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Original Research

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

Adam M. Gonzalez[b], *Jay R. Hoffman*[a,*], *Adam R. Jajtner*[a], *Jeremy R. Townsend*[a], *Carleigh H. Boone*[a], *Kyle S. Beyer*[a], *Kayla M. Baker*[a], *Adam J. Wells*[a], *David D. Church*[a], *Gerald T. Mangine*[a], *Leonardo P. Oliveira*[a,c], *Jordan R. Moon*[d], *David H. Fukuda*[a], *Jeffrey R. Stout*[a]

[a] *Institute of Exercise Physiology and Wellness, Sport and Exercise Science, University of Central Florida, Orlando, FL, USA*
[b] *Department of Health Professions, Hofstra University, Hempstead, NY, USA*
[c] *Department of Internal Medicine, College of Medicine, University of Central Florida, Orlando, FL, USA*
[d] *Sports Science Institute, MusclePharm, Corp, Denver, CO, USA*

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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

Abbreviations: 1H, 1 hour postexercise; 1-RM, maximal strength; 2H, 2 hours postexercise; 30P, 30 minutes postexercise; 5H, 5 hours postexercise; Akt, protein kinase B; BL, baseline; GH, growth hormone; HPL, Human Performance Laboratory; IGF-1, insulin-like growth factor-1; IP, immediately postexercise; LDH, lactate dehydrogenase; mTOR, mammalian/mechanistic target of rapamycin; mTORC1, mammalian/mechanistic target of rapamycin complex 1; p70S6k, ribosomal S6 kinase 1; PL, placebo; Rag, GTPases Ras-related guanosine triphosphatases; Rheb, Ras homolog enriched in brain; RPS6, ribosomal protein S6; SUPP, supplement; TSC2, tumor sclerosis complex 2; USG, urine-specific gravity.

* *Corresponding author at:* Sport and Exercise Science, College of Education & Human Performance, University of Central Florida, PO Box 161250, Orlando, FL 32816-1250, USA. Tel.: +1 407 823 1272.
E-mail address: Jay.Hoffman@ucf.edu (J.R. Hoffman).

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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 resistance 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 protein regulating several downstream signaling molecules that promote protein synthesis, including ribosomal S6 kinase 1 (p70S6k) and ribosomal protein S6 (RPS6) [3,6]. The mTORC1 signaling promotes anabolic and inhibits catabolic cellular functions 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

Table 1 – Characteristics of study participants

n	10
Weight (kg)	90.1 ± 11.3
Height (cm)	176.0 ± 4.9
Age (y)	24.7 ± 3.4
Body fat (%)	14.1 ± 6.1
Resistance training experience (y)	6.7 ± 4.6
Barbell back squat 1-RM (kg)	172.7 ± 25.2

All data are reported as means ± SD.