Muscle Protein Synthetic Responses to Exercise: Effects of Age, Volume, and Intensity

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We explored the relationships between resistance exercise volume/intensity and muscle myofibrillar protein synthetic (MPS) responses in young and older men. In a crossover design, four groups of six young (24±6 years) and older (70±5 years) men performed two volumes of resistance exercise: either 40% one repetition maximum (1RM) (3×14, then 6×14 repetitions) or 75% 1RM (3×8, then 6×8 repetitions), such that at the same volume, work was identical between intensities. Muscle biopsies were taken 0, 1, 2, and 4 hours after exercise to measure MPS via myofibrillar bound [1,2-13C2]leucine and indices of mammalian target of rapamycin signaling by immunoblotting. In younger men, doubling exercise volume produced limited added effects, whereas in older men, it resulted in greater MPS and p70S6 kinase (p70S6K) phosphorylation at both intensities, that is, MPS area under the curve: 75% (1×volume: 0.07±0.01 vs 2×volume: 0.14±0.02% protein synthesized/4 hours (p<.001). Doubling exercise volume is a valid strategy to maximize postexercise MPS in ageing.

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MOST previous work on the response of myofibrillar protein synthetic (MPS) responses to resistance exercise shows that it is stimulated at exercise of high intensity with a large number of sets/repetitions, for example, 6–20 sets with 8–12 repetitions at 70%–80% one repetition maximum (1RM), in fasted or fed conditions (1–7). However, the relationship between intensity and volume of exercise for the stimulation of MPS is still not clear because in previous studies, the effect of volume was not explicitly examined, and many results are the aggregate effects of feeding and exercise together. Furthermore, earlier work has mostly examined the responses of the synthesis of mixed muscle proteins (1–3), whereas it is now becoming clear that myofibrillar protein responses to exercise are usually relatively greater and sustained for longer than those of sarcoplasmic fractions (8–10).

The control of increases in MPS after exercise has been the subject of intense research. Although the proximal pathways involved are complex and diffuse, there is little doubt that activation of the mammalian target of rapamycin (mTOR) and its downstream effectors, p70S6 kinase (p70S6K) and eukaryotic initiation factor 4E binding protein 1 (4EBP1) (11–14), ultimately co-ordinate the synthesis of skeletal muscle protein in response to exercise and nutrition. This involvement of mTOR was elegantly confirmed in a study demonstrating that administration of rapamycin suppressed postexercise increases in MPS in humans (14). Accordingly, the degree of increase in the phosphorylation of the mTOR substrate p70S6K after a bout of resistance exercise has been closely associated with that of exercise intensity, MPS, and hypertrophy in both rat and human muscle (7,11,15). However, it remains unclear whether downstream signaling proteins such as p70S6K and 4EBP1 are differentially activated in response to resistance exercise of different volumes.

We recently showed that an acute bout of resistance exercise at higher intensities (60%–90% 1RM) increases MPS in the postabsorptive state over 1–2 hours postexercise to a greater extent than that at lower intensities (20%–40% 1RM) but with a larger response in young than older men (7). In that study, we kept the volume of exercise and total external work output at different exercise intensities (ie, percentage of 1RM × number of repetitions × number of sets) constant such that we remain uninformed of how the volume of exercise influences MPS in the postabsorptive state in either young or old muscle. Thus, expanding on this previous work (7), we sought to investigate the effect of two different volumes of exercise, three versus six sets of unilateral leg extension, at two different exercise intensities, a lower intensity (40% 1RM) and a higher intensity (75% 1RM), on MPS in healthy young and old men. We...
purposely decided to study subjects in the postabsorptive state to distinguish the exercise-mediated responses from those coupled to increased nutrient availability (7). We hypothesized that there would exist a greater anabolic sensitivity in young than older muscle such that in muscle of young men, at a given intensity, there are modest thresholds for responses of MPS and anabolic signaling beyond which any additional volume of work would not induce further increases in them and increasing the volume of exercise would overcome “exercise desensitization” in muscles of older men.

METHODS

Recruitment and Screening

The study was approved by the University of Nottingham Ethics Committee and complied with the Declaration of Helsinki. Written informed consent was obtained from the subjects after explaining the study procedure and associated risks. Twenty-four men, both young (n = 12; mean ± standard error of mean [SEM], 24 ± 6 years, and older (n = 12; mean ± SEM, 70 ± 5 years) men, were recruited through newspaper and web advertisements. They were recreationally active, physically independent, and healthy overall, but not currently or previously engaged in any formal resistance training. Screening of the subjects, performed by an experienced clinician, included a clinical history, a physical examination, and an electrocardiogram. Blood was tested for full blood count, coagulation profile, fasting blood glucose, and markers of liver, kidney, and thyroid function. Subjects were excluded if they had a history of cardiac, pulmonary, liver, kidney, vascular or autoimmune disease, clotting disorders, uncontrolled hypertension, diabetes, thyroid disorders, obesity, anemia, cancer, alcohol abuse, obvious muscle wasting, corticosteroid use, or inability to discontinue aspirin therapy. Older subjects who had mild hypertension (<140/90 mm Hg) controlled by medication were included in the study, but did not take medication on the day of the study. The maximal strength of the subjects’ dominant leg was measured approximately 1 week before the study on a free-weight leg extension machine (ISO leg extension, Leisure Lines [GB] Ltd), and they were familiarized with the study exercise protocols. Whole-body composition was measured by dual-energy X-ray absorptiometry (GE Lunar Prodigy II, GE Healthcare).

Study Design

Subjects were randomly assigned to perform, at 3-month intervals, first three and then six sets of an isotonic, unilateral leg extension and flexion exercise at one of two different exercise intensities (either 40% or 75% of 1RM (six per group and per intensity) in a randomized and balanced fashion. Although the work was matched between intensities at the same volume, the protocols differed only in volume of work. All subjects were studied after an overnight fast. They were asked to refrain from any heavy exercise for 72 hours before the study day. On the morning of the study (approximately 9 AM), subjects had 18-g polyethylene catheters inserted in the antecubital veins of both arms, one for tracer infusion and the other for venous blood sampling. Blood samples were taken according to the protocol (Figure 1). Muscle biopsies were taken from the m. vastus lateralis under sterile conditions using the conchotome biopsy technique (16) with 1% lignocaine as local anesthetic. The muscle tissue was washed in ice-cold...
saline before blood, visible fat, and connective tissue were removed, and then, it was immediately frozen in liquid nitrogen and then stored at −80°C until further analysis. A primed, continuous infusion (0.7 mg.kg⁻¹, 1 mg.kg.h⁻¹) of [1,2-¹³C₂]leucine tracer (99 atoms %; Cambridge Isotopes Ltd, Cambridge, MA) was started (at 0-hours) after the first biopsy and maintained until the end of the study. After taking biopsies at rest, t = 0 and 2.5 hours in the postabsorptive pre-exercise state, the subjects performed unilateral leg extensions at a moderate contraction velocity (1–2 seconds concentric, 1–2 seconds eccentric), with 3-minute rest between sets. Subjects who performed unilateral leg extension at 40% 1RM subjects completed 3 sets × 14 repetitions or 6 sets × 14 repetitions, and those who performed at 75% 1RM completed 3 sets × 8 repetitions or 6 sets × 8 repetitions. Four further muscle biopsies were obtained from the exercised leg immediately after and at 1, 2, and 4 hours following exercise. After the study, cannulae were removed, and the subjects were fed (a sandwich, chocolate bar, and drink of their choice) before being allowed home.

**Measures of MPS**

Muscle tissue (approximately ~25 mg) was minced with scissors in ice-cold homogenization buffer containing 50 mM Tris–HCl (pH 7.4), 1 mM EGTA, 1 mM EDTA, 10 mM β-glycerophosphate, 50 mM NaF, 0.5 mM activated sodium orthovanadate (all Sigma-Aldrich, Poole, UK), and a complete protease inhibitor cocktail tablet (Roche, West Sussex, UK). The resulting homogenate was centrifuged at 3000g at 4°C for 20 minutes to precipitate the myofibrillar fraction. The myofibrillar pellet was then solubilized with 0.3 M NaOH and centrifuged at 3000g for 20 minutes to separate it from the insoluble collagen fraction. The soluble myofibrillar protein was precipitated using ice-cold 1M perchloric acid, the resulting pellet was washed twice with 70% ethanol and collected by centrifugation. Protein-bound amino acids were released by acid hydrolysis in a Dowex H⁺ resin slurry (0.05M HCl) at 110°C overnight. The amino acids were then purified by ion exchange chromatography on Dowex H⁺ resin. The amino acids were derivatized as their n-acetyl-N-propyl esters as previously described (8,17). Incorporation of [1,2-¹³C₂]leucine into protein was determined by gas chromatography–combustion–isotope ratio mass spectrometry (Delta plus XP, Thermo Fisher Scientific, Hemel Hempstead, UK) using our standard techniques (18). The fractional synthesis rate (FSR) of myofibrillar protein was determined from the incorporation of [1,2-¹³C₂]leucine, using the precursor labeling of venous α-KIC between subsequent muscle biopsies as previously described (19). Using venous KIC labeling to reflect precursor labeling, that is, the leucyl-t-RNA is the standard approach for this method (13,20), and it has been demonstrated to closely reflect the leucyl-t-RNA labeling in the postabsorptive state. In other experiments (unpublished observations), we have established that the KIC enrichment from the femoral vein on the exercised leg is in steady state; thus, KIC labeling accurately reflects the intracellular leucine labeling also in the exercising leg. The rate of myofibrillar FSR (MPS) was calculated using the standard precursor-product method: fractional protein synthesis (kₛ, % h⁻¹) = ΔLₘ/L₀ × 100, where ΔLₘ is the change in protein labeling between two biopsy samples, L₀ is the mean value over time of venous α-KIC labeling, and t is the time between biopsies in hours.

**Measures of Protein Phosphorylation**

Immunoblotting was performed using our standard methods as previously described (20,21). Briefly, after finely mincing the muscle tissue in ice-cold homogenization buffer containing the appropriate phosphatase and protease inhibitors, sarcoplasmic proteins were separated from myofibrillar proteins by centrifugation at 3000g at 4°C. Proteins were separated by electrophoresis at 200 V. h⁻¹ for approximately 1 hour and transferred to 100% methanol permeabilized 0.2-mm polyvinylidene difluoride membranes for 45 minutes at 100V. Membranes sections were then blocked for 1 hour with 5% BSA before overnight exposure at 4°C, with gentle agitation, to a primary antibodies for p70S6K1 (Thr389) and (UK New England Biolabs) at 1:2000. The next morning, membranes were exposed to antirabbit IgG secondary at 1:2000 (UK New England Biolabs) before exposure and densitometric quantification using the Chemidoc XRS system (Bio-Rad Laboratories, Inc. Hercules, CA).

**Statistical Analysis**

All data are reported as means ± SEM. All analyses were made using GraphPad Prism version 5.0 or SPSS. Area under the curve (AUC) data is presented as Rate of myofibrillar protein synthesis × Time or percentage of total myofibrillar protein synthesized in the period. One-way analysis of variance (ANOVA) with repeated measures was used to determine temporal differences in MPS at specific time points. Area under the MPS and signaling time course curves (AUC) were calculated and used to perform a three-way ANOVA comparing cumulative AUC across age, intensity, and workload. Significance was accepted as p < .05.

**RESULTS**

**Subject Characteristics**

The participants in this study were all healthy, recreationally active individuals (but engaged in no formal training program) with no differences presenting between groups in body weight (young, 72 ± 11 kg; older, 72 ± 16 kg), body mass index (young, 22 ± 3 kg/m²; older, 23 ± 4 kg/m²), height (young, 1.80 ± 0.07 m; older, 1.74 ± 0.06 m), DXA-derived lean mass (young, 60 ± 11 kg; older, 52 ± 7 kg), or DXA-derived fat mass (young, 15 ± 7 kg; older, 19 ± 9 kg).
Thus, the only characteristics by which our groups could be distinguished were age (young, 24 ± 6 years; older, 70 ± 5 years; p < .05) and 1RM leg extension force, which was significantly diminished in our older subjects (young, 750 ± 225N; older, 392 ± 196 N; p < .05).

MPS and Anabolic Signaling in Younger Men

As previously reported (7), although the muscle of the young subjects elicited little response to exercise at 40% 1RM (no significant increases in MPS at any time-point), increasing the intensity at both workloads did significantly elevate MPS above basal, 1–2 hours postexercise (ie, 75%: 0.12% ± 0.02% hours⁻¹; p < .05; Figure 2B). Taking the entire postexercise period together, the AUC for 2× volumes of exercise was greater at 75% than 40% 1RM (Figure 2B; p < .05) while increasing the volume of exercise at each intensity did not supply any additional effect: 40% (AUC 1× volume: 0.07±0.02 versus 2× volume 0.06% ± 0.02% protein synthesized in 4 hours) and 75% (AUC 1× volume: 0.09±0.03 versus 2× volume 0.14% ± 0.03% protein synthesized in 4 hours; Figure 2B). In terms of signaling, although there was no effects on the phosphorylation responses of p70S6K to resistance exercise at different volumes at 40% 1RM, increasing exercise volume from three sets to six sets at 75% 1RM did result in a greater AUC (p < .05) and sustained elevation of phosphorylated p70S6K in young men, up to the last time point (4 hours postexercise; p < .05; Figures 3A and B).

MPS and Anabolic Signaling in Older Men

Despite there being no changes in MPS throughout the time course at 1× volume during 40% or 75% 1RM exercise, increasing to 2× volumes elicited significant increases between 0–2 hours after exercise at both intensities (Figure 4A). This also translated into significant differences in the AUC (Figure 4A and 4B), whereby increasing the volume of exercise at both 40% and 75% 1RM from three to six sets significantly enhanced the MPS responses 40% (AUC 1× volume: 0.01±0.01 versus 2× volume 0.13% ± 0.03% protein synthesized in 4 hours; p < .01) and 75%
AUC 1× volume: 0.07 ± 0.01 versus 2× volume 0.14% ± 0.02% protein synthesized in 4 hours; $p < .001)$. In terms of signaling time course, although phosphorylated p70S6K was unaffected by 1× volume of exercise, it was elevated at 1 and 4 hours ($p < .05$) and 1 and 2 hours after exercise ($p < .05$) in response to 2× volumes of 40% and 75% 1RM, respectively. In addition, the extent of increases in postexercise p70S6K phosphorylation was significantly higher after six sets of exercise at both intensities in older men (AUC both $p = .05$; Figure 5B). Finally, there were no differences between AUC in response to 2× volumes of exercise at 40% and 75% 1RM.

Differences in AUC of MPS Between Young and Older Men

The AUC for MPS was greater in young versus older men at 1× volume of 40% 1RM exercise, ($p < .05$), although not at 75% 1RM. At 2× volume exercise, the older men displayed a trend for greater MPS versus the young men at 40% 1RM ($p < .1$). There were no differences in AUC between young and older men at 75% 1RM.

**DISCUSSION**

We report novel information concerning the responses of MPS in young and older men to different volume of resistance exercise at two different intensities in the postabsorptive state. Increasing the volume of exercise from three to six sets at a given intensity robustly enhances postexercise MPS responses in older men, whereas it has relatively minimal (ie, nonsignificant approximately 50% increase in AUC versus 110% in older men at 75% 1RM) additional effect in young men. It has been previously demonstrated that “anabolic blunting” is a pervasive feature of ageing muscle, which is revealed in a reduced sensitivity to the anabolic effects of both feeding (22-25) and exercise (7,25). These lower synthetic responses of older muscle have been associated with inability to fully activate mTOR...
signaling when compared with young muscle (7,23). We, therefore, expected that the less sensitive muscle of older men would require a greater anabolic stimulus (ie, to perform more work) to activate the protein synthetic machinery sufficiently to achieve MPS rates comparable to those seen in younger men. In concordance with this, we observed that doubling the exercise volume at both 40% and 75% of 1RM, enhanced MPS rates such that they equaled the young at 75% and even surpassed them at 40%.

The older men were weaker in terms of strength-related performance (1RM young, 750 ± 225 N vs older, 392 ± 196 N) such that they were lifting considerably less weight per unit cross-sectional area (given equal muscle mass by DXA). Although this may explain the lack of sensitivity at 1× volume of 75% 1RM exercise, why would MPS be potentiated in older but not younger muscle after resistance exercise at a lower intensity, such as 40%? According to Henneman's size principle, during low-intensity muscular contraction, slow twitch fibers with small motor units are primarily recruited, whereas increasing muscular force gradually recruits increasing numbers of type II fibers (26). However, several studies have shown that in hypoxic or fatiguing conditions, early recruitment of type II fibers can occur with the effect of maintaining the muscular force during low-intensity resistance exercise (27–29). In comparison to young muscle, older muscle has reduced fatigue resistance (30,31) such that a higher number of repetitive muscular contractions even at low intensity (40% 1RM) cause heightened metabolic stress (28) and additional recruitment of type II fibers. Thus, increased recruitment of larger type II fiber containing motor units can perhaps explain increases in MPS in older, but not younger men at 2× volumes of 40% 1RM exercise (32,33).

In terms of intramuscular “anabolic” signaling, the dissociation between p70S6K phosphorylation and MPS at 75% in the younger men further underlines the potential for discordance between amplitude of signaling protein phosphorylation and biological end-points such as MPS (17).
That said, we provide evidence for the existence of a link between exercise volume and phosphorylation of p70S6K, which is increased after resistance exercise to an extent depending on the exercise volume at higher intensity in the young group, and at both lower and higher intensities in the older group.

In conclusion, we have found that in young men doubling the volume of exercise at 40% had no additional effects and at 75% 1RM only small additional effects, on the response of MPS in the postabsorptive state; however, in older men, it resulted greater responses at both intensities. The results suggest that there is increased latency of response to exercise in muscle of older men and increasing the volume of exercise enhances the stimulatory effect of resistance exercise on MPS, even at relatively low intensities, that is, 40% of 1RM. Although we accept our sample sizes are small and give limited resolution of the existence of small cross-sectional differences in responses between age-groups afforded by other studies, that is (25), our present one-way findings (particularly, intensity crossover within the older-groups) are clear and may have important implications for the exercise recommendations for elderly people, and the frail elderly in particular, for whom muscle maintenance is of crucial importance and who may not be capable of undertaking high-intensity exercise (>70% 1RM). Therefore, although further work is needed to define the application of such an exercise strategy in overtly sarcopenic groups, we conclude that older people would perhaps benefit from doing moderate-intensity exercise of a higher volume in order to help maintain/increase their muscle mass.

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**References**


