

HIGHLIGHTED TOPIC | *Regulation of Protein Metabolism in Exercise and Recovery***Exercise training and protein metabolism: influences of contraction, protein intake, and sex-based differences****Nicholas A. Burd, Jason E. Tang, Daniel R. Moore, and Stuart M. Phillips***Exercise Metabolism Research Group, McMaster University, Hamilton, Ontario Canada*

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Burd NA, Tang JE, Moore DR, Phillips SM. Exercise training and protein metabolism: influences of contraction, protein intake, and sex-based differences. *J Appl Physiol* 106: 1692–1701, 2009. First published November 26, 2008; doi:10.1152/jappphysiol.91351.2008.—Muscle contraction during exercise, whether resistive or endurance in nature, has profound effects on muscle protein turnover that can persist for up to 72 h. It is well established that feeding during the postexercise period is required to bring about a positive net protein balance (muscle protein synthesis – muscle protein breakdown). There is mounting evidence that the timing of ingestion and the protein source during recovery independently regulate the protein synthetic response and influence the extent of muscle hypertrophy. Minor differences in muscle protein turnover appear to exist in young men and women; however, with aging there may be more substantial sex-based differences in response to both feeding and resistance exercise. The recognition of anabolic signaling pathways and molecules are also enhancing our understanding of the regulation of protein turnover following exercise perturbations. In this review we summarize the current understanding of muscle protein turnover in response to exercise and feeding and highlight potential sex-based dimorphisms. Furthermore, we examine the underlying anabolic signaling pathways and molecules that regulate these processes.

resistance exercise; endurance

DURING A WEEK, IN WHICH A person does not exercise and consumes adequate daily meals, skeletal muscle protein mass remains essentially unchanged. This effect is achieved by normal counterbalanced fluctuations in muscle protein synthesis (MPS) and muscle protein breakdown (MPB) that contribute algebraically to defining the overall net protein balance (NPB = MPS minus MPB) (81, 84). Feeding and exercise are potent stimuli for MPS, which is the variable in healthy individuals that contributes the most to NPB equation (91). It should be noted, however, that the feeding-induced stimulation of MPS is transient and cannot account for muscle protein accretion alone. Similarly, exercise, aerobic and more so resistive, improve NPB; however, the consumption of protein during postexercise recovery is necessary to shift the balance in favor of muscle protein accumulation (94). Thus feeding and exercise must be utilized concurrently to manifest a positive NPB, which will ultimately summate into skeletal muscle hypertrophy when performed habitually (Fig. 1).

The cellular mechanisms that regulate muscle protein turnover, which include gene transcription, cell signaling to initiate protein synthesis, and enzymes involved in several proteolytic pathways, are beginning to be elucidated (7, 27, 63, 119, 123).

These mechanisms appear to be responsive to both exercise and feeding, which function through separate but convergent pathways (25, 26, 35). A general concept becoming increasingly evident is that exercise is capable of turning on anabolic signaling cascades and that feeding potentiates this effect (25, 28, 38, 52).

The focus of this brief review will be on our current understanding of muscle protein metabolism following different muscle contraction modalities, including both resistive type and endurance type, and the influential effects of postexercise feeding on MPS response as it relates to healthy adult humans. Furthermore, the influence of exercise and feeding on anabolic signaling during postexercise recovery will also be examined.

RESISTANCE EXERCISE AND GENERAL CHANGES IN MUSCLE PROTEIN SYNTHESIS

The anabolic potency of resistance exercise is well established, having been reported to stimulate MPS between ~40 and 150% above rested levels following a single acute bout (3, 19, 86, 87). Despite a marked stimulation of MPS in the postexercise period, muscle NPB remains negative in the fasted state due to a concomitant elevation of MPB (Fig. 1). Although the change in MPB after resistance exercise in the fasted state is smaller in magnitude than the change in MPS, this obligatory increase in MPB provides amino acids necessary to support the exercise-induced increase in MPS. The mechanism by which MPB is elevated after exercise in the

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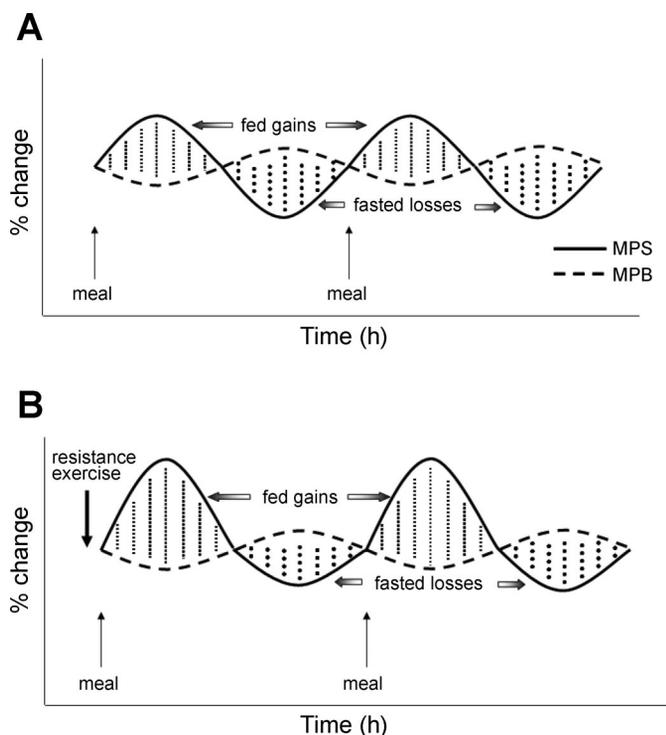


Fig. 1. *A*: changes in muscle protein synthesis (MPS) and muscle protein breakdown (MPB) in response to feeding (i.e., amino acids). *B*: changes in MPS and MPB in response to resistance exercise and feeding. Chronic application of these anabolic stimuli, as in *B*, results in muscle hypertrophy.

fasted state is currently unknown but likely involves one or all of the major proteolytic pathways (calpains, caspases, lysosomes, and ubiquitin-proteasomal) present in muscle (63). We speculate that while fasted, the elevated MPB with resistance exercise is likely confined to the more rapidly turning over sarcoplasmic pool, which may provide a source of amino acids to support the synthesis of myofibrillar proteins. This supposition is based on the observation the sarcoplasmic protein fraction turns over at an approximately twofold greater rate than myofibrillar proteins in the fasted state, demonstrating its labile nature (22, 74).

The rise in MPB is attenuated and net balance consequently becomes positive when amino acid availability is increased by the provision of an exogenous source of essential amino acids (EAA), either intravenously (4) or orally (111) after exercise (Fig. 1). The magnitude and duration of changes in MPS with resistance exercise point to a change in this variable in determining the extent of muscle protein accretion after exercise. More importantly, the acute resistance exercise-induced increase in MPS, even in the absence of feeding, is sustained for up to 48 h (86), suggesting that feeding at any time during this “window of anabolic opportunity” should stimulate a greater muscle protein synthetic response compared with feeding at rest (Fig. 2). In support of this, data from a recent study within our laboratory demonstrated that the acute increase in muscle protein synthesis after ingestion of a suboptimal dose of whey protein isolate [i.e., 15 g of protein or the equivalent of ~6 g (EAA) (22)] was greater 24 h after a bout of resistance exercise compared with at rest suggesting exercise enhances the nutrient sensitivity of the muscle for up to 24 h (Burd NA, Staples AW, Dan WD, West AD, Moore DR, Holwerda AM, Baker SK and

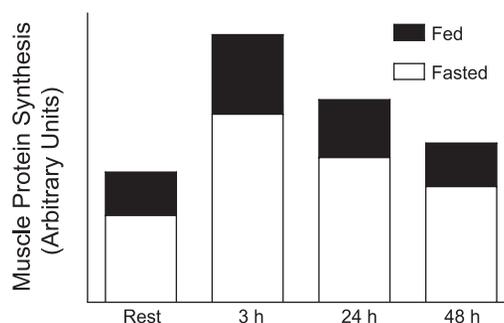


Fig. 2. Resistance training induces a sustained increase in MPS for up to 48 h, which likely sensitizes the muscle to feeding. As a result, feeding during this time should result in a greater MPS response compared with feeding at rest.

Phillips SM, unpublished data); indicating the “window of anabolic opportunity” may be extended up to 24 h after resistance exercise; however, it appears that feeding early may offer some additional advantages because this is when MPS is stimulated to the greatest extent (43, 86, 92). When discussing the effect of feeding there are many considerations, including the protein source (i.e., animal- vs. plant-based proteins), quantity, the timing of postexercise ingestion, and the presence of carbohydrate to stimulate insulin release. In the absence of feeding, MPS may also be influenced by training status (Fig. 3) and/or training overload (intensity, volume, frequency) (20, 68, 75, 107). A valid question is what is the value of the short-term changes we observe within hours in predicting a long-term chronic change leading to a differing phenotype? Some evidence exists to show that in carefully controlled studies short-term changes in MPS and MPB (124) are qualitatively predictive of long-term changes (43). Thus acute changes in protein turnover during postexercise recovery can be, at least qualitatively, predictive of chronic adaptations to different training or feeding interventions.

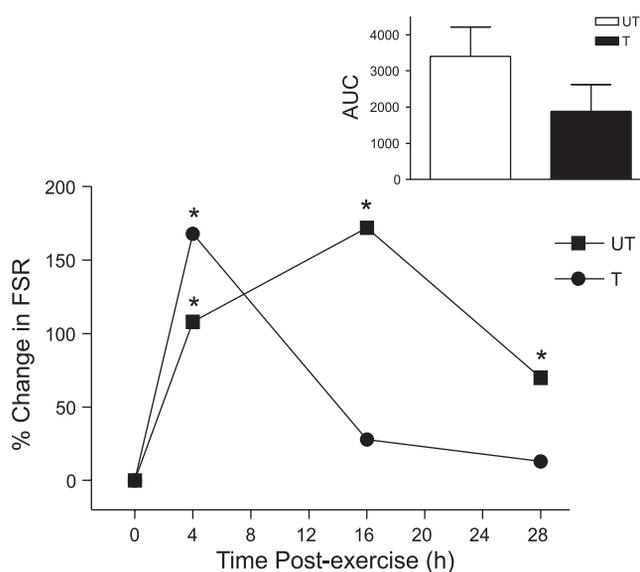


Fig. 3. Time course of the elevation in MPS after a single bout of resistance exercise. *Inset*, area under the curve (AUC) for % change in fractional synthetic rate (FSR). T, trained; UT, untrained. The 16-h time point is taken from Ref. 48. *Significantly different from rest, $P < 0.01$. [From Tang et al. (107).]

Training status. Training status can also affect the postexercise stimulation of MPS. For example, training alters the extent and duration of the postexercise stimulation of protein synthesis to feeding as evidenced by a greater early (i.e., 1–4 h) postexercise stimulation of mixed muscle protein synthesis that returns to basal more quickly (i.e., with 28 h) in the trained state (Fig. 3) (107). These data indicate that postexercise feeding is critically important in the trained state as it may improve the timing of amino acid delivery to the muscle at a time when cell signaling involved in the initiation of protein synthesis is most sensitive (35, 51); this notion is partially supported by data demonstrating an attenuation in the training-induced increase in lean mass when protein feeding is delayed as little as 2 h after exercise in young men (43).

Recent data suggest that the MPS response to resistance exercise becomes more “refined” with training such that available substrates are preferentially directed toward the synthesis of different protein fractions depending on the exercise stimulus (48, 123). For example, our laboratory recently demonstrated that in untrained individuals the performance of an acute bout of resistance exercise, and to a lesser extent aerobic exercise, stimulates global protein synthesis (48, 123). These data suggest that a novel stimulus disrupts homeostasis such that the signal to the protein synthetic machinery is unrefined and turns on all muscle proteins (myofibrillar and mitochondrial). Following training, however, the acute synthetic response becomes more finely tuned to the specific mode of exercise in that resistance exercise preferentially stimulates the synthesis of myofibrillar proteins whereas aerobic exercise stimulates the synthesis of the mitochondrial fraction (48, 123). How this is accomplished is uncertain since changes in phosphorylation of many commonly studied signaling proteins did not shed insight into mechanistic underpinning of the response.

Muscle contraction intensity. A relatively understudied variable is the dose-response relationship between muscle contraction intensity and protein turnover. To date, the majority of the studies have examined changes in the protein synthesis response following an exercise intensity of 70–80% of maximal effort or ~8–12 repetitions (3, 19, 26, 48, 67, 86, 87, 121), because high intensities (>70% maximal effort) are reportedly required to induce any significant gains in muscle mass (58). Incompletely understood, however, is the effect of low intensity contractions on MPS. It has been demonstrated that exercise intensities at 20% maximal effort are ineffective at stimulating a measurable protein synthetic response (34, 60). Interestingly, the occlusion of the limb at this same contraction intensity stimulates mixed MPS (34), which is consistent with the ability of this form of resistive exercise to induce increases in muscle strength and hypertrophy (105, 106). What then is the mechanism of such an effect? Certainly an appealing paradigm is one relating to motor unit recruitment and their associated muscle fibers (i.e., the size principle). For example, the greater metabolic stress (secondary to reduced blood flow) and the associated fatigue have been shown to enhance muscle activation of low-force contractions suggesting a greater recruitment of normally type II muscle fibers, which would not normally be recruited at such a low exercise intensity (77). This finding may be important for the postexercise stimulation of MPS because type II fibers have been shown to hypertrophy with training to a greater extent than type I fibers (56, 115). Therefore, it seems plausible that performing both high (>70%

of maximal effort) and lower intensity (30–50% maximal effort) exercises to failure may produce similar elevations in MPS, which may translate into comparable increases in muscle hypertrophy and lean mass. In fact, recent data have demonstrated that resistance training at an intensity of only ~15% of maximal effort is capable of inducing small gains in thigh cross-sectional area ($3 \pm 1\%$), although inferior to the gains experienced for the exercise leg trained at 70% maximal effort ($8 \pm 1\%$) (44). These data suggest that low intensity exercise could stimulate a small rise in MPS postexercise that is capable of inducing hypertrophy, premised on the idea that the acute MPS response is predictive, at least qualitatively, of muscle protein accrual over time (43, 124). It should be noted, however, that to satisfy that the argument the of amount of work (contractions \times load, assuming the same range of motion per contraction) performed may be important in determining the degree of muscle hypertrophy (39), these researchers equated total work between the low- and high-intensity legs and consequently lost the benefit of recruiting fast twitch fiber as occurs with training to fatigue. Recent data support this thesis that training until fatigue may be more important than the intensity of the contraction performed on maximizing the MPS response (60).

Amino acid type and amount. Several studies have demonstrated that of the physiological amino acids, only the EAA appear necessary for the stimulation of MPS (13, 102, 111, 113). Moreover, EAA appear to stimulate MPS in a dose-dependent manner at rest and possibly after exercise (13, 22, 73). To fully elucidate the dose response of MPS to ingested dietary protein, our laboratory has recently examined the response of MPS to increasing amounts of protein (0, 5, 10, 20, and 40 g) after resistance exercise. Data from this study indicated that 20 g of intact dietary protein (the equivalent of ~8.6 g of EAA) maximally stimulated MPS after resistance exercise, with only a slight increase in amino acid oxidation above rest (76). Thus we proposed that the dose of dietary amino acids necessary to maximize protein anabolism after resistance exercise is similar to what is required at rest (22) or ~8–10 g of EAA.

Amino acid source. A series of studies have identified protein digestibility as an independent variable that modulates protein metabolism (8, 14, 23, 24, 33). For example, proteins that are digested quickly, such as whey (8, 23, 24) and soy (14), result in a large but relatively transient hyperaminoacidemia, and that stimulates an increase in whole body protein synthesis. Conversely, slowly digested proteins such as casein (8, 23, 24) or milk (4:1 casein-whey protein by content) (33) result in a modest hyperaminoacidemia and exert their effect on protein metabolism by primarily inhibiting whole body protein breakdown.

With respect to muscle protein metabolism, our laboratory has shown that milk protein promotes greater muscle protein accretion than soy protein after resistance exercise (43, 123). Moreover, our laboratory has recently measured MPS in young men after consuming specific (whey or casein) or a vegetable-based protein (soy) after resistance exercise (Tang JE, Moore DR, Kujbida GW, and Phillips SM, unpublished observations). The results indicated that whey stimulates MPS to a greater degree than either casein or soy protein both at rest and after resistance exercise. It is not clear at present why this would be the case, but we speculate that this may be due to whey's higher leucine content and rapid digestion rate. which provide

a greater stimulus for MPS. Tipton and colleagues (110) have also reported that whey and casein protein equally improve muscle protein net balance after resistance exercise, which taken together with our laboratory's recent findings on specific milk protein suggest that casein may improve muscle net balance after resistance exercise by primarily inhibiting muscle protein breakdown to a greater degree than fast protein such as whey. Collectively, these studies illustrate how differences in amino acid availability following protein consumption affect not only whole body protein metabolism but also MPS at rest and after exercise.

Timing of amino acid ingestion. The timing of amino acid provision with respect to an exercise bout may be an important consideration. Tipton and colleagues (109) reported no difference in MPS with whey protein ingestion either before or after exercise. Moreover, Rasmussen et al. (91) noted no differences in postexercise MPS with protein consumption either 1 or 3 h after the exercise bout. Although these studies indicate that the timing of amino acid provision does not appear to affect MPS in the acute scenario, some evidence to the contrary exists with respect to chronic training. For example, delaying protein ingestion after exercise by up to 2 h in young men results in a reduction in the training-induced increase in muscle fiber hypertrophy and lean mass compared with immediate protein consumption (43). Additionally, a study by Esmarck and colleagues (30) showed that delaying provision of a drink containing protein, carbohydrate, and fat by 2 h postexercise resulted in significantly lower strength gains in elderly individuals. However, these findings are not universal; there was no difference in the increase in strength, muscle thickness (a proxy marker of muscle mass), or lean tissue mass gains when protein timing was manipulated in older persons (17). Collectively, these findings suggest that an increase in amino acid availability in close temporal proximity to the exercise stimulus appears beneficial and may even be necessary, especially in the elderly, to support resistance training-induced muscle adaptations. Although not established following exercise, it has been suggested at rest that upon consumption of a suboptimal amount of EAAs (6.7 g) that the elderly may benefit from an increased proportional of the branched-chain amino acid leucine to elicit a similar MPS response as the young (47). However, if adequate protein is consumed, the additional leucine does not appear to be necessary (53, 55, 79, 104).

Insulin in the regulation of protein metabolism. Insulin is a known regulator of muscle protein metabolism; however, the manner by which insulin promotes anabolism in human skeletal muscle is unresolved. Available data show that insulin activates several proteins [e.g., phosphatidylinositol-3-kinase (PI3K)] that cause downstream phosphorylation of factors known to play key roles in regulating protein and glycogen synthesis (50). Moreover, insulin has been shown to attenuate ubiquitin proteolysis (95), which is believed to be responsible for the degradation of the bulk of muscle proteins (116), and can also, via calpain or caspase activation, degrade myofibrils, which constitute the majority of proteins in skeletal muscle (91).

Few studies have directly examined the effect of insulin on protein metabolism with resistance exercise. While fasted, there appears to be little additional effect of insulin on MPS after resistance exercise (5, 41). This may be due to a reduced availability of intracellular amino acids because insulin atten-

uates the resistance exercise-induced increase in MPB (5). Studies that have provided carbohydrates after resistance exercise have also reported an apparent attenuation of MPB, with no effect on MPS (11, 54, 73). For example, Borsheim et al. (12) found that provision of a drink containing 100 g of carbohydrates 1 h after a bout of resistance exercise improved muscle protein balance such that it was not different from zero, but balance did not become positive when measured for up to 3 h after consuming the drink. Thus, without an increase in amino acid availability, the antiproteolytic effect of insulin alone is insufficient to achieve protein accretion (i.e., positive protein balance) after resistance exercise.

Some have suggested the loss of muscle mass commonly associated with the aging process is a relative resistance to insulin-stimulated amino acid uptake and stimulation of MPS (118). Given the recent data of Greenhaff et al. (41) showing the dissociation between insulin and its effects, or lack thereof, on protein synthesis, we propose that any age-related decline in insulin action and its mediation of amino acid-stimulation of MPS is not likely to be a direct mechanism of insulin on protein kinetics (41, 82). Instead, we suggest that insulin would mediate changes in microvascular flow (117) that are likely impaired in older persons (99). The effect therefore would be a relatively poorer delivery of amino acids to an aged muscle and thus an impaired protein synthetic response.

AEROBIC EXERCISE AND GENERAL CHANGES IN MUSCLE PROTEIN TURNOVER

The influence of endurance exercise on muscle protein turnover remains relatively understudied. This may be related to the general observation that such exercise does not typically result in significant gains in muscle size. However, changes in MPS following endurance exercise are relevant for tissue repair and remodeling as well as changes in synthesis of protein fractions that do not contribute to muscle hypertrophy such as the mitochondrial proteins (cf. myofibrillar proteins). Currently, differences in exercise mode and intensity confound our current understanding of how aerobic exercise affects MPS and limits our ability to make comparisons between studies (18, 66, 72, 112).

Initial studies examining the acute response of MPS to treadmill walking at 40% maximal O₂ uptake in untrained subjects established that low load exercise is capable of stimulating an increase MPS (18, 97). Tipton et al. (112), however, observed that a high-intensity swim routine was incapable of stimulating a significant response in MPS in trained female swimmers. These opposing results may be related to the muscle studied (vastus lateralis vs. deltoid), the mode of exercise, or the subjects' training status. Certainly, the latter has considerable influence on the results as it has been shown chronic aerobic training results increased rates of basal MPS (88, 98).

Using a unique model of endurance exercise, one-legged kicking exercise on a modified Krogh ergometer was shown to stimulate sarcoplasmic and myofibrillar protein synthesis for 48 and 72 h, respectively (72). Endurance exercise, however, is not commonly characterized by skeletal muscle hypertrophy, as would be expected with such a robust increase in myofibrillar protein synthesis. Therefore, the "aerobic" nature of single-legged exercise may be more appropriately labeled a low-intensity resistive-type exercise. Our laboratory has recently

examined the specific responses of individual proteins (myofibrillar and mitochondrial) residing within skeletal muscle following single leg cycling for 45 min at 75% maximal O_2 uptake in both the untrained and trained states. We observed, regardless of training status, a robust increase in mitochondrial protein synthesis. This was in contrast to no observed increase in myofibrillar protein synthesis (123). Thus mitochondrial and to some extent saroplasmic proteins are the primarily proteins contributing to increased mixed MPS after endurance exercise.

CELL SIGNALING RESPONSES TO EXERCISE AND FEEDING

The rapid increases in muscle protein synthesis following exercise and an increase in amino acid availability suggest that these changes are mediated through posttranscriptional mechanisms (19). Exercise and amino acids appear to stimulate muscle protein synthesis through separate but convergent signaling pathways, with maximal stimulation of translation initiation and protein synthesis requiring basal levels of insulin. Although a detailed examination of the control of transcription is beyond the scope of this review (6, 10, 28, 49), the key regulatory steps involved in the signaling pathways that respond to resistance exercise and feeding will be briefly discussed. Noteworthy is recent evidence highlighting the dissociation between the activation of signaling molecules and changes in MPS (41). We propose that when the stimuli are adequate, that several signaling molecules [e.g., mammalian target of rapamycin (mTOR)] respond in a near-maximal fashion to exercise and amino acids; the result is a coordinated increase in MPS. However, as insulin is permissive for MPS and not modulatory (41, 93), we propose that large increases in insulin enhance anabolic signaling molecule activation (i.e., presumably reflective in phosphorylation) but do not further increase MPS (Fig. 4).

RESISTANCE EXERCISE AND CELL SIGNALING

The exact mechanism by which the contractile signal from the cytoskeleton of the cell is translated to the protein synthetic

machinery has yet to be fully elucidated. Data in rodents (31, 32, 40) and cell culture (126) suggest that focal adhesion kinase (FAK), a load sensing protein, is a potential link in the mechanotransduction of a loading stimulus to the stimulation of MPS. Recently, it has been demonstrated that FAK phosphorylation remains unchanged 6 h following an acute bout of resistance exercise (38). It was speculated that FAK phosphorylation is transient and occurs in close immediacy to exercise or in response to chronic unloading (38). Support for this notion is that following 10 wk of both endurance and resistance training FAK phosphorylation was significantly greater than before training in humans suggesting a chronic stimulus may be required (123).

To date, the underlying subsarcolemma cellular mechanisms that initiate the protein synthetic response are gradually becoming elucidated (7, 90). Indeed, the activation of primary mediators of protein synthesis such as protein kinase B (Akt), mTOR, and mTOR's downstream effectors, 4E binding protein 1 (4E-BP1), 70-kDa S6 protein kinase ($p70^{S6K}$), and ribosomal protein S6 (rpS6) have been shown to be active in the immediate acute (1–4 h) postexercise period (21, 26, 29, 46, 56, 125). This response would be expected given that their activation would be necessary to initiate a protein synthesis response. What is lesser known, however, is the degree of activation at later time points following an exercise bout. Recent data from our laboratory demonstrated 6 h following resistance exercise in the fasted state that mTOR is no longer phosphorylated to a significant degree; however, its downstream effectors $p70^{S6K}$ remained robustly increased (38).

An additional variable understudied is the influence of chronic resistance training on cell signaling pathways. It has been established that training can influence the extent to which individual classes of muscle proteins (myofibrillar or mitochondrial) are synthesized (123). Therefore, it might be expected the activity of key initiating protein factors/kinases in controlling this response may also be altered. It was recently demonstrated that following 10 wk of resistance training that Akt, eukaryotic initiation factor 4E (eIF4E), FAK, and glycogen synthase kinase- β (GSK- β) manifested increased resting phosphorylation. Furthermore the duration of their activation was reduced compared with the untrained state (123). These data suggest that 10 wk of exercise training modifies the activation state of anabolic signaling molecules such that they are more readily responsive to initiate protein synthesis in response to training stimuli. However, the response to chronic training (8–9 yr) of a particular training discipline (resistance training or endurance) may suppress anabolic signaling (21) and is consistent with the idea that exercise training is an adaptive process and that an increase training stress is needed to achieve greater training effects (e.g., overload principle) (58).

ENDURANCE EXERCISE AND CELL SIGNALING

Endurance exercise activates proteins involved in regulation of MPS that are similar to those in resistance exercise (e.g., mTOR) (69). One of the most conspicuous adaptations to endurance exercise, however, is an increase in the aerobic capacity of skeletal muscle, which is primarily effected by changes in mitochondrial content. Thus specific protein synthetic changes in mitochondrial (rather than myofibrillar) protein are of particular interest with respect to endurance exercise. One path-

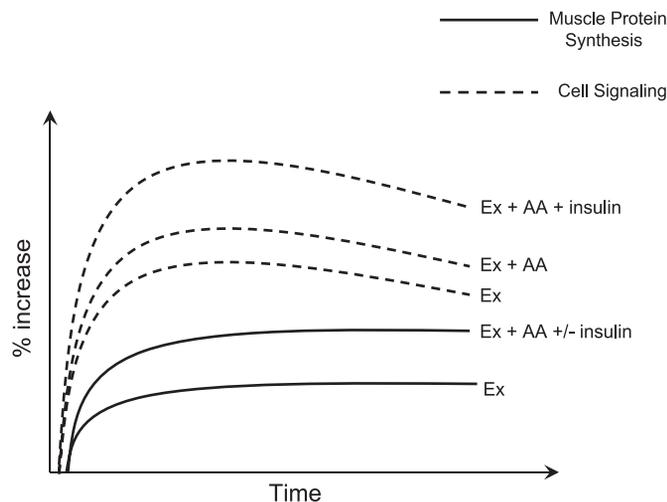


Fig. 4. A proposed model illustrating how exercise (Ex) and amino acids (AA) activate anabolic signaling proteins to initiate the MPS response. Once this response is maximized, further increases in anabolic signaling protein activation (mostly assumed to be phosphorylation) are likely mediated by insulin, but they are not associated with further increases in MPS.

way of considerable focus has been the AMP-activated protein kinase (AMPK)-peroxisome proliferator-activated receptor- γ coactivator-1 α (PGC-1 α) signaling cascade and its role in mitochondrial biogenesis. Acute endurance exercise increases the transcription and mRNA content of PGC1 α , an effect that is further potentiated with training (89).

FEEDING AND CELL SIGNALING

When examining the regulation of protein synthesis by feeding, the main effectors are the hormone insulin and amino acids. Insulin, in the absence of an increase in amino acids, does not stimulate MPS (41). However, insulin does signal through several intracellular pathways involved in translation initiation and protein synthesis and consequently is involved in the modulation of these cellular responses. Insulin binding to its receptor activates PI3K, which initiates a signaling cascade through Akt/protein kinase B (PKB). As discussed after exercise, Akt/PKB phosphorylates mTOR, whose downstream targets include p70^{S6k1} as well as 4E-BP1, ultimately leading to translation initiation and increased protein synthesis.

Amino acids also stimulate several proteins involved in translation initiation, including mTOR (22), p70^{S6k1} (22, 64), as well as 4E-BP1 (65). However, amino acids do not activate PI3K or Akt/PKB, and therefore the amino acid-induced stimulation of mTOR must occur through a different upstream input than insulin. Initially it was thought that the amino acid activation of mTOR was mediated through proteins such as the tuberous sclerotic complexes (TSC1/2), G protein-like subunit- β (G β L), regulatory associated protein of mTOR (raptor), or Ras homolog enriched in brain (Rheb) (9). However, recent evidence suggests that amino acids stimulate mTOR through a class 3 PI3K, the human vacuolar protein sorting 34 (hVps34) (16, 78).

SEX-BASED DIFFERENCES IN PROTEIN METABOLISM

Sex-based differences in protein metabolism are likely small compared with those often observed in lipid and carbohydrate metabolism (96, 108). Nonetheless, some studies have been conducted in which men and women have been compared and have shown, in general, that women rely less on protein as a substrate during aerobic exercise than their matched male counterparts (61, 62, 83). A body of data also suggests that across the menstrual cycle there are relatively small changes in protein kinetics, indicating that acute differences in estrogen and progesterone do not appear to exert a tremendous influence on the turnover of whole body or muscle proteins (70). Moreover, in the basal state men and women, at least when protein kinetic rates are normalized to lean mass, are virtually identical in their muscle protein turnover rates (36).

In response to feeding and resistance exercise, young men and women appear to respond in a qualitatively and quantitatively similar manner since studies in which men and women have been used as subjects can show little difference between the two sexes (26, 92, 114). To date and to our knowledge, however, no systematic sex-based comparison has been done in men and women to examine their responses to both feeding alone and resistance alone or in combination with feeding. If, however, we examine data looking at chronic adaptations to resistance exercise in men and women, the data suggest that whereas quantitative differences in hypertrophy and muscle

gain exist that relative changes are similar (1, 45, 57). Thus young women have the capacity to hypertrophy their muscle fibers in response to resistance training (103, 120), despite 10-fold lower testosterone concentration than men, which is consistent with the notion that local, rather than circulating systemic androgen hormone, mechanisms are dominant in promoting increases in MPS and fiber hypertrophy.

There is some evidence to suggest that the tendons in women have a potentially lesser capacity for adaptation in response to exercise (71, 122). Interestingly, this lower capacity for adaptation in tendon may be exacerbated by oral contraceptive use (42). Given that women suffer a greater number of ligament and tendon strains than men this clearly is an area for unique research into sex-based differences.

Although young men and women may show little difference in protein turnover the situation appears to be different for older men and women where basal differences in MPS have been reported to exist (101). Perhaps more importantly, older women also demonstrated an inability to increase MPS in response to a protein feeding (101). Older women also have a reduced capacity for hypertrophy in response to resistance exercise (2, 15, 57). Recent unpublished data, corroborating the idea that hypertrophic potential is impaired in older women, also indicates that older women show a smaller increase in MPS in response to resistance exercise than men (100). A reduced capacity and responsiveness in anabolic signaling might be the reason for these changes (37, 80, 101); however, more research is required here to say definitively how aging and sex interact with anabolism as it relates to feeding and resistance exercise.

SIGNIFICANCE AND PERSPECTIVES

Over the past several years, our general understanding of the regulation of muscle protein metabolism in exercise and recovery has become better defined. It is increasingly apparent that the MPS response is highly regulated and that the magnitude of the response can be manipulated by multiple factors related both to the exercise itself as well as nutrition. Sex-based differences in protein turnover related to exercise appear to exist in young men and women, but these differences are relatively minor. By contrast, older women may develop an anabolic resistance to both feeding and exercise, the cause of which is not known. Resolution of responses of specific muscle protein sub-fractions (i.e., myofibrillar, mitochondrial, and sarcoplasmic), and in the future specific proteins themselves, will yield insights into different exercise perturbations and how these lead to diverse muscular adaptations and ultimately phenotypic changes associated with the plasticity of skeletal muscle.

There are some research gaps, however, that are limiting the complete understanding of the regulation of protein turnover following exercise and thus further research is warranted. For example, in humans a dissociation between signaling proteins that regulate the initiation of protein synthesis and directly measure rates of protein synthesis does exist (41). It is likely that the molecular signaling changes that regulate MPS following exercise occur very rapidly. Thus a detailed time course of the signaling and muscle protein response in the immediate postexercise period is required. Currently, most data have been reported for a single time point at postexercise (3–5, 48, 85, 87, 115). We propose that different mechanisms are in place for

the rise in MPS at later time points (i.e., 24–72 h) (72, 86), which is needed to drive muscle remodeling and the successive adaptations to the exercise stimulus. As such, studies in which examinations of protein metabolism at later periods than the immediate acute postexercise period of 1–4 h, but shorter than chronic training adaptations, are warranted. All in all, it is worth highlighting that most muscle protein metabolism studies investigating the effects of nutrition and exercise are performed under highly controlled conditions to help delineate the specific mechanistic responses to different interventions and therefore may not be directly applicable to free-living individuals. These investigations often employ exercise models not commonly prescribed and/or utilized during everyday practice. However, these studies provide the obligatory scientific framework from which these nutritional and exercise interventions can subsequently be prescribed to different populations and applied in a more “real world.” Finally, although it is not the primary regulating variable, changes in protein breakdown after exercise (especially in the 24- to 72-h time period) have been understudied. The methodological shortcomings for measuring fractional breakdown rate, a direct measure of muscle protein breakdown, in skeletal muscle largely precludes its ability to be utilized in the fed state and therefore is leaving an incomplete understanding of muscle protein turnover following exercise.

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