Protein supplementation does not alter intramuscular anabolic signaling or endocrine response after resistance exercise in trained men


ARTICLE INFO

Article history:
Received 24 June 2015
Revised 4 September 2015
Accepted 8 September 2015

Keywords:
mTORC1
mTOR pathway
Sports nutrition
Amino acids
Leucine
Hormones

ABSTRACT

The mammalian/mechanistic target of rapamycin complex 1 (mTORC1) signaling pathway appears to be the primary regulator of muscle protein synthesis. A variety of stimuli including resistance exercise, amino acids, and hormonal signals activate mTORC1 signaling. The purpose of this study was to investigate the effect of a protein supplement on mTORC1 signaling following a resistance exercise protocol designed to promote elevations in circulating hormone concentrations. We hypothesized that the protein supplement would augment the intramuscular anabolic signaling response. Ten resistance-trained men (age, 24.7 ± 3.4 years; weight, 90.1 ± 11.3 kg; height, 176.0 ± 4.9 cm) received either a placebo or a supplement containing 20 g protein, 6 g carbohydrates, and 1 g fat after high-volume, short-rest lower-body resistance exercise. Blood samples were obtained at baseline, immediately, 30 minutes, 1 hour, 2 hours, and 5 hours after exercise. Fine-needle muscle biopsies were completed at baseline, 1 hour, and 5 hours after exercise. Myoglobin, lactate dehydrogenase, and lactate concentrations were significantly elevated after resistance exercise (P < .0001); however, no differences were observed between trials. Resistance exercise also elicited a significant insulin, growth hormone, and cortisol response (P < .01); however, no differences were observed between trials for insulin-like growth factor-1, insulin, testosterone, growth hormone, or cortisol. Intramuscular anabolic...
signaling analysis revealed significant elevations in RPS6 phosphorylation after resistance exercise ($P = .001$); however, no differences were observed between trials for signaling proteins including Akt, mTOR, p70S6k, and RPS6. The endocrine response and phosphorylation status of signaling proteins within the mTORC1 pathway did not appear to be altered by ingestion of supplement after resistance exercise in resistance-trained men.

**1. Introduction**

The mammalian/mechanistic target of rapamycin complex 1 (mTORC1) signaling pathway appears to be the primary regulator of muscle protein synthesis and growth [1–3]. A variety of stimuli including exercise, amino acids, and hormonal signals activate mTORC1 signaling regulating messenger RNA translation initiation, the rate-limiting step in muscle protein synthesis [4,5]. The protein kinase mammalian/mechanistic target of rapamycin (mTOR) serves as a critical point of convergence providing a biochemical mechanism for controlling processes related to muscle remodeling [3].

Although several upstream mediators have been identified to initiate mTORC1 signaling through distinct mechanisms, they appear to converge on increasing the activity of Ras homolog enriched in brain (Rheb) [7]. Tumor sclerosis complex 2 (TSC2) negatively regulates the GTP-loading state of Rheb, and upon phosphorylation, TSC2 is sequestered away from Rheb allowing mTORC1 to be activated [7,8]. Resistance exercise and growth factors including insulin and insulin-like growth factor-1 (IGF-1) lead to the phosphorylation of TSC2 [9–11]. When insulin and/or IGF-1 binds to its membrane receptors, TSC2 is subsequently phosphorylated via protein kinase B (Akt) [10,11], whereas resistance exercise–induced activation of mTORC1 appears to be Akt independent [12]. Because the end result of both resistance exercise and growth factors is the movement of TSC2 away from Rheb via different upstream kinases, resistance exercise and transient exercise–induced elevations in circulating hormones may not offer an additive effect [13–16]. However, amino acids (eg, leucine) promote mTORC1 in a parallel fashion, independent of TSC2, allowing for a synergistic effect of amino acid ingestion on muscle protein synthesis after resistance exercise [7,17]. Several mediators of amino acid signaling have been identified to lie upstream of mTORC1 [18]. Specifically, Ras-related guanosine triphosphatases (Rag GTPases) are activated by amino acids, subsequently translocating mTORC1 to the surface of the lysosomal membrane which contains the mTORC1 activator, Rheb [18,19].

An acute bout of resistance exercise appears to increase mTORC1 signaling [20–23] and muscle protein synthesis [24–27]. Protein supplementation combined with resistance exercise appears to further augment intramuscular anabolic signaling [28–32] and muscle protein synthesis [33,34] in untrained men. Muscle protein synthesis appears to increase in a dose-dependent manner with protein intakes up to ~20 g of high-quality protein (~10 g essential amino acids) in participants with recreational weight-lifting experience [35,36]. Protein supplementation also appears to enhance mTORC1 signaling in a similar dose-dependent manner in untrained men [37,38]. However, several studies have suggested that experienced, resistance-trained individuals may have an attenuated signaling response to resistance exercise [20,39,40]. Whether the attenuation of the anabolic signaling response occurs when protein supplementation is combined with resistance exercise in trained men is not well understood.

Protein supplementation may also alter metabolic and hormonal responses after resistance exercise by attenuating the cortisol response and augmenting the insulin and growth hormone (GH) response [41–43]. However, recent evidence has indicated that systemic hormonal concentrations may not promote a more favorable intramuscular anabolic environment [15,44]. The molecular links between mTORC1 and stimuli including resistance exercise, amino acids, and hormonal signals form a complicated signaling network controlling cellular growth. Thus, the purpose of this study was to investigate the effect of a protein supplement on mTORC1 signaling after a lower-body resistance exercise protocol designed to promote elevations in circulating hormone concentrations in resistance-trained men. We hypothesized that the protein supplement would augment the intramuscular anabolic signaling response.

**2. Methods and materials**

**2.1. Participants**

Ten resistance-trained men were recruited to participate in this randomized, crossover design research study. Characteristics of study participants are presented in Table 1. Inclusion criteria required participants to be between the ages of 18 and 35 years, a minimum of 1 year of resistance training experience, and the ability to squat a weight equivalent to their body mass. Participants had 6.7 ± 4.6 years of resistance training experience with an average maximum barbell back squat 1-RM of 172.7 ± 25.2 kg.

**Table 1 – Characteristics of study participants**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>n</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>10</td>
<td>90.1 ± 11.3</td>
</tr>
<tr>
<td>Height (cm)</td>
<td></td>
<td>176.0 ± 4.9</td>
</tr>
<tr>
<td>Age (y)</td>
<td></td>
<td>24.7 ± 3.4</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td></td>
<td>14.1 ± 6.1</td>
</tr>
<tr>
<td>Resistance training experience (y)</td>
<td></td>
<td>6.7 ± 4.6</td>
</tr>
<tr>
<td>Barbell back squat 1-RM (kg)</td>
<td></td>
<td>172.7 ± 25.2</td>
</tr>
</tbody>
</table>

All data are reported as means ± SD.
squats of 172.7 ± 25.2 kg. All participants were free of any physical limitations that may affect performance. In addition, all participants were free of any medications and performance-enhancing drugs, as determined by a health and activity questionnaire. After an explanation of all procedures, risks, and benefits, each participant provided his informed consent prior to participation in this study. The research protocol was approved by the New England Institutional Review Board prior to participant enrollment.

2.2. Maximal strength testing

Prior to experimental trials, participants reported to the Human Performance Laboratory (HPL) to establish maximal strength (1 repetition maximum, or 1-RM) on all lifts involved in the exercise protocol. Prior to 1-RM testing, participants performed a standardized warm-up consisting of 5 minutes on a cycle ergometer against a light resistance, 10 body weight squats, 10 body weight walking lunges, 10 dynamic walking hamstring stretches, and 10 dynamic walking quadriceps stretches. The 1-RM test for the barbell back squat and leg press was performed using methods previously described by Hoffman [45]. Briefly, each participant performed 2 warm-up sets using a resistance of approximately 40% to 60% and 60% to 80% of his perceived maximum, respectively. For each exercise, 3 to 4 subsequent attempts were performed to determine the 1-RM. A 3- to 5-minute rest period was provided between each attempt. For all other exercises, the 1-RM was assessed using a prediction formula based on the number of repetitions performed to fatigue using a given weight [46]. Attempts not meeting the range of motion criteria for each exercise or where proper technique was not used were discarded.

2.3. Experimental trials

On the morning of each trial, participants reported to the HPL after a 10-hour overnight fast and having refrained from all forms of moderate to vigorous exercise for the previous 72 hours. Experimental trials were performed in a counterbalanced, randomized order, and each experimental trial was separated by a minimum of 1 week to ensure adequate recovery. Each participant performed experimental trials at the same time of day to avoid diurnal variations. Participants provided a urine sample upon arrival to the HPL for analysis of urine-specific gravity (USG) by refractometry to ensure an adequate hydration status (USG ≤ 1.020 defined as euhydration).

During each experimental trial, participants performed the standardized warm-up discussed above followed by a lower-body resistance exercise protocol depicted in Table 2. The resistance exercise protocol used a load of 70% 1-RM for sets of 10 to 12 repetitions with a 1-minute rest period length between sets and exercises. The protocol included 6 sets of barbell back squats and 4 sets of bilateral leg press, bilateral hamstring curls, bilateral leg extensions, and seated calf raises. The protocol was similar to what one may see being used by an athlete focusing on muscle hypertrophy [47]. During each experimental trial, participants were verbally encouraged to complete each set. If the participant was unable to complete the desired number of repetitions, spotters provided assistance until the subject completes the remaining repetitions. Subsequently, the loads were adjusted so that participants were able to perform the specific number of repetitions for each set.

Following each resistance exercise protocol, participants remained in the laboratory for all postexercise assessments. Blood samples were obtained at 6 time points over the course of the study: baseline (BL), immediately postexercise (IP), 30 minutes postexercise (30P), 1 hour postexercise (1H), 2 hours postexercise (2H), and 5 hours postexercise (5H). Fine-needle muscle biopsies were completed at BL, 1H, and 5H.

To control for diet, participants were provided a standardized low-protein, low-carbohydrate breakfast bar (Atkins Nutritionalts, Inc, Denver, CO, USA; 190 calories, 7 g protein, 3 g carbohydrate, and 13 g fat) after BL assessments. To control for diet, participants were provided a standardized low-protein, low-carbohydrate breakfast bar (Atkins Nutritionalts, Inc, Denver, CO, USA; 190 calories, 7 g protein, 3 g carbohydrate, and 13 g fat) after BL assessments.

Table 2 – Resistance exercise protocol

<table>
<thead>
<tr>
<th>Exercise</th>
<th>Sets × repetitions</th>
<th>Intensity load</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barbell back squat</td>
<td>6 × 10−12</td>
<td>70% 1-RM</td>
</tr>
<tr>
<td>Bilateral leg press</td>
<td>4 × 10−12</td>
<td>70% 1-RM</td>
</tr>
<tr>
<td>Bilateral hamstring curls</td>
<td>4 × 10−12</td>
<td>70% 1-RM</td>
</tr>
<tr>
<td>Bilateral leg extensions</td>
<td>4 × 10−12</td>
<td>70% 1-RM</td>
</tr>
<tr>
<td>Seated calf raises</td>
<td>4 × 10−12</td>
<td>70% 1-RM</td>
</tr>
</tbody>
</table>

The rest interval between each set and between all exercises was 1 minute. For each exercise, the initial load was 70% of the participant’s 1-RM. If the participant was unable to complete the desired number of repetitions, spotters provided assistance until the subject completed the remaining repetitions. Subsequently, the load was adjusted so that participants were able to perform 10 to 12 repetitions for each set.

Table 3 – Amino acid composition of the SUPP

<table>
<thead>
<tr>
<th>Essential amino acids (g/serving)</th>
<th>Essential amino acids (g/serving)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serine 1.08</td>
<td>Glutamic acid 4.07</td>
</tr>
<tr>
<td>Proline 1.85</td>
<td>Glycine 0.36</td>
</tr>
<tr>
<td>Alanine 0.67</td>
<td>Aspartic acid 1.51</td>
</tr>
<tr>
<td>Tyrosine 0.95</td>
<td>Tryptophan 0.29</td>
</tr>
<tr>
<td>Lysine 1.59</td>
<td>Arginine 0.72</td>
</tr>
<tr>
<td>Cystine 0.13</td>
<td>Total 11.34</td>
</tr>
</tbody>
</table>

Participants were encouraged to complete each set. If the participant was unable to complete the desired number of repetitions, spotters provided assistance until the subject completes the remaining repetitions. Subsequently, the loads were adjusted so that participants were able to perform the specific number of repetitions for each set.

Table 2 – Resistance exercise protocol

<table>
<thead>
<tr>
<th>Exercise</th>
<th>Sets × repetitions</th>
<th>Intensity load</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barbell back squat</td>
<td>6 × 10−12</td>
<td>70% 1-RM</td>
</tr>
<tr>
<td>Bilateral leg press</td>
<td>4 × 10−12</td>
<td>70% 1-RM</td>
</tr>
<tr>
<td>Bilateral hamstring curls</td>
<td>4 × 10−12</td>
<td>70% 1-RM</td>
</tr>
<tr>
<td>Bilateral leg extensions</td>
<td>4 × 10−12</td>
<td>70% 1-RM</td>
</tr>
<tr>
<td>Seated calf raises</td>
<td>4 × 10−12</td>
<td>70% 1-RM</td>
</tr>
</tbody>
</table>

The rest interval between each set and between all exercises was 1 minute. For each exercise, the initial load was 70% of the participant’s 1-RM. If the participant was unable to complete the desired number of repetitions, spotters provided assistance until the subject completed the remaining repetitions. Subsequently, the load was adjusted so that participants were able to perform 10 to 12 repetitions for each set.

Table 3 – Amino acid composition of the SUPP

<table>
<thead>
<tr>
<th>Nonessential amino acids (g/serving)</th>
<th>Nonessential amino acids (g/serving)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serine 1.08</td>
<td>Glutamic acid 4.07</td>
</tr>
<tr>
<td>Proline 1.85</td>
<td>Glycine 0.36</td>
</tr>
<tr>
<td>Alanine 0.67</td>
<td>Aspartic acid 1.51</td>
</tr>
<tr>
<td>Tyrosine 0.95</td>
<td>Tryptophan 0.29</td>
</tr>
<tr>
<td>Lysine 1.59</td>
<td>Arginine 0.72</td>
</tr>
<tr>
<td>Cystine 0.13</td>
<td>Total 11.34</td>
</tr>
</tbody>
</table>

Participants were encouraged to complete each set. If the participant was unable to complete the desired number of repetitions, spotters provided assistance until the subject completes the remaining repetitions. Subsequently, the loads were adjusted so that participants were able to perform the specific number of repetitions for each set.

Table 2 – Resistance exercise protocol

<table>
<thead>
<tr>
<th>Exercise</th>
<th>Sets × repetitions</th>
<th>Intensity load</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barbell back squat</td>
<td>6 × 10−12</td>
<td>70% 1-RM</td>
</tr>
<tr>
<td>Bilateral leg press</td>
<td>4 × 10−12</td>
<td>70% 1-RM</td>
</tr>
<tr>
<td>Bilateral hamstring curls</td>
<td>4 × 10−12</td>
<td>70% 1-RM</td>
</tr>
<tr>
<td>Bilateral leg extensions</td>
<td>4 × 10−12</td>
<td>70% 1-RM</td>
</tr>
<tr>
<td>Seated calf raises</td>
<td>4 × 10−12</td>
<td>70% 1-RM</td>
</tr>
</tbody>
</table>

The rest interval between each set and between all exercises was 1 minute. For each exercise, the initial load was 70% of the participant’s 1-RM. If the participant was unable to complete the desired number of repetitions, spotters provided assistance until the subject completed the remaining repetitions. Subsequently, the load was adjusted so that participants were able to perform 10 to 12 repetitions for each set.

Table 3 – Amino acid composition of the SUPP

<table>
<thead>
<tr>
<th>Essential amino acids (g/serving)</th>
<th>Essential amino acids (g/serving)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serine 1.08</td>
<td>Glutamic acid 4.07</td>
</tr>
<tr>
<td>Proline 1.85</td>
<td>Glycine 0.36</td>
</tr>
<tr>
<td>Alanine 0.67</td>
<td>Aspartic acid 1.51</td>
</tr>
<tr>
<td>Tyrosine 0.95</td>
<td>Tryptophan 0.29</td>
</tr>
<tr>
<td>Lysine 1.59</td>
<td>Arginine 0.72</td>
</tr>
<tr>
<td>Cystine 0.13</td>
<td>Total 11.34</td>
</tr>
</tbody>
</table>

Participants were encouraged to complete each set. If the participant was unable to complete the desired number of repetitions, spotters provided assistance until the subject completes the remaining repetitions. Subsequently, the loads were adjusted so that participants were able to perform the specific number of repetitions for each set.

To control for diet, participants were provided a standardized low-protein, low-carbohydrate breakfast bar (Atkins Nutritionalts, Inc, Denver, CO, USA; 190 calories, 7 g protein, 3 g carbohydrate, and 13 g fat) after BL assessments. To control for diet, participants were provided a standardized low-protein, low-carbohydrate breakfast bar (Atkins Nutritionalts, Inc, Denver, CO, USA; 190 calories, 7 g protein, 3 g carbohydrate, and 13 g fat) after BL assessments. To control for diet, participants were provided a standardized low-protein, low-carbohydrate breakfast bar (Atkins Nutritionalts, Inc, Denver, CO, USA; 190 calories, 7 g protein, 3 g carbohydrate, and 13 g fat) after BL assessments.
2.4. Blood measurement

During each experimental trial, all blood samples were obtained using a Teflon cannula placed in a superficial forearm vein using a 3-way stopcock with a male luer lock adapter and plastic syringe. The cannula was maintained patent using an isotonic saline solution (Becton Dickinson, Franklin Lakes, NJ, USA). Baseline blood samples were obtained after a 15-minute equilibration period, and IP blood samples were taken within 1 minute of exercise cessation. Participants were instructed to lie in a supine position for 15 minutes prior to 3P, 1H, 2H, and 5H blood draws.

All blood samples were collected into three 6-mL Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ, USA). Blood samples were drawn into either plain, sodium heparin, or K$_2$EDTA-treated tubes. A small aliquot of whole blood was removed and used for determination of hematocrit and hemoglobin concentrations. The blood in the plain tube was allowed to clot at room temperature for 30 minutes and subsequently centrifuged at 3000 × g for 15 minutes, along with the remaining whole blood from the other tubes. The resulting serum and plasma were placed into separate microcentrifuge tubes and frozen at −80°C for later analysis.

2.5. Biochemical analysis

Blood lactate concentrations were analyzed from plasma using an automated analyzer (Analox GM7 enzymatic metabolite analyzer; Analox Instruments USA, Lunenburg, MA, USA). Hematocrit concentrations were determined from whole blood via microcentrifugation (CritSpin, Westwood, MA, USA) and microcapillary technique. Hemoglobin concentrations were analyzed from whole blood using an automated analyzer (HemoCue, Cypress, CA, USA). Plasma volume shifts were calculated using the formula established by Dill and Costill [48,49]. To eliminate interassay variance, all samples were analyzed in duplicate by a single technician. Coefficient of variation for each assay was 1.4% for blood lactate, 0.4% for hematocrit, and 0.6% for hemoglobin.

Circulating concentrations of IGF-1, insulin, testosterone, GH, and cortisol were assessed via enzyme-linked immuno-sorbent assays (ELISAs) and a spectrophotometer (BioTek Eon, Winooski, VT, USA) using commercially available kits. Myoglobin concentrations were determined via ELISA (Calbiotech, Spring Valley, CA, USA) and a spectrophotometer. Lactate dehydrogenase (LDH) concentrations were determined via ELISA (Sigma-Aldrich, St Louis, MO, USA) and a spectrophotometer. To eliminate interassay variance, all samples for each assay were thawed once and analyzed in duplicate in the same assay run by a single technician. Coefficient of variation for each assay was 6.5% for IGF-1, 8.1% for insulin, 4.8% for testosterone, 4.9% for GH, 5.3% for cortisol, 4.1% for myoglobin, and 4.8% for LDH.

2.6. Fine-needle muscle biopsy procedure

Fine-needle muscle biopsies were performed on the vastus lateralis muscle of the participant’s dominant leg using a spring-loaded, reusable instrument with 14-gauge disposable needles and a coaxial introducer (Argon Medical Devices Inc, Plano, TX, USA). After local anesthesia with 2 mL of 1% lidocaine applied into the subcutaneous tissue, a small incision to the skin was made and an insertion cannula was placed perpendicular to the muscle until the fascia was pierced. The biopsy needle was inserted through the cannula and a muscle sample was obtained by the activation of a trigger button, which unloaded the spring and activated the needle to collect a muscle sample. Multiple biopsy passes at each time point were made with the cannula in place, thus avoiding repeated skin punctures. Each muscle sample was removed from the biopsy needle using a sterile scalpel and was subsequently placed in a cryotube, rapidly frozen in liquid nitrogen, and stored at −80°C. A licensed physician performed all muscle biopsies.

2.7. Intramuscular anabolic signaling analysis

Tissue samples were thawed and kept on ice for preparation and homogenization. A lysis buffer with protease inhibitor (EMD Millipore, Billerica, MA, USA) was added to each sample at a rate of 500 μL per 10 mg of tissue. Samples were homogenized using a Teflon pestle and sonication (Branson, Danbury, CT, USA). Tissue samples were then placed on a plate shaker (Thermo Fisher Scientific Inc, Waltham, MA, USA) for 10 minutes at 4°C and subsequently centrifuged at 10000g for 5 minutes. The supernatant was aspirated and used for analysis.

Multiplex ELISA was used to quantify the phosphorylation status of proteins specific to the mTORC1 signaling pathway using MAGPIX (Luminex, Austin, TX, USA) and a multiplex signaling assay kit (EMD Millipore) according to the manufacturer’s guidelines. Multiplex ELISA has been validated [49] and previously used to determine the phosphorylation status of proteins in the mTORC1 signaling pathway [50–52]. Samples were analyzed for phosphorylation of IGF-1 receptor at Tyr 1135/1136, insulin receptor at Tyr 1162/1163, insulin receptor substrate 1 at Ser 636, TSC2 at Ser 939, Akt at Ser 473, p70S6k at Thr 412 and the splice variant p70S6k II at Thr 412, and RPS6 at Ser 235/236. The specificity of the p70S6k antibody recognized p70S6k I phosphorylated on Thr412 and the splice variant p70S6k II phosphorylated on Thr389. Total protein quantification was conducted using a detergent compatible protein assay kit (Bio-Rad, Hercules, CA, USA). Homogenized samples were diluted prior to being loaded and results are reported as fluorescence intensity expressed relative to total protein content. To eliminate interassay variance, all tissue samples were thawed once and analyzed in duplicate in the same assay run by a single technician. The average coefficient of variation for phospho-protein analysis was 8.4%.

2.8. Dietary logs

Participants were instructed to maintain their normal dietary intake leading up to experimental trials. Participants were
then instructed to record as accurately as possible everything they consumed during the 24 hours prior to the first experimental trial. For the following experimental trial, participants were required to duplicate the content, quantity, and timing of their daily diet during the 24 hours prior. Participants were instructed not to eat or drink (except water) within 10 hours of reporting to the HPL for experimental trials.

2.9. Statistical analyses

Prior to statistical procedures, all data were assessed for normal distribution, homogeneity of variance, and sphericity. If the assumptions of sphericity were violated, a Greenhouse-Geisser correction was applied. Biochemical changes were analyzed using a 2-factor (trial × time) analysis of variance with repeated measures on time. In the event of a significant F ratio, least significant difference post hoc tests were used for pairwise comparisons. Area under the curve (AUC) was also calculated for biochemical measures using a standard trapezoidal technique. Area under the curve analysis was performed via paired-samples t tests. For effect size, partial η² statistics were calculated, and according to Green et al [53], 0.01, 0.06, and 0.14 were interpreted as small, medium, and large effect sizes (ESs), respectively. Significance was accepted at an α level of P ≤ .05, and all data are reported as means ± SD. Using the equation: ES = (experimental mean – control mean)/ [the pooled SD of peak testosterone levels], the study by Hoffman et al [54] had an ES of 1.14. Power was set at 80 with an α level of .05 using an ES of 1.14. We estimated that a minimum of 9 participants was needed for the study.

3. Results

3.1. Resistance exercise protocol

All participants were adequately hydrated (USG ≤ 1.020) prior to each trial, and no significant differences were noted between trials for BL USG (P = .35). No significant differences were noted for water consumption during each trial (P = .81). No significant differences were noted for workout volume (P = .35) between PL (45300 ± 13920 kg) and SUPP (46888 ± 12705 kg).

3.2. Biochemical analysis

3.2.1. Myoglobin

Significant time effects were observed for myoglobin (F = 45.4, P = .0001, η² = 0.72). When collapsed across trials, myoglobin concentrations were significantly elevated from BL at all time points (P < .0001). However, no significant differences (F = 0.1, P = .83, η² = 0.01) were noted between trials. In addition, AUC analysis revealed no significant difference between trials (P = .10; Fig. 1).

3.2.2. Lactate dehydrogenase

Significant time effects were observed for LDH (F = 19.7, P = .0001, η² = 0.52). When collapsed across trials, LDH concentrations were significantly elevated from BL at all time points (P < .0001). However, no significant differences (F = 1.1, P = .33, η² = 0.06) were noted between trials. In addition, AUC analysis revealed no significant difference between trials (P = .63; Fig. 2).

Fig. 1 – Myoglobin concentration after resistance exercise. Ten resistance-trained men received either a PL or a SUPP (20 g protein, 6 g carbohydrates, and 1 g fat) after resistance exercise. Groups: SUPP and PL. Time points: BL, IP, 30P, 1H, 2H, and 5H. Inset: AUC. All data are reported as means ± SD. #Significant difference from BL (P < .05).

Fig. 2 – Lactate dehydrogenase concentration after resistance exercise. Ten resistance-trained men received either a PL or a SUPP (20 g protein, 6 g carbohydrates, and 1 g fat) after resistance exercise. Groups: SUPP and PL. Time points: BL, IP, 30P, 1H, 2H, and 5H. Inset: AUC. All data are reported as means ± SD. #Significant difference from BL (P < .05).

Fig. 3 – Lactate concentration after resistance exercise. Ten resistance-trained men received either a PL or a SUPP (20 g protein, 6 g carbohydrates, and 1 g fat) after resistance exercise. Groups: SUPP and PL. Time points: BL, IP, 30P, 1H, 2H, and 5H. Inset: AUC. All data are reported as means ± SD. #Significant difference from BL (P < .05).
3.2.3. Lactate
Significant time effects were observed for lactate ($F = 279.6$, $P = .0001$, $\eta^2 = 0.94$). When collapsed across trials, lactate concentrations were significantly elevated from BL at all time points ($P < .0001$). However, no significant differences ($F = 0.4$, $P = .71$, $\eta^2 = 0.02$) were noted between trials. In addition, AUC analysis revealed no significant difference between trials ($P = .71$; Fig. 3).

3.2.4. Insulin-like growth factor-1
Significant time effects were observed for IGF-1 ($F = 4.3$, $P = .002$, $\eta^2 = 0.19$). When collapsed across trials, IGF-1 concentrations were significantly elevated from BL at IP ($P = .001$) and 30P ($P = .001$), and 1H ($P = .001$). No significant differences ($F = 0.3$, $P = .63$, $\eta^2 = 0.02$) were observed between trials. In addition, AUC analysis revealed no significant difference between trials ($P = .23$; Fig. 4A).

3.2.5. Insulin
Significant time effects were observed for insulin ($F = 12.1$, $P = .001$, $\eta^2 = 0.40$). When collapsed across trials, insulin concentrations were significantly elevated from BL at IP ($P = .01$), 30P ($P = .001$), and 1H ($P = .001$). No significant differences ($F = 0.3$, $P = .63$, $\eta^2 = 0.02$) were observed between trials. In addition, AUC analysis revealed no significant difference between trials ($P = .23$; Fig. 4B).

3.2.6. Testosterone
Significant time effects were observed for testosterone ($F = 6.3$, $P = .002$, $\eta^2 = 0.26$). When collapsed across trials, testosterone concentrations were significantly decreased from BL at 2H ($P = .04$). No significant difference were noted ($F = 0.9$, $P = .42$, $\eta^2 = 0.05$) between trials. In addition, AUC analysis revealed no significant difference between trials ($P = .29$; Fig. 4C).

Fig. 4 – IGF-1 (A), insulin (B), testosterone (C), GH (D), and cortisol (E) concentration after resistance exercise. Ten resistance-trained men received either a PL or a SUPP (20 g protein, 6 g carbohydrates, and 1 g fat) after resistance exercise. Groups: SUPP and PL. Time points: BL, IP, 30P, 1H, 2H, and 5H. Inset: AUC. All data are reported as means ± SD. *Significant difference from BL ($P < .05$).
3.2.7. Growth hormone

Significant time effects were observed for GH ($F = 109.8, P = .0001, \eta^2 = 0.86$). When collapsed across trials, GH concentrations were significantly elevated from BL at IP, 30P, and 1H ($P < .0001$), and significantly decreased from BL at 5H ($P = .0001$). No significant differences ($F = 0.4, P = .78, \eta^2 = 0.02$) were observed between trials. In addition, AUC analysis revealed no significant difference between trials ($P = .92$; Fig. 4D).

3.2.9. Plasma volume shifts

Relative to BL, plasma volume decreased at IP ($-8.8\% \pm 6.4\%$), increased at 30P ($0.7\% \pm 7.6\%$), increased at 1H ($7.4\% \pm 12.0\%$), increased at 2H ($5.0\% \pm 5.4\%$), and decreased at 5H ($-0.7\% \pm 5.0\%$). However, the difference between trials was not significant. Blood variables were not corrected for plasma volume shifts due to the importance of molar exposure at the tissue receptor level.

3.3. Intramuscular anabolic signaling

No significant changes from BL were observed in the phosphorylation of IGF-1 receptor ($F = 1.8, P = .18, \eta^2 = 0.09$), insulin receptor ($F = 0.2, P = .79, \eta^2 = 0.01$), insulin receptor substrate 1 ($F = 0.6, P = .55, \eta^2 = 0.03$), Akt ($F = 0.4, P = .62, \eta^2 = 0.02$), and p70S6k ($F = 2.9, P = .07, \eta^2 = 0.14$). In addition, no significant differences between trials were noted in these signaling molecules ($P > .05$).

Significant time effects were observed for phosphorylation of both TSC2 ($F = 5.8, P = .01, \eta^2 = 0.25$) and mTOR ($F = 4.2, P = .02, \eta^2 = 0.19$). When collapsed across trials, the phosphorylation of both TSC2 and mTOR was significantly decreased from BL at 5H ($P = .001$ and $P = .004$, respectively). However, no significant differences were noted between trials for phosphorylation of TSC2 ($F = 0.1, P = .87, \eta^2 = 0.01$; Fig. 5A) and mTOR ($F = 0.3, P = .71, \eta^2 = 0.02$; Fig. 5B).

Significant time effects were observed for phosphorylation of RPS6 ($F = 11.4, P = .0001, \eta^2 = 0.39$). When collapsed across trials, phosphorylation of RPS6 was significantly elevated from BL at 1H ($P = .001$) and 5H ($P = .001$). However, no significant differences were noted between trials for phosphorylation of RPS6 ($F = 0.1, P = .88, \eta^2 = 0.01$; Fig. 5C).

4. Discussion

This study examined the effect of a protein SUPP on intramuscular anabolic signaling and endocrine response after a high-volume, short-rest resistance exercise protocol in experienced, resistance-trained men. Resistance exercise significantly elevated markers of muscle damage (ie, myoglobin and LDH) and lactate concentrations; however, no differences were observed between trials. Resistance exercise also elicited a significant elevation in the insulin, GH, and cortisol response; however, no differences were observed between trials for IGF-1, insulin, testosterone, GH, or cortisol. The resistance exercise protocol resulted in significant elevations in RPS6 phosphorylation; however, no differences between trials. In addition, AUC analysis revealed no significant difference between trials ($P = .72$; Fig. 4D).
were noted between the trials for the signaling proteins Akt, mTOR, p70S6k, and RPS6. Therefore, we reject the research hypothesis. The results of this current investigation indicated that the endocrine response and phosphorylation status of signaling proteins within the mTORC1 pathway were not altered by ingestion of the protein SUPP after resistance exercise in experienced, resistance-trained men.

Resistance exercise can induce significant muscle damage and metabolic stress [55,56]. Myoglobin and LDH concentrations are often used as markers of muscle damage [57–59]. The high-volume resistance exercise protocol used in this study appeared to result in some degree of muscle damage, as evidenced by the significant elevation in both myoglobin and LDH. The similar response between the 2 trials in these muscle damage markers suggests that the postexercise protein ingestion was unable to attenuate the damage response. Previous studies have indicated that postexercise ingestion of a protein and carbohydrate drink may attenuate exercise-induced elevations of myoglobin in untrained men [60,61]. However, indirect markers of muscle damage have not been shown to be a consistent indicator of exercise-mediated adaptation, and muscle hypertrophy has been observed in the relative absence of muscle damage [55,62]. Hence, the role of exercise-induced elevations of muscle damage markers for promoting skeletal muscle adaptation remains unclear. An elevation in blood lactate concentrations has also been observed after high-intensity resistance exercise and is used as a marker of metabolic stress [56,63,64]. In the current study, ingestion of the protein SUPP did not appear to alter lactate concentrations after resistance exercise. This is consistent with previous investigations reporting that protein supplementation had no effect on blood lactate concentrations [60,65]. Lactate production may contribute to mTORC1 activation [66]; however, the mechanisms by which metabolic stress influences anabolic signaling are not fully elucidated and warrant further investigation.

Resistance exercise appears to be a potent stimulus for acute increases in circulating anabolic hormones. High-volume, short-rest resistance exercise protocols are associated with elevations of GH [64,67], testosterone [56,68], and cortisol [56,64,68,69]. In the current study, resistance exercise elicited significant elevations in GH, cortisol, and insulin; however, ingestion of SUPP did not appear to alter the endocrine response after resistance exercise. Postexercise nutritional interventions have previously been demonstrated to augment the endocrine response after resistance exercise by potentially reducing the rise in cortisol while increasing the insulin and GH response [41–43]. However, the total amount of protein and carbohydrate ingested by the subjects in these studies exceeded the amount provided in this investigation. The lower dose of protein and carbohydrates in SUPP did not augment the cortisol, insulin, or GH response after resistance exercise. Nevertheless, recent evidence suggests that transient hormonal responses may not promote a more favorable intramuscular anabolic environment [15,44]. The mechanisms of exercise-mediated muscle hypertrophy have been suggested to be solely an intrinsic process, which may not be influenced by transient changes in circulating hormones [13–16].

Resistance exercise also appears to elicit activation of mTORC1 signaling in both untrained [1,70] and resistance-trained men [52,71]. Muscle protein synthesis is regulated by mTORC1 signaling to several downstream effectors, including p70S6k and RPS6 [3,6]. The phosphorylated state of p70S6k has been shown to be a proxy marker of myofibrillar protein synthesis rates [15,72]. RPS6 is a downstream target of p70S6k with the potential to regulate protein synthesis and is commonly used as an indirect marker of mTORC1 activation [3]. In the current study, resistance exercise significantly elevated RPS6 phosphorylation after resistance exercise. Interestingly, the phosphorylation of TSC2 and mTOR was significantly decreased after resistance exercise. Previous investigations have also indicated that resistance exercise may inhibit upstream signaling while concomitantly augmenting downstream signaling proteins [73]. The lack of any significant change in p70S6k phosphorylation after resistance exercise may be related to the greater training experience and muscle strength of the participants [52]. Participants with greater experience in resistance training appear to have an attenuated response to resistance exercise–induced intramuscular anabolic signaling compared with less experienced participants [20,39,40]. Although speculative, it is possible that the effect of a protein SUPP on the anabolic signaling process may also be attenuated in experienced, resistance-trained men. The results of this present study suggest that the regulation of signaling proteins within mTORC1, including Akt, mTOR, p70S6k, and RPS6, was not augmented by ingestion of the protein SUPP. This is in contrast to other investigations that have demonstrated superior intramuscular anabolic signaling when protein is ingested after resistance exercise in untrained men [28–31,74]. Only a limited number of studies have investigated the influence of protein supplementation in a trained population [71,75]. Areta and colleagues [75] reported elevations in p70S6k phosphorylation in response to 3 different feeding patterns of 80 g protein after resistance exercise in trained men, whereas Glover et al [71] reported elevations in the phosphorylation of p70S6k and RPS6 in response to 3 mixed meal drinks (10 g protein, 41 g carbohydrate, 4 g fat) provided every 1.5 hours into recovery from resistance exercise in trained men. The total protein ingested by the subjects in those studies exceeded the amount of protein provided in this investigation.

A recent review by Moore et al [76] suggested that 0.24 g of high-quality protein per kilogram of body weight is required to maximally stimulate muscle protein synthesis in healthy young men. The stimulation of muscle protein synthesis is dependent on protein quality (ie, leucine content), quantity, and sensitivity of the skeletal muscle to the subsequent hyperaminoacidemia [76]. Leucine is the key amino acid that triggers mTORC1 signaling and a rise in muscle protein synthesis [77], and it has been suggested that 0.05 g of leucine per kilogram of body weight is required to maximize the anabolic response [78]. In the current study, an average of 0.22 g of protein per kilogram of body weight was provided to the participants. The total leucine content of the SUPP was 1.86 g (0.02 g/kg of bodyweight), which may not have been sufficient to maximally stimulate muscle protein synthesis in this sample of experienced, resistance-trained men.

To further augment intramuscular anabolic signaling after resistance exercise, a greater dose of protein (and leucine) may be required to support a more robust post-prandial
stimulation of signaling proteins within mTORC1. Although the stimulation of muscle protein synthesis appears to require mTORC1 activation [79–81], a dissociation between anabolic signaling and muscle protein synthesis may exist [82,83]. Muscle protein synthesis has shown to be maximally stimulated without augmenting phosphorylation of p70S6k and RPS6 after protein ingestion [35], suggesting that the stimulation of postexercise muscle protein synthesis may also be primarily related to amino acid availability. In addition, the SUPP in the current study contained milk protein, which is ~80% casein. The greater proportion of the slowly digested protein in the SUPP may have also limited the acute postprandial anabolic response in comparison to a rapidly digested protein such as whey [84,85].

Athletes with increased training experience possess a potentially lower adaptive ability to resistance exercise which may, in part, be attributable to an attenuation of exercise-induced intramuscular anabolic signaling responses in comparison to untrained individuals [20,40,52,86]. Therefore, the results of this study are specific to experienced, trained individuals. We also recognize that the methods of studying intramuscular signaling in vivo in humans are accompanied with inherent limitations as it requires repeated biopsy sampling of a small population of muscle fibers at a few, distinctive time points after exercise and the analyzed tissue is assumed to be representative of the entire muscle.

In conclusion, high-volume, short-rest lower-body resistance exercise significantly elevated myoglobin, LDH, and lactate concentrations; elicited a significant insulin, GH, and cortisol response; and stimulated significant elevations in RPS6 phosphorylation. However, the endocrine response and phosphorylation status of signaling proteins within the mTORC1 pathway did not appear to be altered by ingestion of SUPP after resistance exercise in resistance-trained men.

Conflict of Interest

This study was funded through a grant from MusclePharm, Corp. A.M.G., J.R.H., A.R.J., J.R.T., C.H.B., K.S.B., K.M.B., A.J.W., D.D.C., G.T.M., L.P.O., D.H.F., and J.R.S. declare no competing interests. At the time of the study, J.R.M. was employed by MusclePharm, Corp.

Acknowledgment

The authors wish to thank Alyssa N. Varanoske, Ran Wang, Michael B. LaMonica, Mattan W. Hoffman, and Josh J. Riffe for their assistance in data collection.

REFERENCES


