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Acute resistance exercise augments integrative myofibrillar protein synthesis

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ABSTRACT

The purpose of this study was to determine whether an acute bout of high-intensity resistance exercise (RE) would augment integrative mixed muscle and myofibrillar protein fractional synthesis rates (FSRs) when total energy and macronutrient intake was controlled. Twelve healthy young men were studied over 24 hours and performed an acute bout of exhaustive (5 sets until volitional failure of their 85% 1-repetition maximum) unilateral leg press and knee extension exercise, such that one leg was exercised (EX) and the other served as a control (CON). ^2H_2O (70%) was provided to measure mixed muscle and myofibrillar FSR, and muscle biopsies (vastus lateralis) were collected from the EX and CON legs 16 hours following the RE session. ^2H-labeling of body water over the course of the experiment was 0.32 ± 0.01 mole percent excess. Interestingly, integrative mixed muscle FSR (percent per hour) was similar between the CON (0.76% ± 0.08%) and EX (0.69% ± 0.06%) legs. In contrast, upon determination of myofibrillar FSR, there was an RE effect (EX, 0.94% ± 0.16% vs CON, 0.75% ± 0.08%; $P < .05$). High-intensity RE without prior training impacts integrative myofibrillar 24-hour FSR, perhaps without altering total responses.

1. Introduction

Resistance exercise (RE) in the postabsorptive state stimulates fractional synthesis rates (FSRs) of mixed [1-5], myofibrillar [3,6,7], and sarcoplasmic [6] muscle proteins from 1 to 72 hours following a single session. The addition of feeding to RE (particularly just after exercise) has been reported to optimize this anabolic effect [8-12]. Results from experiments using a primed-continuous infusion over a 2- to 6-hour period are suggestive of what occurs over the course of a day. Although appropriate for directly determining the effect of RE and feeding on acute FSR in muscle, the use of relatively invasive procedures make "integrative" assessments for longer periods of time problematic. Specifically, subjects are in the laboratory under strict dietary/activity control during periods of assessment; thus, variables such as activities of daily living, stress, time of day, and sleep are generally excluded or tightly controlled.

Little is known regarding the effects of RE and feeding on human integrative muscle protein synthesis (MPS). The purpose of this study was to determine whether an acute bout of high-intensity RE remains anabolic when an integrative measurement of mixed muscle and myofibrillar protein FSR is made under free-living conditions over 24 hours.

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2. Methods

2.1. Subjects

Twelve healthy (nonsmokers), recreationally active young men (21.6 ± 0.4 years, 81.9 ± 3.2 kg, 1.80 ± 0.2 m; means ± SEM) volunteered to participate in this study. All experimental procedures were approved by the Institutional Review Board of Texas A&M University; and participants were informed of the study purpose, procedures, and risks associated with the study before participation.

2.2. Experimental protocol

On the day of the study, subjects reported to the laboratory at 8:00 AM to receive their first of 4 boluses (300 mL total) of 70% ^2H_2O (Cambridge Isotopes, Andover, MA) to achieve approximately 0.4% to 0.5% ^2H-labeling of body water (Fig. 1). In addition, participants received their first of 6 meals (a combination of Boost Plus and Boost; Novartis Medical Nutrition, Fremont, IN), which supplied the subjects with 8037 kJ (based on daily intake over 72-hour recall), 52% carbohydrate, 20% protein, and 28% fat. Six hours after initiating the study, subjects performed a 10-minute warm-up on a stationary cycle ergometer followed by an acute bout of dynamic unilateral leg press and knee extension (Keiser, Fresno, CA) RE (5 sets each; 85% of 1-repetition maximum) until exhaustion (EX), whereas the contralateral leg served as control (CON). Subjects rested for 2 minutes between sets and 5 minutes between exercises. Within hours after exercise, protein (Gatorade Nutrition Shake; Gatorade, Chicago, IL) and carbohydrate-lipid supplements (85% Waximaize Fruit Punch; Innovative Delivery Systems, Oviedo, FL, and 15% peanut oil, commercially available) were administered based on body mass to provide an additional 3935 ± 103 kJ, 71% carbohydrate, 11% protein (0.3 g/kg body mass), and 18% fat. In total, subjects consumed 12 223 ± 133 kJ, 56% carbohydrate, 15% protein (1.4 ± 0.05 g/kg body mass), and 29% fat during the experiment. Energy (kilojoules) and macronutrient intake (grams) did not differ among subject's baseline, pre–study day, and study day intakes.

The following morning, subjects returned to the laboratory (~7:00 AM) for the final blood draw and vastus lateralis muscle biopsies, obtained from both the EX and CON legs using a 5-mm Bergström biopsy needle with local anesthesia (1% lidocaine) and sterile procedures. Fat, connective tissue, and blood were removed from the samples; frozen with liquid N_2; and stored at −80°C until analyzed.

2.3. Analysis

^2H-labeling of body water and protein-bound alanine was determined as previously described [13]. Briefly, 20 μL of plasma was reacted with 2.0 μL of 10 N NaOH and 4.0 μL of a 5% (vol/vol) solution of acetone in acetonitrile for 24 hours. Acetone was removed by the addition of 0.6 mL of chloroform and 0.5 g Na_2SO_4. For determination of ^2H-labeling of protein-bound alanine, approximately 25 mg of wet muscle was homogenized on ice in 0.3 mL of a 10% (wt/vol) tricarboxylic acid and centrifuged at 3750 rpm at 4°C for 15 minutes × 4 cycles before dissolving the protein pellet in 6 N HCl (0.1 mL/0.030 g tissue) and reacting at 100°C for 18 hours [14]. To determine myofibrillar FSR, approximately 40 mg of wet muscle was homogenized on ice in 0.4 mL of a 1× kinase buffer (25 mmol/L Tris-HCl, 5 mmol/L β-glycerophosphate, 2 mmol/L dithiothreitol, 0.1 mmol/L Na_3VO_4, 10 mmol/L $MgCl_2$; Cell Signaling Technologies, Danvers, MA) and 0.01% Triton. Homogenates were centrifuged at 14 000 rpm at 4°C for 30 minutes, the supernatant containing the cytosolic and membrane portion was discarded, and the precipitate representing myofibrillar protein was prepared as above for mixed muscle. An aliquot (0.100 mL) of the hydrolysate was freeze-dried for 24 hours. A 3:2:1 ratio (0.1 mL) of *N*,*N*-dimethylformamide dimethyl acetal (Methyl-8 reagent; Pierce, Rockford, IL), methanol, and acetonitrile was added to the residue to determine the ^2H-labeling of alanine on its methyl-8 derivative. All samples were analyzed using an Agilent 5973N-MSD (Agilent Technologies, Santa Clara, CA) equipped with an Agilent 6890 GC system and a DB17-MS capillary column (30 m × 0.25 mm × 0.25 μm).

2.4. Statistics

A paired *t*-test was used to determine differences of means (^2H-labeling and FSR) between the CON and EX legs.

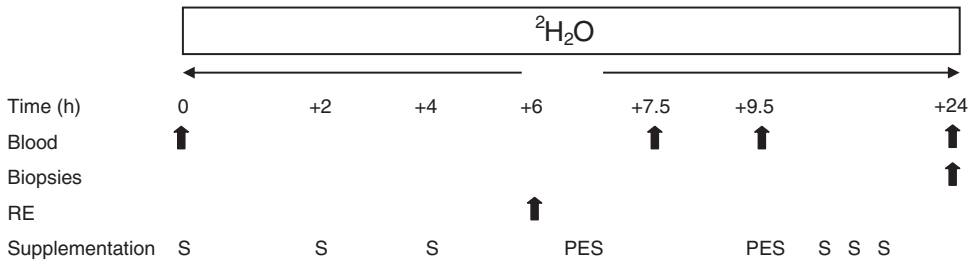

Fig. 1 – Study design. Subjects reported to the laboratory at 8:00 AM to start the 24-hour experiment. Time points for blood sampling, bilateral muscle biopsies, and the unilateral RE session are indicated by the arrows. Subjects received a combination (6 in total) of Boost Plus and Boost drinks (S) throughout the day and postexercise drinks (PES) based on body mass to remove variability associated with diet and maximize their anabolic potential. Subjects were allowed to leave the laboratory and perform their normal daily routines; however, they were instructed to refrain from exercising or eating. At 6:00 PM, subjects were dismissed for the evening with their final 2 Boost drinks and encouraged to consume the final beverage at 10:00 PM. At 7:00 AM, subjects returned to the laboratory for their final blood draw and bilateral muscle biopsies.

3. Results

Body water ^2H enrichment over 24 hours was approximately 0.32 mol percent excess (MPE) (Table 1). Because of low levels of ^2H (<0.5% body water enrichment), we amplified the detection by integrating the initial 20% region of the chromatographic peaks [15] to determine ^2H-labeling of protein-bound alanine (Table 1). Resistance exercise did not increase mixed muscle FSR, but resulted in a 20% elevation in myofibrillar FSR ($P < .05$) (Table 1). Because we did not have adequate sample quantities to measure the myofibrillar protein fraction in all subjects, we also statistically assessed the mixed fraction of this specific subset of individuals (excluding the other subjects) to determine if the mixed fraction of this group was affected differently as compared with all subjects. We did not observe an effect of RE ($P < .05$) on the mixed fraction in that subset.

4. Discussion

The main objective of this study was to determine if high-intensity RE stimulates integrative mixed muscle and myofibrillar protein FSR when measured over 24 hours. The most important finding from this study is that a single bout of exhaustive RE is sufficient to stimulate myofibrillar protein FSR without altering the total anabolic response of the mixed muscle pool. This may have important implications toward our understanding of selective protein synthesis at the onset of training.

Myofibrillar protein (primarily actin and myosin) constitutes the majority of skeletal muscle protein (>70%) [16], so it would seem that changes within this fraction would be observed in the mixed muscle measurement. However, it should be noted that skeletal muscle contains proteins that possess very different renewal rates; and previous investigations that measured both mixed muscle and myofibrillar protein FSR following unilateral RE at 6- to 24-hour time points reported differences between myofibrillar and mixed MPS [3,17,18]. Elevations the mixed and myofibrillar protein pools without observable changes in the total pool may be attributed to the hypothesis that cellular energy dictates the capacity of protein renewal in any cell, so that if one subset is augmented, another must be compromised. Therefore, the assessment of specific proteins may be more applicable for our understanding of specific changes in protein metabolism under varying conditions, especially when the study may introduce perturbations of normal physiology, such as RE.

Tipton et al [19] also assessed mixed muscle protein FSR over 24 hours using [$^{13}C_6$]-phenylalanine in young adults following an acute bout of RE and administration of essential amino acids. Because of methodological constraints, the subjects were primarily in the supine position for 24 hours and were provided 2 meals that constituted 80% of their habitual energy intake. That study demonstrated that acute RE with essential amino acids stimulated muscle protein FSR over 24 hours compared with resting conditions [19]. By using an alternative tracer methodology to assess MPS (see Gasier et al [20] for a detailed description), we were able to account for normal biological and environmental factors that are generally "methodologically" excluded. Although consistent with the findings of Tipton et al, the FSR values obtained herein were substantially higher (~10-fold) than previously reported, which may be because of our ability to capture the ongoing synthesis of pools with shorter half-lives, as well as provide us with a greater chance of tracer incorporation in fractions with very long half-lives, which may be impossible to detect using methodologies that do not value long-term integrative approaches.

In conclusion, an acute bout of high-intensity RE stimulates myofibrillar protein FSR, which was not obvious in the mixed fraction; and measures of integrative anabolic response (24 hours) may result in higher MPS than what has been previously reported. We acknowledge that a limitation to this investigation was a small sample size, but we are hopeful that the results from this experiment will stimulate research incorporating integrative assessments of MPS to elucidate how normal daily living interacts with exercise and feeding.

Table 1 – ^2H-labeling of plasma (precursor) and protein-bound alanine (product), and calculated FSR

Group	Plasma (MPE)	Protein-bound Ala (MPE)	FSR (%·h^{-1})
Mixed muscle protein			
Control	0.32 ± 0.01	0.21 ± 0.02	0.76 ± 0.08
Exercise	0.32 ± 0.01	0.20 ± 0.02	0.69 ± 0.06
Myofibrillar protein			
Control	0.32 ± 0.02	0.21 ± 0.01	0.75 ± 0.08
Exercise	0.31 ± 0.02	0.25 ± 0.01 *	0.94 ± 0.16 *

Values are expressed as means ± SEM for integrative mixed muscle (n = 12) and myofibrillar (n = 5) protein FSRs determined over 24 hours. Fractional synthesis rate was calculated as the rate at which ^2H-labeled alanine is incorporated into muscle protein(s) relative to the total abundance of the alanine pool per unit of time ($[(MPE_{Ala})/(3.7 \times MPE_{BW} \times t)] \times 100$), where, MPE_{Ala} represents the total ^2H-labeling of protein-bound alanine, 3.7 represents the exchange of ^2H between body water and free alanine, MPE_{BW} (area under the curve) represents the ^2H-labeling of body water over the course of 24h, and t is time.

* Significantly different from control ($P < .05$).

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Conflict of Interest

The authors declare no conflicts of interest.

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