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ABSTRACT

During a traditional set configuration of resistance exercise (TRD), characterized by a continuous completion of repetitions, a decrease in power output tends to occur throughout a set of repetitions. Inclusion of intra-set rest, otherwise known as a cluster set configuration (CLU), counteracts this power decline. However, the effect of a CLU configuration on post-exercise myofibrillar protein synthesis rates (MPS) and anabolic signaling has not been investigated.

PURPOSE: We aimed to determine if any mechanistic differences exist between TRD and CLU signaling events associated with muscle anabolism. METHODS: In randomized crossover trials, eight resistance-trained participants (23±1 years, 81±4.7 kg, body fat: 18±1.9 %; 1 repetition maximum (1RM): 150±9.1 kg) performed an acute bout of CLU (4 sets × (2×5) repetitions, 30s intra-set rest, 90s inter-set rest) and TRD (4 sets×10 repetitions, 120s inter-set rest) barbell back squats at ~70% 1RM with total volume load equated during primed continuous L-[ring-\(^{13}\)C\(_6\)]phenylalanine infusions. Blood and muscle biopsy samples were collected at rest and after exercise at 0, 2, and 5 h. RESULTS: There was no difference in post-exercise MPS between the CLU and TRD condition (\(P>0.05\)) and no changes in phosphorylation of mTORC1 downstream targets (p70S6K and 4EBP1). Total and phosphorylated YAP on Ser127 transiently increased (\(P<0.01\)) immediately after exercise (\(t=0\)) in CLU (~2.1 fold) and TRD condition (~2.2 fold).

CONCLUSION: Our results show that CLU is a viable anabolic option by preserving power output with similar MPS stimulation when compared to the TRD condition in trained young adults. Key words: Cluster set, Yes-associated protein, muscle power, anabolic signaling, ERK1/2

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INTRODUCTION

Resistance exercise manipulations of load (1, 2), volume (3, 4) and the time that muscle is experiencing loaded tension (5) have been shown to modify the magnitude and duration of the post-exercise muscle protein synthetic response. Based on this work, it has been suggested that common characteristics within resistance exercise prescription to promote maximal post-exercise muscle protein synthesis rates, primarily within the myofibrillar protein fraction, is to ensure each exercise set is performed to volitional fatigue (proxy indicator for bulk muscle fiber recruitment (6)) and with sufficient volume load (3). Indeed, hypertrophic muscle protein remodeling through resistance exercise training supports long-term muscle health and physical independence by expanding the myofibril protein pool and facilitating the removal of damaged proteins with the replacement of new muscle proteins (7). These past studies, however, that assessed the influence of contractile manipulation on the regulation of post-exercise myofibrillar protein synthesis rates did not specifically seek to assess other important performance-related characteristics such as improvement in power.

Intra-set rest is a variable that is manipulated within a resistance exercise prescription to prevent power decrements throughout an exercise session in more advanced training programs (8), but also has relevance to the general population (9, 10). Specifically, the incorporation of cluster sets (CLU), whereby a small period of intra-set rest is incorporated between ‘clusters’ of repetitions has been shown to produce greater velocity and power during resistance exercise when compared to training with a continuous completion of repetitions (traditional set configuration; TRD) (10). As such, resistance exercise prescriptions that promote long-term functional status through power preservation (11, 12) as well as hypertrophic muscle protein remodeling may optimize muscle health and physical performance for both athletes and the
general population. Despite the potential benefit of the CLU configuration on the skeletal muscle adaptive response, the effect of intra-set rest on the post-exercise regulation of muscle protein synthesis rates has not been investigated.

The mechanisms that underpin the stimulation of post-exercise muscle protein synthesis rates have largely been attributed to the activation of signaling molecules within the mTOR (mammalian target of rapamycin) pathway (13). Past efforts have shown that the activation of the mTORC1 pathway is modifiable based on exercise intensity (% of 1 repetition maximum; 1RM) (14) and volume load (repetitions × load) in healthy adults (1, 4). However, this hallmark of the post-exercise activation of the mTOR pathway seems to be diminished by chronic resistance exercise training (15, 16), consequently there is a need to better understand alternative anabolic mechanisms that may be involved in the regulation of changes in post-exercise muscle protein synthesis rates. Interestingly, Yes-associated protein (YAP) has recently been implicated as an mTORC1-independent mechanosensor involved in hypertrophic muscle protein remodeling in mice (17). However, the extent that resistance exercise mediates YAP expression and/or how intra-set rest manipulations impacts mTORC1 pathway activation has not been investigated in humans.

Therefore, the purpose of the study was to examine the extent to which intra-set rest manipulations consisting of CLU or TRD set configurations matched for total volume load alters mTORC1 and YAP mediated anabolic signaling events and post-exercise myofibrillar protein synthesis rates in resistance trained young adults. Given that CLU configurations are mainly applied with compound exercises in the field of strength and conditioning (10), we utilized barbell back squats as the form of resistance exercise to address the research question. We hypothesized that CLU or TRD conditions would both stimulate post-exercise myofibrillar
protein synthesis rates to a similar extent when matched for total volume load. We also hypothesized that both CLU and TRD conditions would phosphorylate mTORC1 targets (i.e., p70S6K and 4EBP1) and promote YAP expression during recovery from resistance exercise.

METHODS

Ethical Approval. The study was approved by the University of Illinois Institutional Review Board and conformed to standards for the use of human participants in research as outlined in the Declaration of Helsinki. This trial is registered at clinicaltrials.gov as NCT04028726. Each participant was informed of the purpose of the study, the experimental procedures, and all of the potential risks prior to providing their written consent to participate.

Participants. Eight resistance-trained participants (n = 7 males and 1 female; 23 ± 1 years, 81 ± 4.7 kg, BMI=26.6 ± 1 kg·m⁻², body fat: 18 ± 1.9%) volunteered to participate in this study. Participants were regularly engaged in resistance exercise (mean ± SEM: 5 ± 0.1 sessions per week) for the past 8 ± 1 years. All participants performed barbell squats frequently in their training with minimum strength eligibility assessed by 1RM ≥ 1.5 × body mass for study inclusion. All participants were considered healthy based on a self-reported medical screening questionnaire and had no prior history of participating in stable isotope amino acid tracer experiments. Volunteers who reported lower body musculoskeletal injury/surgery within the year prior, consumption of dietary supplements and/or ergogenic aids, within the 6 months prior, or any previous use of non-food anabolic agents, were also excluded from participation.

Baseline measurements. At least 72 hours prior to the first infusion trial, participants reported to the laboratory having refrained from all activities outside of daily living for at least 72 hours. Upon arrival, body weight and height were measured followed by body composition assessment via dual energy x-ray absorptiometry (QDR 4500A; Hologic, Marlborough, MA, USA). Finally,
the participants were familiarized with exercise equipment prior to determination of their one-repetition maximum (1RM) for the barbell back squat exercise (mean ± SEM: 150 ± 9.1 kg) using standardized procedures (8).

**Experimental control.** Participants were instructed to maintain their regular exercise habits between trials and refrain from vigorous physical activity for the 72 h before each infusion trial. In addition, participants were instructed to consume their habitual diet. The Automated Self-Administered Recall System (ASA24 version 2016; National Cancer Institute, Rockville, MD, USA) was used to record their intake for the 48 h prior to each trial. Recorded dietary intakes before each trial were similar for the TRD (30±4 kcal/kg body weight, 2.7±0.4 g carbohydrate/kg, 1.1±0.19 g fat/kg, and 2.4±0.4 g protein/kg) and CLU condition (31±1 kcal/kg body weight, 3.4±0.3 g carbohydrate/kg, 1.1±0.1 g fat/kg, and 2.1±0.3 g protein/kg). All participants were provided with a standardized meal of the same composition to be consumed the evening before each infusion trial. On completion of the first trial, a copy of the diet records were returned to participants, which they were instructed to replicate for the 48 h leading up to the second trial. Participants were randomly assigned in a counterbalanced fashion to perform the TRD or CLU condition for their first trial. The time between crossover trials was ~7 d.

**Infusion protocol.** The protocol for the infusion trials is presented in Fig. 1. On the infusion trial days, participants reported to the laboratory at 0600 after an overnight fast. A Teflon catheter was inserted into a dorsal vein in the hand for baseline blood sample collection, after which a primed continuous infusion of L-[ring-13C6]phenylalanine [prime: 2 μmol • kg lean body mass (LBM)⁻¹; 0.05 μmol • kg LBM • min⁻¹] was initiated and maintained until the end of the trial. A second Teflon catheter was placed in a contralateral heated dorsal hand vein and kept patent by a 0.9% saline drip for repeated arterialized blood sampling. To minimize the number of biopsies
collected during the trials and to provide a reference value for the assessment of postabsorptive myofibrillar protein synthesis rates (18), we collected a single resting biopsy after 3 h ($t=-60$) of infusion. Afterwards, participants consumed 25 g of protein (2.6 g of leucine) dissolved in 350 mL of water prior to the performance of TRD or CLU conditions as represented in Fig. 1. The TRD condition consisted of 4 sets of 10 repetitions (4×10) with a 120s rest between sets. The CLU condition consisted of 4 sets of 2×5 clustered repetitions (4×(2×5)) with 30s of rest between clusters and 90s between sets. Barbell load for both sessions was set at 70% 1RM. Volume load (repetitions × load) was matched between the TRD (4059 ± 244 kg) and CLU (4059 ± 244 kg) conditions ($P=1.00$). Barbell kinematic data (e.g., mean and peak concentric velocity) were assessed using a commercially-available optical encoder (GymAware®; Kinetic Performance Technology, Caberra, Australia). Validity and methods of data acquisition for this unit are reported elsewhere (19).

RPE and affective responses were also determined after the completion of each exercise set. Participants completed the Feeling Scale [FS, a single item scale ranging from -5 (very bad) to +5 (very good)], to assess affective valence (20, 21) along with Borg’s (22). Rating of Perceived Exertion scale [RPE; a single-item scale ranging from 6 (no exertion) to 20 (maximal exertion)], was used to assess perceptions of exertion during the exercise intervals. Participants were also asked to complete the Physical Activity Enjoyment Scale (PACES) following (5-minutes post) each conditions. The PACES is an 18-item (Likert-style with bipolar anchors) questionnaire that has been deemed a valid tool for measuring activity enjoyment (23). Upon completion of the exercise bout, a muscle biopsy sample was collected ($t=0$) followed by the ingestion of a second drink containing 25 g of protein (2.6 g of leucine). All drinks were enriched to 4% with tracer according to the measured phenylalanine content of the whey protein
to minimize disturbances in the precursor pools (24). Additional muscle biopsies were collected at 120 and 300 min of the post-exercise recovery period. Muscle biopsies were collected from the middle region of the vastus lateralis (∼15 cm above the patella) with a Bergström needle modified for manual suction under local anesthesia (2% Xylocaine). All muscle biopsy samples were freed from any visible blood, adipose, and connective tissue, immediately frozen in liquid nitrogen, and stored at −80°C until subsequent analysis. Arterialized blood samples were drawn every 30 or 60 min during the post-absorptive and postprandial-states. Blood samples (8 mL) were collected in EDTA-containing tubes and centrifuged at 3000× g at 4°C for 10 min. Aliquots of plasma were frozen and stored at −80°C until subsequent analysis.

**Blood Analyses.** Glucose and lactate were analyzed with whole blood using an automated biochemical analyzer (YSI 2300 Stat Plus; YSI, Yellow Springs, OH, USA). Plasma insulin concentrations were determined using a commercially available enzyme-linked immunosorbent assay (ELISA) (ALPCO Diagnostics, Salem, NH, USA). For plasma amino acid enrichments and concentrations, the Amino Acid standard solution (AAS18, Sigma, USA), containing 2.5 μmol•mL⁻¹ each of L-alanine, L-arginine, L-aspartic acid, L-glutamic acid, glycine, L-histidine, L-isoleucine, L-leucine, L-lysine-HCl, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tyrosine and L-valine, and 1.25 μmol•mL⁻¹ L-cystine and a custom mixture containing 2.5 μmol•mL⁻¹ each of L-tryptophane, L-glutamile, L-asparagine, L-citrulline, L-cysteine were used for the calibration curve. Plasma samples (200 μL) were deproteinized with ACN:IPA:Water (3:3:2 v/v), centrifuged with following supernatant evaporation in vacuum and re-suspended in 1 mL of 0.1% formic acid in water before instrument injection. Twenty μL of internal standard (ε-aminocaproic acid, 1 mg•mL⁻¹) was added to each sample and standard solution. Samples were analyzed by Thermo Altis Triple Quadrupole LC/MS/MS.
system. Software TraceFinder 4.1 was used for data acquisition and analysis. The LC separation was performed on a Thermo Accucore Vanquish C18+ column (2.1 x 100mm, 1.5μm) with mobile phase A (0.1% formic acid in water) and mobile phase B (0.1% formic acid in acetonitrile) and the flow rate was 0.2 mL/min. The linear gradient was as follows: 0-0.5 min, 0% B; 0.5-3.5 min, 60% B; 3.5-5.5 min, 100% B; 5.5-7.5 min, 0% B. The autosampler and HPLC column chamber were set at 10°C, 50°C, respectively. The injection volume was 1 μL. Mass spectra was acquired under positive electrospray ionization (ESI) with the ion spray voltage of 3500 V. Selected reaction monitoring (SRM) used for the amino acid quantitation.

**Muscle Analyses.** The myofibrillar protein-enriched fraction was extracted from ~50 mg of wet muscle using an ice-cold homogenizing buffer (10 μl•mg⁻¹; RIPA) supplemented with protease and phosphatase inhibitor tablets (cOmplete and PhosSTOP, Roche Applied Science, Mannheim, Germany). Myofibrillar proteins were isolated by the differential centrifugation whereby a slow speed spin (700×g) is used to pellet the myofibrillar and collagen proteins from the muscle homogenate. Subsequently, the myofibrillar proteins were solubilized by adding 0.3 M NaOH and heating at 37°C for 30 min as described previously (25). The remaining muscle homogenate was stored at −80°C for subsequent western blot analyses. Isolated myofibrillar protein fractions were hydrolyzed overnight in 6 m HCl at 110°C. The resultant free amino acids were purified using cation exchange chromatography (Dowex 50W-X8-200 resin; Acros Organics, Geel, Belgium) and dried under vacuum. Free amino acids were re-suspended in 60% methanol and centrifuged before analysis using 5500 QTRAP (Sciex, Redwood City, CA, USA) liquid chromatography–tandem mass spectrometry in accordance with a previously reported methodology (26). The L-[ring\(^{13}\)C₆]phenylalanine enrichments were determined by multiple reaction monitoring at m/z 166.0 → 103.0 and 172.0 → 109.0 for unlabeled and labelled L-[ring-
\(^{13}\)C\(_6\)phenylalanine, respectively. Analyst V1.6.2 (Sciex) was used for data acquisition and analysis.

**Calculations.** The fractional synthetic rate (FSR) of myofibrillar proteins were calculated using standard precursor-product methods by dividing the increment in tracer enrichment in the myofibrillar protein fraction by the enrichment of the plasma precursor pool over time as described previously (27). The recruitment of ‘tracer-naive’ participants allowed us to use the single biopsy approach to estimate post-absorptive muscle protein FSR during the first infusion trial as described in detail in our past efforts (18).

**Western Blotting.** Muscle homogenate supernatants from myofibrillar pelleting were stored at \(-80^\circ\text{C}\) for subsequent western blot analyses. Total protein concentrations of the muscle homogenate was determined using BCA assays (Pierce, Rockford, IL). Equal amounts of protein from each sample were mixed with loading buffer, denatured at 95°C for 5 minutes, separated on 7.5% or 10% (w/v) polyacrylamide gels, and then electrophoretically transferred to polyvinylidene fluoride membranes (PVDF, MilliporeSigma, St. Louis, MO). Membranes were blocked with non-fat milk or bovine serum albumin diluted in Tris-buffered saline with Tween solution (TBS-T) for 1 hour at room temperature before overnight incubation in primary antibodies at 4°C. Proteins of interest were detected with primary antibodies as follows: rabbit anti-\(\alpha\)-tubulin (1:1000; Abcam Cat# ab4074, RRID:AB_2288001), rabbit anti-phospho-AMPK\(\alpha\) (Thr172) (1:1000; Cell Signaling Technology Cat# 2535, RRID:AB_331250), rabbit anti-AMPK\(\alpha\) (1:1000; Cell Signaling Technology Cat# 5831, RRID:AB_10622186), rabbit anti-phospho-p70S6K (Thr389) (1:500; Cell Signaling Technology Cat# 9205, RRID:AB_330944), rabbit anti-p70S6K (1:500; Cell Signaling Technology Cat# 9202, RRID:AB_331676), rabbit anti-phospho-4E-BP1 (Thr37/46) (1:1000; Cell Signaling Technology Cat# 9459,
RRID:AB_330985), rabbit anti-4E-BP1 (1:1000; Cell Signaling Technology Cat# 9452, RRID:AB_331692), mouse anti-phospho-Erk1/2 (Thr202/Tyr204) (1:1000; Cell Signaling Technology Cat# 9106, RRID:AB_331768), rabbit anti-Erk1/2 (1:1000; Cell Signaling Technology Cat# 9102, RRID:AB_330744), rabbit anti-phospho-YAP (Ser127) (1:1000; Cell Signaling Technology Cat# 4911, RRID:AB_2218913), and rabbit anti-YAP (1:1000; Cell Signaling Technology Cat# 4912, RRID:AB_2218911). After primary incubation, blots were exposed to horseradish-peroxidase-conjugated horse anti-mouse IgG (1:2000; Cell Signaling Technology Cat# 7076, RRID:AB_330924) or goat anti-rabbit IgG (1:2000-1:10,000; Abcam Cat# ab6721, RRID:AB_955447) and detected using the ECL Western Blotting Substrate (Thermo Scientific, Waltham, MA) and the ChemiDoc XRS+ Imaging System (Bio-Rad Laboratories, Hercules, CA). Bands were quantified using ImageJ (National Institute of Health) and then normalized to α-tubulin as the internal control, and a control sample included on each blot to account for inter-blot variability.

Statistics. Based on previous research (27, 28), our power analysis showed that a sample size of 8 participants was sufficient to detect differences in post-exercise muscle protein synthesis between conditions when using a 2-sided statistical test (P < 0.05, 80% power, f = 1.2; G*power version 3.1.9.4). Differences in blood metabolites, muscle protein synthesis rates, and intramuscular signaling were tested using a two-factor (condition × time) analysis of variance (ANOVA) with repeated measures on time. Acute exercise variables (repetitions, load, velocity and power) were analyzed using a one-factor ANOVA. Where significant interactions were identified in the ANOVA, Tukey's post hoc test was performed to determine differences between means for all significant main effects and interactions. Pearson's r product moment correlation was used to examine the relationship between different variables (i.e., muscle protein synthesis
and anabolic signaling molecules). Linear regression lines were fitted to plasma enrichments to assess the existence of any deviation in enrichment indicated by lines with a significant positive or negative slope. The level of statistical significance was set at $P<0.05$ for all analyses. The data are expressed as mean ± standard error of the mean (SEM). For RPE and affective responses, Cohen’s d (d) was calculated as a measure of effect size (0.20=small effect size; 0.50=medium effect size; 0.80=large effect size) for these variables.

RESULTS

**Acute resistance exercise variables.** There were no differences in repetitions (40 ± 0) performed or load lifted in sets 1-4 between TRD or CLU conditions ($P=1.00$). CLU condition resulted in greater mean (Fig. 2a) and peak concentric velocity (Fig. 2b) for sets 1-4 when compared to the TRD condition (main effect of condition: $P < 0.001$). Similarly, CLU produced greater average and peak power for sets 1-4 when compared to the TRD condition (Fig. 2; main effect of condition: $P < 0.001$).

**RPE and affective responses.** Perceptions of exertion, based on RPE responses, were significantly different between conditions ($P = 0.047$) and across time ($P= 0.004$), but the Condition × Time interaction was not significant ($P = 0.17$). RPE responses were higher in the TRD condition (16.3) compared to the CLU condition (15.8) and increased over time in both conditions (14.6 to 17.4). Although the interaction was not significant, following the second set, perceptions of exertion were ~1 unit less in the CLU condition (17.3 vs 16.1, 17.9 vs 16.9 following the 3rd and 4th sets, respectively). There were no significant Condition ($P = 0.29$), Time ($P = 0.21$), or Condition × Time interaction ($P = 0.11$) effects for the affective valence measures (which is a measure of how pleasant or unpleasant the individual feels). Overall, affect was slightly worse in TRD (1.6) than CLU (1.9), but it stayed fairly stable in TRD (~1.5 after each
set); for CLU, affect started higher (2.6) and then declined, with the largest decline after the 2nd set compared to after the 3rd set. Finally, when the participants were asked to rate their enjoyment of the two approaches, while not significant, enjoyment was greater for the CLU (100.8) than for the TRD (96.3) (Cohen’s $d$ effect size = 0.51).

**Blood Analysis.** Blood glucose concentration increased above baseline immediately after resistance exercise with no differences between TRD or CLU conditions (Table 1; main effect of time: $P=0.03$). Blood lactate concentration was higher after TRD when compared to CLU during recovery from resistance exercise (Table 1; main effect of condition: $P < 0.001$). Plasma insulin concentration reached peak values ($P=0.01$) at 30 minutes after the ingestion of the second protein bolus and returned to basal values by 1.5 h irrespective of condition (Table 1). Plasma EAA concentrations reached peaked values at 1 h ($P < 0.001$) after the ingestion of the second protein bolus and returned to baseline values by 2 h of the post-exercise period irrespective of condition (Table 1). Plasma phenylalanine concentrations reached peak values of 70.8±3.6 and 70.2±3.8 μM at 0.5 h after the ingestion of the second protein bolus and returned to baseline values of 61.0±3.7 and 58.4±3.5 μM at 1.5 h of the post-exercise period in both the TRD and CLU conditions, respectively. Basal plasma L-[ring-$^{13}$C$_6$]phenylalanine enrichments did not differ between the TRD (averaged 0.053±0.006 tracer·tracee$^{-1}$; TTR) or CLU (0.048±0.007 TTR) conditions ($P>0.05$). Moreover, postprandial plasma L-[ring-$^{13}$C$_6$]phenylalanine enrichments did not differ between the TRD or CLU conditions ($P=0.26$). Specifically, plasma L-[ring-$^{13}$C$_6$]phenylalanine enrichments increased ($P<0.05$) after the ingestion of the 2nd protein bolus during the late post-exercise phase (2-5 h) in the TRD (0.072±0.007 TTR) and CLU condition (0.073±0.008 TTR). Linear regression analysis indicated that the slopes of the plasma enrichments were not significantly different from zero, regardless of condition, during the early
(0-2 h) or late phase (3-5 h) of the post-exercise period, demonstrating isotopic plateau during these measurement periods.

Muscle anabolic signaling. Phosphorylated, total, or phosphorylated-to-total ratios of AMPKα, P70S6K, and 4E-BP1 were unaffected by both conditions and did not increase from baseline values at any time point during recovery (Fig. 3; all \( P > 0.05 \)). Phosphorylated (Fig. 4a) and total expression of YAP (Fig. 4b) were elevated \( (P < 0.05) \) immediately post-exercise regardless of condition (main effect for time: \( P = 0.006 \) and 0.009, respectively) with no change to the phosphorylated-to-total ratio \( (P = 0.134; \text{Fig } 4c) \). Similarly, phosphorylation of ERK1/2 \( \text{Thr}^{202}/\text{Tyr}^{204} \) and phosphorylated-to-total ERK1/2 ratio were significantly elevated immediately post-exercise with no differences between condition (main effect for time: \( P = 0.004 \) and 0.006, respectively; Fig. 3b).

Myofibrillar protein synthesis. Myofibrillar protein synthesis rates increased above baseline by ~4- and 3-fold during the early phase (0-2 h) of post-exercise recovery for the TRD and CLU conditions, respectively (both \( P < 0.001 \); Fig. 5a). TRD tended \( (P = 0.096) \) to increase the post-exercise myofibrillar protein synthetic response to a greater extent during the early phase of recovery when compared to the CLU condition. However, the increase in the cumulative myofibrillar protein synthetic response calculated over the entire 5 h post-exercise phase did not differ \( (P = 0.48) \) between the TRD \( (0.074 \pm 0.013 \% \cdot \text{h}^{-1}) \) or CLU \( (0.063 \pm 0.018 \% \cdot \text{h}^{-1}) \) condition.

DISCUSSION

Our study is the first to characterize the impact of intra-set rest on the post-exercise regulation of myofibrillar protein synthesis rates and anabolic signaling in trained young adults. We show that the CLU configuration resulted in higher power output and a similar stimulation of the
cumulative (0-5 h) post-exercise myofibrillar protein synthetic response when compared to TRD with total volume load equated. In addition, we show that resistance exercise, regardless of condition, induced an increase in total and phosphorylated YAP expression without altering mTORC1-related signaling events (i.e., p70S6k and 4EBP1 phosphorylation).

It is indeed interesting that our results demonstrated a tendency ($P=0.09$) for the post-exercise myofibrillar protein synthetic response to be greater during the early (0-2 h) phase of recovery in the TRD compared to the CLU condition (Fig. 5a). We speculate this could be related to the decrease in concentric velocity (Fig. 2) resulting in greater time the muscle is experiencing loaded tension (10), which likely would have increased surface electromyography (EMG) activity in the TRD when compared to CLU condition, had it been measured in our study (29). This notion is consistent with the thesis that motor unit recruitment and activation modulates the post-exercise stimulation of myofibrillar protein synthesis rates (3, 5). What is noteworthy is that the post-exercise myofibrillar protein synthetic response, irrespective of condition, returned to the basal-state by 3-5 h of recovery. The short-lived nature of the post-exercise myofibrillar protein synthetic response is likely linked to the trained status of the volunteers. For example, it has been shown that the stimulation of post-exercise muscle protein synthesis rates is attenuated both in terms of amplitude and duration in trained vs. novice weight lifters (30-32).

The influence of acute resistance exercise on the activation-status of the mTORC1 pathway has been shown to be intensity (%1RM) (14) and volume load (1) sensitive in humans. Here, we show that the mTORC1 pathway, as noted by the extent p70SK1 and 4E-BP1 phosphorylation, remained unchanged from pre- to post-exercise regardless of the intra-set rest manipulation. Similarly, we show AMPK phosphorylation, a cellular energy sensor (33), was
not altered during recovery from resistance exercise. Again, these findings are likely related to the trained nature of the participants whereby the phosphorylation of these proteins have been shown to be diminished by regular resistance exercise (15, 16). Moreover, it is also possible that other relevant mTORC1 regulatory events, such as protein-protein interactions or intracellular redistribution to the sarcolemma, occurred and were not captured by our more traditional Western blot readout of protein phosphorylation (34).

Given that training status (15) and regular resistance exercise training (16) have been shown to reduce mTORC1 pathway activation, we specifically sought to investigate other potential anabolic mechanisms that may modulate the post-exercise stimulation of muscle protein synthesis rates in trained volunteers. Recently YAP has been implicated as a mechanosensor involved in hypertrophic muscle protein remodeling (17). In this study, we show that total and phosphorylated YAP on Ser127 is increased immediately after acute resistance exercise irrespective of condition. Our results are consistent with those of Goodman et al. (17) who demonstrated that mechanical loading increased total and phosphorylated YAP on Ser112 (corresponding to Ser127 in humans) in hypertrophic mice muscle. Indeed, it could be speculated that the immediate intramuscular adjustment of total YAP protein implies it is translationally regulated and/or stabilized through reduction of YAP degradation rates, thereby leading to YAP accumulation. In any case, the underlying mechanism in which YAP transduces mechanical signals to regulate muscle mass has not been completely deciphered (17, 35), but it has been speculated that YAP expression is not involved in the contraction-induced activation of mTORC1 as originally assumed (17).

Interestingly, we observed a relationship between total \((r=0.67; P<0.0001)\) and phosphorylated \((r=0.58; P<0.0001)\) human YAP with Erk1/2 phosphorylation immediately
after resistance exercise regardless of the TRD or CLU configuration. The increase in Erk1/2 phosphorylation in our study is consistent with past efforts that have shown the MAPK pathway is sensitive to acute resistance exercise (36-38); however, our results suggest there may be a connection between hippo/YAP signaling and Erk1/2 phosphorylation in human skeletal muscle as shown in other cell models (39). This notion is consistent with results that have shown Erk1/2 phosphorylation is mTORC1 independent in rodents (40, 41). From our study design it is not possible to decipher the influence of training status per se, but it is possible that mTORC1 dependent and independent events are differentially modulated in trained vs. untrained muscle in response to acute resistance exercise (42). Moreover, it is possible that YAP localization, rather than phosphorylation, may be more relevant as a mediator of the anabolic response as suggested by a cell-based assay (43). These are points for future work to explore.

Overall, our results implicate the CLU configuration as an easily manipulated resistance exercise variable to maximize power development as well as facilitate muscle protein remodeling as indicated by similar increases in the cumulative (0-5 h) post-exercise myofibrillar protein synthetic response between the TRD and CLU conditions with equal total volume load. In support, Oliver et al. (2013) showed that 12 weeks of training with CLU or TRD configurations resulted in similar increases lean body mass in trained young men. From a psychological perspective, the CLU approach was perceived as less effortful, particularly on the later sets, yet rated the same affectively and was somewhat more enjoyable. This would seem to suggest that either the TRD or CLU approach could be used depending on the individual’s preference without detrimental affective or perceptual consequences. Certainly future efforts would be required to directly determine the translation of our findings into other populations (e.g., aging) and other
exercise selections (e.g., leg extension) that are often commonplace within a program of resistance exercise training, especially in the general population.

In conclusion, intra-set rest manipulations of CLU or TRD configurations stimulated a similar increase in cumulative (0-5 h) myofibrillar protein synthesis rates. This result was primarily driven by the early (0-2 h) stimulation of myofibrillar protein synthesis rates as the response waned in the later phase (3-5 h) of recovery from resistance exercise. These results demonstrate that intra-set rest is an exercise variable that can be manipulated to augment power output and support the acute skeletal muscle adaptive response without altering total volume load or the duration of the exercise bout. Finally, our findings support the notion that YAP may be involved in resistance exercise-induced muscle protein remodeling in healthy trained young adults.
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CONFLICT OF INTEREST. No authors have any conflicts of interest, financial or otherwise, to declare. The results of the present study do not constitute endorsement by the American College of Sports Medicine and are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.
REFERENCES


**Figure Legends**

**Figure 1.** Experimental infusion and exercise protocols. Participants consumed 25 g of protein at each feeding time point.

**Figure 2.** Peak and mean concentric velocity (A and B) and power (C and D) during the resistance exercise session consisting of traditional (TRD) or cluster-set (CLU) configurations. Panels A and B represent the average of all repetitions within a set while C and D represent the average of all repetitions within the bout. *Significant main effect for condition between CLU and TRD (P < 0.001). Values are presented as mean ± SEM.

**Figure 3.** Phosphorylated-to-total protein ratio of AMPKα (A), ERK1/2 (B), P70S6K (C), and 4E-BP1 (D) at rest and after the performance of the cluster-set (CLU; open circles) and traditional set configurations (TRD; filled circles). n=8 per condition. Values are presented as mean ± SEM. *Significantly (P<0.05) different from rest. †Significantly (P<0.05) different from 5 h.

**Figure 4.** Phosphorylated (A), total (B), and phosphorylated-to-total protein ratios (C) of YAP at rest and after the performance of the cluster-set (CLU; open circles) and traditional set configurations (TRD; filled circles). n=8 per condition. Values are presented as mean ± SEM. *Significantly (P<0.05) different from rest. †Significantly (P<0.05) different from 5 h.

**Figure 5.** Temporal (A) and cumulative (B) myofibrillar protein synthesis rates at rest and after the performance of the cluster-set (CLU; open circles) and traditional set configurations (TRD;
filled circles). $n=8$ per condition. Values are presented as mean ± SEM. *Significantly (P<0.05) elevated above basal. †Significant (P<0.10) difference between TRD and CLU
Figure 1

[Diagram showing primer-continuous L-\([\text{ring-}^{13}\text{C}_{6}]\text{phenylalanine infusion}\) with time points and biological samples.

- Time (min): -240, -180, -120, -60, 0, 60, 120, 180, 240, 300
- Blood samples at -240, -180, -120, -60, 60, 120, 180, 240, 300
- Biopsy samples at -60, 60, 120, 180, 240, 300
- Protein intake arrows at -240, -180, -120, -60, 60, 120, 180, 240, 300

Traditional Set:
- Rep 1, 2, 3, 4, 5
- Rep 6, 7, 8, 9, 10

Cluster Set:
- Rep 1, 2, 3, 4, 5
- Rep 6, 7, 8, 9, 10

Rotation angles: 120° and 90° with repetition count '4x'.
Table 1. Plasma amino acids, blood glucose, and plasma insulin concentrations during experimental infusion trials.

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Values are means (SEM) (n=8). Resistance exercise protocol was performed between -60 to 0 minutes. ΣEAA are sum of His, Ile, Leu, Lys, Met, Phe, Thr, Val. a Significantly different from fast.*Significantly different from TRD (P<0.05). †Tendency for difference from TRD (P<0.10).