁵/²³⁶ (D) at rest after 5 days of energy balance (45 kcal · kg⁻¹ FFM · day; EB), after 5 days of energy deficit (30 kcal · kg⁻¹ FFM · day; ED) and following a bout of leg press (6 sets × 8 repetitions at 80% one repetition maximum) and post-exercise ingestion of a placebo (PL), 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. a, EB; b, ED; c, PL 1.5 h; d, PL 4.5 h; f, 15 g 4.5 h; h, 30 g 4.5 h (P < 0.05).

**Figure 6.** Representative blots for signaling proteins.

**Figure 7.** MuRF-1 (A), Atrogin-1 (B), SLC38A2/SNAT 2 (C) and SLC7A5/LAT1 (D) mRNA abundance at rest after 5 days of energy balance (45 kcal · kg⁻¹ FFM · day; EB), after 5 days of energy deficit (30 kcal · kg⁻¹ FFM · day; ED) and following a bout of leg press (6 sets × 8 repetitions at 80% one repetition maximum) and post-exercise ingestion of a placebo (PL), 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. a, EB; b, ED; c, PL 1.5h; d, PL 4.5 h; e, 15g 1.5 g; g, 30 g 1.5 h (P < 0.05).

**Figure 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.
**A**

![Graph showing the relationship between myofibrillar FSR (% • h⁻¹) and g prot • kg BM⁻¹.]

- Males (△)
- Females (●)

$r^2 = 0.4313$

$P < 0.001$

**B**

![Graph showing the relationship between myofibrillar FSR (% • h⁻¹) and g prot • kg FFM⁻¹.]

$r^2 = 0.4177$

$P < 0.001$
α-tubulin
p-Akt Ser473

α-tubulin
p-mTOR Ser2448

α-tubulin
p-p70S6k Thr389

α-tubulin
p-rpS6 Ser235/236
A

\[ \text{MuRF-1/GAPDH mRNA expression (Arbitrary units)} \]

- Males
- Females

B

\[ \text{Atrogin-1/GAPDH mRNA expression (Arbitrary units)} \]

C

\[ \text{SLC38A2/GAPDH mRNA expression (Arbitrary units)} \]

D

\[ \text{SLC7A5/GAPDH mRNA expression (Arbitrary units)} \]

- EB
- ED
- PL
- PL 1h
- PL 4h
- 15g
- 15g 1h
- 15g 4h
- 30g
- 30g 1h
- 30g 4h