Exercise, Amino Acids, and Aging in the Control of Human Muscle Protein Synthesis

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ABSTRACT

WALKER, D. K., J. M. DICKINSON, K. L. TIMMERMAN, M. J. DRUMMOND, P. T. REIDY, C. S. FRY, D. M. GUNDERMANN, and B. B. RASMUSSEN. Exercise, Amino Acids, and Aging in the Control of Human Muscle Protein Synthesis. Med. Sci. Sports Exerc., Vol. 43, No. 12, pp. 2249–2258, 2011. In this review, we discuss recent research in the field of human skeletal muscle protein metabolism characterizing the acute regulation of mammalian target of rapamycin complex (mTORC) 1 signaling and muscle protein synthesis (MPS) by exercise, amino acid nutrition, and aging. Resistance exercise performed in the fasted state stimulates mixed MPS within 1 h after exercise, which can remain elevated for 48 h. We demonstrate that the activation of mTORC1 signaling (and subsequently enhanced translation initiation) is required for the contraction-induced increase in MPS. In comparison, low-intensity blood flow restriction (BFR) exercise stimulates MPS and mTORC1 signaling to an extent similar to traditional, high-intensity resistance exercise. We also show that mTORC1 signaling is required for the essential amino acid (EAA)–induced increase in MPS. Ingestion of EAAs (or protein) shortly after resistance exercise enhances MPS and mTORC1 signaling compared with resistance exercise or EAAs alone. In older adults, the ability of the skeletal muscle to respond to anabolic stimuli is impaired. For example, in response to an acute bout of resistance exercise, older adults are less able to activate mTORC1 or increase MPS during the first 24 h of postexercise recovery. However, BFR exercise can overcome this impairment. Aging is not associated with a reduced response to EAAs provided the EAA content is sufficient. Therefore, we propose that exercise combined with EAA should be effective not only in improving muscle repair and growth in response to training in athletes, but that strategies such as EAA combined with resistance exercise (or BFR exercise) may be very useful as a countermeasure for sarcopenia and other clinical conditions associated with muscle wasting. Key Words: SARCOPENIA, PROTEIN TURNOVER, MTORC1, ESSENTIAL AMINO ACIDS, LEUCINE

Skeletal muscle represents 50%–75% of all body proteins and approximately 40% of total body weight (72). In addition to sheer volume, muscle possesses numerous vital functions such as force generation, temperature regulation, energy metabolism, amino acid reserves, immune function, and the ability to grow and regenerate (68). Consequently, decrements in skeletal muscle mass and function can introduce complications, which become especially apparent during treatment and rehabilitation for various clinical conditions such as cancer cachexia, chronic heart failure, forced inactivity (i.e., bed rest), acquired immunodeficiency syndrome, etc. In addition, the loss of muscle mass with advancing age (sarcopenia) is quickly becoming recognized as a major health concern as it has been linked to increased functional disability, loss of independence, and decreased life expectancy (12,22,76). Considering this link to various debilitating clinical conditions, strategies are needed to counteract the loss of muscle mass and function to improve quality of life. The purpose of this review was to summarize recent research on the role of exercise and nutrition in human muscle protein metabolism. Such research elucidating the cellular mechanisms regulating muscle mass seeks the development of evidence-based interventions to prevent muscle wasting in aging and other clinical conditions.
Resistance exercise stimulates an increase in the rate of skeletal muscle protein synthesis (MPS) (17,24,82). The increase in MPS occurs within the first hour after exercise (24) and can persist for 24 to ~48 h (82). Concomitant with the increase in protein synthesis, resistance exercise performed in the fasted state also elicits an increase in muscle protein breakdown (MPB) (67,82). However, changes in MPS seem to be much more responsive to an exercise stimulus (82). Consequently, skeletal muscle protein turnover is increased, and net protein balance (difference between protein synthesis and protein breakdown) becomes less negative after an acute bout of resistance exercise and accumulation of these acute changes in protein metabolism are believed to provide the foundation for increased muscle mass and strength after resistance exercise training.

The molecular mechanisms that lead to acute increases in MPS after resistance exercise have been linked to enhanced messenger RNA (mRNA) translation. Studies in rodent and cell models (6,8,89) have identified the mammalian target of rapamycin complex (mTORC) 1 pathway as a critical regulator of mRNA translation and MPS. This pathway is described in Figure 1, showing a simplified diagram of the key signal transduction steps leading to mTORC1 activation and, subsequently, enhanced mRNA translation. Other reviews are available for a more comprehensive description of the regulation of mRNA translation (59,85).

Table 1 provides a review of the literature examining the postexercise mTORC1 signaling responses in fasted, untrained humans in response to an acute bout of resistance exercise. The variability in responses is likely due to different exercise protocols, time of measurement, and intrasubject variability. However, the one consistent theme is that an acute resistance exercise–induced increase in MPS is associated with enhanced mTORC1 signaling (13,20,24,25,34, ...
<table>
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<tr>
<th>Reference</th>
<th>Sets x Reps; Mode</th>
<th>Intensity</th>
<th>Time Course</th>
<th>AMPK (Thr172)</th>
<th>TSC2 (Thr1462)</th>
<th>Akt (Ser473)</th>
<th>Akt (Thr308)</th>
<th>mTOR (Ser2448)</th>
<th>S6K1 (Thr421/Ser424)</th>
<th>4EBP1 (Thr37/46)</th>
<th>eEF2 (Ser539)</th>
<th>rpS6 (Ser240/244)</th>
<th>rpS6 (Ser235/236)</th>
<th>ef2B (Ser57)</th>
<th>GSK-3β (Ser9)</th>
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<td>Apro and Blomstrand (4)</td>
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<td>Apro and Blomstrand (4)</td>
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<td>Dreyer et al. (23)</td>
<td>11 x 10; KE</td>
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<td>Dreyer et al. (24)</td>
<td>11 x 10; KE</td>
<td>70% 1RM</td>
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<td>Eliasson et al. (34)</td>
<td>4 x 6; Con LP</td>
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<td>Fry et al. (36)</td>
<td>8 x 10; KE</td>
<td>70% 1RM</td>
<td>3, 6, and 24 h</td>
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<td>Fujita et al. (40)</td>
<td>11 x 10; KE</td>
<td>70% 1RM</td>
<td>0, 1, and 2 h</td>
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<td>Glover et al. (42)</td>
<td>4 x 10; LP</td>
<td>10RM</td>
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<td>Holm et al. (50)</td>
<td>10 x 36; KE or 10 x 8; KE</td>
<td>16% or 70% 1RM</td>
<td>30 min and 3 and 5.5 h</td>
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<td>Hulmi et al. (52)</td>
<td>5 x 10; LP</td>
<td>10RM (~75% 1RM)</td>
<td>1 and 48 h</td>
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<td>Karlsson et al. (54)</td>
<td>8 x 10; LP</td>
<td>80% 1RM</td>
<td>0, 1, and 2 h</td>
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<td>Koopman et al. (64)</td>
<td>3 x 9 (60%), or 3 x 8 (75%); LE</td>
<td>75% 1RM</td>
<td>0 and 30 min and 2 h</td>
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<td>Kumar et al. (66)</td>
<td>6 x 3 (90%); LE</td>
<td>60%–90% 1RM</td>
<td>10 min and 1, 2, and 4 h</td>
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<td>Mayhew et al. (73)</td>
<td>3 sets each</td>
<td>8–12RM</td>
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<td>Reitelseder et al. (88)</td>
<td>10 x 8; LE</td>
<td>80% 1RM</td>
<td>1, 3.5, and 6 h</td>
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<td>Terzis et al. (100)</td>
<td>6 x 3, 6 x 5; LP</td>
<td>6RM</td>
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Signaling molecules were recorded above if included within two or more studies. Arrows denote direction of phosphorylation. 
↑, significantly increased; ↓, significantly decreased; --, no change; RM, repetition maximum; LP, leg press; KE, knee extension; S, squat; LE, leg extension; Ecc, eccentric; Con, concentric.
40,42,50,52,54,64,66,73,88,100). Similarly, in trained individuals, a single bout of resistance exercise increases the protein anabolic response but not to the same magnitude as in untrained individuals (18,58,83,98). To determine whether mTORC1 signaling was required for the contraction-induced increase in MPS, we performed a study using rapamycin (a specific mTOR inhibitor). We found that rapamycin administration (using a dose much smaller than typically used in rodents) prevented the increase in MPS (28) while partially blocking mTORC1 and its downstream effectors, S6 kinase 1 (S6K1), ribosomal protein S6 (rpS6), and eukaryotic elongation factor 2 (eEF2) during early postexercise recovery in young men. Although a positive correlation between S6K1 phosphorylation and resistance exercise–induced muscle hypertrophy in humans has been demonstrated (99), it remains to be determined whether mTORC1 signaling and enhanced mRNA translation are directly responsible for changes in muscle growth after resistance exercise training.

Despite the link between mTORC1 signaling and MPS, it is still unclear how muscle contraction stimulates mTORC1 signaling. Recent attention has been drawn to a phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt)–independent mechanism involving mechanical activation of phospholipase D1 and the production of phosphatidic acid, which can directly activate mTOR (78). In addition, the early activation of mTORC1 in skeletal muscle in response to mechanical overload is also independent of PI3K/Akt signaling (75). Furthermore, the importance of amino acid availability through the activation of amino acid transporters (i.e., LAT1/SLC7A5, SNAT2/SLC38A2, PAT1/SLC36A1) (53) and upstream nutrient sensors such as class III PI3K, human vacuolar protein sorting (hVps)-34 (69), and perhaps the Rag proteins (90) may also play a synergistic role in maximal activation of mTOR signaling after resistance exercise. These mechanisms have yet to be extensively examined in human models of resistance exercise; however, we have recently found that human skeletal muscle amino acid transporter expression is upregulated after an acute bout of high-intensity resistance exercise (29).

**BLOOD FLOW RESTRICTION EXERCISE AND THE REGULATION OF MPS**

As previously mentioned, high-intensity resistance exercise (typically >70% one-repetition maximum (1RM)) is a potent stimulus of MPS and hypertrophy (17,65,82,108). However, recent studies have shown that a low-intensity (20%–50% 1RM) resistance exercise, in combination with blood flow restriction (BFR) to the working muscles, produce similar increases in muscle size and strength as a traditional, high-intensity resistance exercise (1,92,97). To determine the effects of BFR exercise on the anabolic response of muscle, we performed an acute study in young adults and observed a 46% increase in MPS, similar to what is observed with traditional, high-intensity resistance exercise (38). The increase in MPS was also associated with the activation of the mTORC1 signaling pathway (37,38). Although several hypotheses have been proposed regarding the mechanism(s) of muscle protein accretion due to BFR exercise, the current literature encompasses primarily descriptive studies. BFR exercise increases limb blood flow, strength, and MVC after 4 wk of BFR training (80), and it has been reported that motor unit activities during the second, third, and fourth sets of BFR exercise were greater than that in nonoccluded exercise (70). In addition, the latter study showed expression of the proteolytic genes, FOXO3A, atrogin, and MuRF-1 to be downregulated 8 h after BFR exercise. In contrast, we reported no differences at 3 h after exercise in growth related or proteolytic genes between BFR exercise and nonrestricted blow flow exercise (30). A potential mechanism for the muscle growth-promoting effects of BFR exercise is that, during exercise, venous return is occluded, resulting in the build up of metabolic end products. Perhaps this altered metabolic milieu plays an important role in motor unit recruitment and subsequent activation of mTORC1 signaling. At this point, the cellular mechanisms responsible for the BFR exercise-induced increase in muscle growth are unclear; however, it is apparent that both high-intensity resistance exercise and BFR exercise stimulate mTORC1 signaling to a similar extent (38).

**AEROBIC EXERCISE AND THE REGULATION OF MPS**

The effect of aerobic exercise on human skeletal muscle protein metabolism has received significant attention in recent years. Acute aerobic exercise has been shown to stimulate MPS in both the fasted (16,48,91) and fed (46,48,51) states, while chronic aerobic exercise seems to elicit an increase in MPS rate at rest (84,93). Examination of various muscle protein subfractions in the fed state suggests that acute aerobic exercise may primarily stimulate mitochondrial protein synthesis while having a minimal influence on myofibrillar protein synthesis (106). However, fed-state myofibrillar protein synthesis has been reported to increase after an acute bout of prolonged one-legged kicking exercise (74), indicating the necessity to more clearly define the response of various protein subfractions to aerobic exercise. Interestingly, aerobic exercise training has recently been reported to elicit a considerable increase in muscle size and strength in older women (47), suggesting that aerobic exercise training can produce a chronic net-positive muscle protein balance and may provide a novel countermeasure to sarcopenia. The ability for aerobic exercise to increase muscle mass in the elderly may be due in part to its ability to sensitize the muscle to the anabolic effects of insulin (41). Despite limited data describing the cellular mechanisms contributing to the increase in MPS, the mTORC1 pathway does seem to have a role in the regulation of muscle protein metabolism after aerobic exercise, as mTORC1 phosphorylation (Ser2448) has been shown to be upregulated in...
response to acute aerobic exercise (7,14,71). However, more research is needed to characterize the cellular mechanisms responsible for the regulation of muscle protein turnover after aerobic exercise in humans, especially in the context of the ability of aerobic exercise to preserve or restore muscle mass and/or function in conditions of muscle wasting.

AGING AND RESISTANCE EXERCISE

The loss of muscle mass associated with aging cannot be explained by detectable age-related differences in postabsorptive skeletal muscle protein metabolism, as healthy, young and older adults seem to have similar resting rates of MPS and MPB (79,86,103). Rather, the muscle loss observed with aging may be, in part, related to the observations that older individuals do not seem to have the same magnitude of anabolic response as younger individuals to an anabolic stimulus. For instance, an acute bout of resistance exercise, which is a very robust anabolic stimulus, has been shown to increase MPS to a greater magnitude in young than in older subjects (27,105). Although some researchers have reported no age-related difference in the anabolic response to resistance exercise (49,108), nonetheless, older men demonstrate a smaller anabolic response to a range of resistance exercise intensities compared with young men (66). In our recent study of young and older adults, we found that aging is associated with an impaired ability to activate mTORC1 signaling and MPS during a 24-h postexercise time course (36). Similar to acute studies, Kosek et al. (65) discovered that 4 months of resistance exercise training (3 d·wk⁻¹) resulted in significantly greater skeletal muscle hypertrophy in young compared with older men and women. Collectively, these data show that both young and older adults can benefit from resistance exercise training. However, aging may result in decreased anabolic responsiveness to resistance exercise and thus potentially contribute to age-related muscle loss.

Recently, we examined whether a novel rehabilitation tool (BFR exercise) would be effective in restoring the contraction-induced increase in MPS in older adults. We found that MPS increased 56% after an acute bout of BFR exercise (37), indicating that this novel treatment was capable of overcoming the impaired MPS response seen with aging in response to traditional resistance exercise. In addition, mTORC1 signaling, as indicated by S6K1 phosphorylation, increased after BFR exercise compared with nonrestricted blood flow exercise. Keeping in mind that BFR exercise increases muscle fiber recruitment (70), it is conceivable that increased muscle fiber recruitment would coincide with greater mTORC1 activation and, subsequently, elevated MPS. These data suggest that BFR exercise could be a potential countermeasure in the treatment in sarcopenia. Furthermore, BFR applications could be extended to other clinical populations who are unable to withstand high-resistance exercise such as conditions of arthritis, osteoporosis, ligament injuries, or post-operation rehabilitation.

ESSENTIAL AMINO ACIDS AND THE REGULATION OF MPS

Amino acids have been shown to stimulate a muscle protein anabolic response (39,79,101). Ingestion of essential and nonessential amino acids significantly increases plasma amino acid concentrations for up to 3 h after ingestion; however, the availability of essential amino acids (EAAs) is the primary stimulator of MPS (101). Regardless of the time course of elevated plasma amino acids, the stimulation of MPS is short-lived, lasting 1–2 h after EAA ingestion (9,44). Of the EAAs, leucine has received considerable attention because of its ability to independently stimulate MPS (2,3,94). In some human studies, ingestion of a high-quality protein or amino acid solution with extra leucine does not further increase MPS rates (44,56,62). However, the added leucine may promote a greater overall anabolic response through a decrease in MPB (44) potentially attenuating muscle loss.

Amino acid availability stimulates MPS partly through activation of mTORC1 signaling (3,5). Our understanding of the exact mechanism(s) of amino acid–induced stimulation of mTORC1 is limited; however, recent findings in animal and cell models have indicated some potential upstream nutrient sensors. Amino acid availability can likely stimulate mTORC1 by activation of hVps34 in a calcium-dependent manner (45,77). 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AGING AND EAAS

In healthy, young and older adults, the ingestion of both intact protein and EAA has been shown to increase MPS and amino acid net balance (79,96,102). Despite similar (63,81) or increased (102) splanchnic extraction of amino acids in older subjects relative to younger subjects, there does not seem to be a significant age-related difference in the muscle protein anabolic response to amino acids, provided that the composition and/or dose are adequate (55,56). For example, MPS is stimulated in older adults after the ingestion of a leucine-enriched supplement (6.7 g of EAAs, 41% Leu), but when EAA (6.7 g of EAAs, 26% Leu) was ingested, no change in MPS was observed (56), whereas young adults experienced a significant increase in MPS with both supplements. Older and younger adults experience a dose-dependent response to EAAs below 10 g, such that MPS plateaus on increasing levels of amino acid ingestion in young and older subjects alike (19). By contrast, after the ingestion of a small bolus of amino acids (7 g of EAAs), older subjects had a smaller muscle protein anabolic response than young subjects (55). A plateau also seems to exist for the anabolic effect of intact protein, as the consumption of a higher protein meal (340 g of lean beef) did not further stimulate MPS, whereas ingestion of a moderately sized protein meal (113 g of lean beef) stimulated MPS equally in young and older adults (95,96). This suggests that the regular consumption of meals containing moderate amounts of protein would support the maintenance of lean tissue better than ingestion of a single high-protein meal. Overall, recent evidence seems to imply that older adults retain the ability to respond to amino acid and protein ingestion, assuming moderate consumption of high-quality protein. Given that older adults are at increased risk for protein malnutrition (15), this may play a more pivotal role in the development of sarcopenia than any age-related differences in amino acid sensitivity.

COMBINING RESISTANCE EXERCISE WITH EAA INGESTION AND THE REGULATION OF MPS

As mentioned previously, resistance exercise and amino acid ingestion independently stimulate MPS; however, net muscle protein balance remains negative when resistance exercise is performed in the fasted state. It has been demonstrated that EAA ingestion after resistance exercise results in greater increases in MPS rates than when EAA are ingested at rest or when resistance exercise is performed in the fasted state (26,107). On the basis of these data, supplying EAA after resistance exercise creates a larger positive protein balance by increasing the difference between the rates of MPS and MPB. For instance, ingestion of 6 g of EAA 1 h after resistance exercise dramatically increased MPS, with minor increases in MPB up to 3 h after ingestion, leading to an overall large positive net protein balance (10). Several other studies have demonstrated similar effects of EAA ingestion on MPS during postresistance exercise recovery (23,27,43,87).

Recent studies (4,23,40,43) have investigated the mechanisms behind the increase in MPS when EAAs are ingested after resistance exercise. The maximal MPS response with EAA given after resistance exercise is attributed to increases in intracellular availability of amino acids, particularly leucine, and subsequently, the activation of mTORC1 signaling (23). For example, ingestion of leucine-enriched EAA and carbohydrate (4,23) and EAA only (27) after a bout of resistance exercise increased phosphorylation of Akt, mTOR, S6K1, and 4EBP1 and decreased phosphorylation of eEF2, reflecting improved translation initiation and elongation, respectively. However, studies examining changes in gene expression after resistance exercise and EAA ingestion found no differences in the mRNA abundance of translationally controlled tumor protein, mTORC1, and S6K1 or the nutrient sensors, hVps34 and MAP4K3 (33). However, we have found that EAA provided after resistance exercise increased the mRNA expression of Rheb and cMyc and decreased the mRNA expression of REDD2, which may also contribute to the regulation of mTORC1 activity (33). It is apparent that ingestion of EAA after a bout of resistance exercise can enhance the muscle protein anabolic response compared with resistance exercise alone or EAA ingestion at rest.

AGING AND EAAS COMBINED WITH RESISTANCE EXERCISE

Muscle loss that accompanies aging has been reported extensively, but the associated physiological mechanisms are still not entirely clear. As previously mentioned, resistance exercise increases MPS in older adults, but to a lesser degree than in young individuals. Nonetheless, ingestion of EAA or protein after a bout of resistance exercise has demonstrated additive effects on MPS. Koopman et al. (60,61) examined the potential age-related differences in the response to combined resistance exercise and carbohydrate + protein + leucine ingestion and reported that MPS and whole-body protein balance were increased in both older and younger subjects with no age-related differences. Similarly, we have shown that EAA ingestion (20 g) given 1 h after resistance exercise resulted in a similar overall increase in MPS in both young and older adults (27). More recently, Pennings et al. (81) demonstrated that ingesting 20 g of intact protein after a bout of resistance exercise resulted in a similar MPS response between younger and older men. Taken together, these studies seem to indicate that young and older subjects demonstrate a similar protein anabolic response to the combined influence of resistance exercise and EAA/protein. More research is needed to determine whether repeated bouts of resistance exercise and EAA ingestion will be an effective countermeasure for sarcopenia.
CONCLUSIONS

In summary, both resistance and aerobic exercise increase human skeletal muscle MPS. In addition, when resistance exercise is performed at lower intensities and blood flow is occluded, a muscle protein anabolic response is achieved similar to that of typical high-intensity resistance exercise. After ingestion of EAA, MPS and mTORC1 signaling is enhanced; however, the muscle protein anabolic response is increased to a greater extent when EAAs are ingested after resistance exercise. Current research suggests that the anabolic signaling and changes in the expression of growth-related genes in response to EAA and/or resistance exercise is mediated through mTORC1 signaling in human skeletal muscle. Moreover, the increase in MPS is blunted in older adults in response to an acute bout of resistance exercise. The age-related differences in the protein anabolic response to ingestion of EAA are unclear, but some data indicate that older individuals may have a blunted response to lower doses of EAA compared with young individuals. However, when ingestion of EAA is combined with resistance exercise, the age-related differences in MPS and anabolic signaling are less apparent. We conclude that, in humans, resistance exercise with EAA ingestion maximally stimulates MPS, primarily via regulation by mTORC1 signaling. Therefore, we propose that BFR exercise or exercise combined with EAA not only should be effective in improving muscle repair and growth in response to training in athletes but may also be a useful countermeasure to sarcopenia and other clinical conditions associated with muscle wasting.

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