Amino Acids and Exercise

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CHAPTER

6

Amino Acids and Exercise: Molecular and Cellular Aspects

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6.1. INTRODUCTION

Skeletal muscle is a critical and often unappreciated organ that not only supports human locomotion but its mass is also a robust predictor for all-cause mortality (Kallman et al., 1990; Metter et al., 2002). Furthermore, given that the human body is 45% skeletal muscle by mass, sustaining skeletal muscle mass throughout life is essential for promoting both athletic performance and longevity. Despite its importance, several of the physiological mechanisms that regulate the size of human muscle mass have only recently been elucidated, and many are still unknown. What is known is that the interaction between amino acid feeding-induced changes in rates of muscle protein synthesis (MPS) and rates of muscle protein breakdown (MPB) ultimately dictate net muscle protein balance (NPB) (Phillips et al., 1997). However, the composition, dose, timing, and daily distribution of protein intake (and subsequent effect on aminoacidemia) throughout the day that induces optimal MPS remains a topic of intense research and debate. Moreover, how these variables influence the cellular and molecular regulators that mediate amino acid-induced increases in MPS, particularly those involved in translation initiation and elongation, remain largely unknown. The aim of this chapter is to provide a critical evaluation of our current understanding of how amino acid ingestion, mainly in the form of intact protein sources, following loading (resistance exercise) influences MPS at both the cellular and molecular level. For the purposes of concision and relevance to the human model, data reported primarily derived from human studies will be cited but, where appropriate, work from other experimental models will be introduced to substantiate points of discussion.

6.2. REGULATION OF THE SIZE OF HUMAN MUSCLE MASS

The size of human skeletal muscle mass is dependent upon the coordinated interaction between changes in rates of MPS and MPB (Phillips et al., 1997). The basal and fasted state rates of MPB are known to exceed those of MPS and thus, skeletal muscle net protein balance (NPB = MPS minus MPB) is negative. Over two decades ago, using stable isotopic infusions, it was demonstrated that a mixed macronutrient meal was capable of increasing rates of MPS above rates of MPB and that the amino acids contained within the meal were primarily responsible for this increase (Bennet et al., 1990; Rennie et al., 1982). However, after approximately 2–3 h rates of MPS are known to decline to postabsorptive levels until the consumption of the next amino acid-containing meal (Areta et al., 2013; Atherton et al., 2010). Interestingly, performing exercise, particularly resistance exercise, prior to consuming amino acids has been shown to potentiate rates of MPS (Phillips et al., 2012; Witard et al., 2009). It is this potentiation by resistance exercise of feeding-induced increases in MPS that is responsible for the hypertrophic phenotype observed with resistance exercise training and protein feeding over time (Fig. 6.1).

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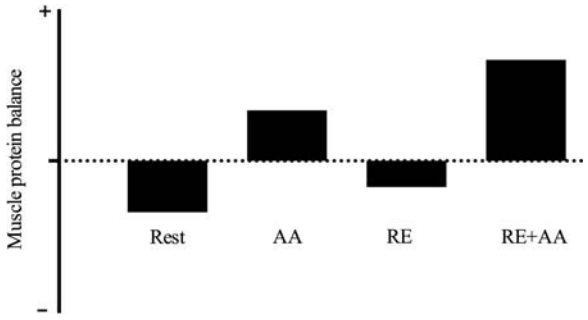

FIGURE 6.1 Changes in net skeletal muscle protein balance at rest, in response to amino acid (AA) consumption, resistance exercise (RE), and when AA ingestion is combined with RE. *Source: Figure redrawn from Phillips (2004) based on original data from Biolo et al. (1995).*

TABLE 6.1 Examples from the literature of rates of muscle protein synthesis captured 4 h following either resistance exercise or endurance exercise

Fractional synthetic rate (%/h)	Rest	RE 4 h	AE 4 h
Mixed	0.04–0.06	0.10–0.16	0.10–0.12
Myofibrillar	0.02–0.05	0.05–0.11	0.03–0.06
Mitochondrial	0.05–0.10	0.10–0.15	0.12–0.15
Sarcoplasmic	0.04–0.06	0.08–0.10	0.08–0.10

Resistance exercise (RE) values obtained from Burd et al. (2010), and aerobic exercise (AE) values obtained from Wilkinson et al. (2008). All mitochondrial protein turnover rates were obtained from Wilkinson et al. (2008).

Importantly, not all amino acids exert the same stimulatory effect of MPS in skeletal muscle either at rest or following exercise, and identifying the relevant amino acids, optimal amino acid/protein dose and/or composition of amino acids to promote gains in muscle size and function is of great scientific interest.

6.3. EXERCISE MODE

While resistance exercise will remain the focus of this chapter it is important to recognize that even aerobic exercise can stimulate MPS (Carraro et al., 1990; Harber et al., 2010) and even result in protein accretion (Harber et al., 2009, 2012). However, the fraction of muscle proteins that are predominantly turned over in response to exercise is specific to the intensity and duration of the exercise bout, as well as the training status of the individual (Burd et al., 2010; Wilkinson et al., 2008). For example, in an untrained state, resistance exercise stimulates an increase in both myofibrillar and mitochondrial MPS; however, in the trained state, resistance exercise only stimulates an increase in myofibrillar MPS (Table 6.1). Endurance exercise on the other hand stimulates an increase in mitochondrial MPS in both the trained and untrained state, but does not result in a stimulation of myofibrillar MPS (Wilkinson et al., 2008). These differences are important considerations when evaluating the efficacy of a given exercise or nutritional stimulus to alter protein turnover as the assessment of mixed MPS can mask any potential protein fraction-specific differences in rates of synthesis (Kim et al., 2005). It is also known that high intensity intermittent exercise training (HIIT), a stimulus that could be considered a "hybrid" of both resistance and endurance exercise, has the capacity to stimulate both myofibrillar and mitochondrial MPS (Bell et al., 2015; Scalzo et al., 2014). Although ingestion of protein (and the subsequent aminoacidemia) is thought to influence the adaptive response to all modes of exercise, the effect is most profound following resistance exercise (Cermak et al., 2012, 2013). As a result, the continuing theme of this chapter will be how protein/amino acid feeding alters the protein synthetic response to resistance exercise with some discussion relating to the role of endurance and HIIT exercise.

6.4. PROTEIN TYPE

A major independent variable that drives MPS is the digestion rate and subsequent aminoacidemia of ingested proteins. This factor can largely be influenced by the quality of intact proteins as defined by various scoring systems such as the Digestible Indispensible Amino Acid Score (for an expanded review see van Vliet et al., 2015). The most widely studied categories of dietary isolated protein are whey, casein and soy, all of which have differing digestion and absorption kinetics as well as amino acid composition. In this regard, isolated whey and soy proteins, since both are acid-soluble, are relatively rapidly digested resulting in a heightened but transient hyperaminoacidemia (Devries and Phillips, 2015). Soy protein however, contains a greater proportion of nonessential amino acids than whey protein (Mahe et al., 1996). Casein in its micellar form (as it exists in milk) is acid insoluble and coagulates in the stomach, which slows transit time into the intestine and thus results in an aminoacidemia that is smaller in amplitude but greater in duration than whey (Boirie et al., 1997).

To date, the influence of protein type on exercise-induced increases in MPS has been understudied. One study has shown that postprandial rates of MPS are greater following the consumption of whey and soy as compared with casein (Tang et al., 2009). Additionally, whey protein consumption has been shown to be superior to both soy and casein for the purposes of stimulating MPS following resistance exercise (Burd et al., 2012; Tang et al., 2009). The superiority of whey to stimulate rates of MPS following resistance exercise is attributed to the high essential amino acid, specifically leucine (Churchward-Venne et al., 2014a) content, the influence of which will be discussed later in this chapter. Another hypothesis is that the rapid increase in blood amino acid concentrations associated with whey protein drives MPS. Indeed, in one study where whey protein was consumed either as a 25 g bolus immediately following resistance exercise or as 10 individual 2.5 g "pulse" drinks separated by 20 min, it was shown that the 25 g bolus of whey induced greater rates of postexercise MPS during a 5 h recovery period (West et al., 2011). When this experiment was repeated, but this time the protein beverages were provided prior to the bout of resistance exercise, there was no difference in postexercise rates of MPS (Burke et al., 2012). However, consuming a single bolus of whey protein following a bout of rigorous exercise as opposed to before is likely a far more practical and attractive strategy to enhance rates of MPS and obviate gastrointestinal stress. Nevertheless, what these data allude to is that the timing, dose, and subsequent hyperaminoacidemia following protein ingestion may be a critical factor modulating the MPS response to resistance exercise.

6.5. DOSE RESPONSE OF MPS TO PROTEIN INGESTION FOLLOWING RESISTANCE EXERCISE

The first study to examine the dose response of MPS to increasing amounts of protein following resistance exercise was conducted by Moore and colleagues who demonstrated that rates of MPS following bilateral lower limb exercises were saturated with the consumption of 20 g of egg protein (Moore et al., 2009). It was also shown that ingestion of 40 g of egg protein resulted in no further enhancement of mixed MPS, but there was a sharp rise in whole-body leucine oxidation indicating oxidative disposal of at least leucine if not other amino acids. Further work on a dose response of MPS with protein ingestion has used a unilateral model of resistance exercise (Witard et al., 2014). These authors reported that rates of both exercise-induced and postprandial myofibrillar MPS were maximally stimulated with 20 g of whey protein (Witard et al., 2014). A unique aspect of this study was that the participants were fed a standard preexercise breakfast meal thus enhancing the practical applicability of the findings to those who consumed food prior to engaging in resistance exercise. Taken together (Moore et al., 2009; Witard et al., 2014), despite the differing sources of protein (egg vs whey), what these data clearly demonstrate is that consuming 20 g of high quality intact protein results in maximal stimulation of both postprandial (Witard et al., 2014) and postresistance exercise (Moore et al., 2009; Witard et al., 2014) rates of MPS in healthy young men.

6.6. TIMING AND DISTRIBUTION

The findings (Moore et al., 2009; Witard et al., 2014) that consumption of protein doses greater than 20 g do not further enhance rates of MPS both at rest and following resistance exercise has lead practitioners of resistance exercise to suggest that frequent small meals may present a feasible strategy to stimulate MPS.

Nonetheless, with prolonged aminoacidemia, lasting approximately 2 h, the rates of MPS are known to decline to postabsorptive values (Atherton et al., 2010) and this effect is apparent whether the amino acids are infused (Bohe et al., 2001) or orally ingested as protein (Atherton et al., 2010). This phenomenon has been coined the "muscle full" effect and has been proposed to be related to the saturation of the translational apparatus (Atherton et al., 2010). However, the characterization of the muscle full effect in these studies was observed in the rested state only. This is an important consideration given that resistance exercise protracts the MPS response to protein feeding (Burd et al., 2011; Churchward-Venne et al., 2012). In fact, as West et al. (West et al., 2011) showed, in a postexercise condition MPS continued to be stimulated despite aminoacidemia having returned back to baseline or despite continued aminoacidemia with oral ingestion. Thus, identification of the optimal pattern and timing of repeated protein feedings to stimulate MPS following a bout of resistance exercise has yet to be determined. Given that consuming 20 g of whey protein is optimal for maximizing postresistance exercise rates of MPS (Moore et al., 2015; Witard et al., 2014), taken together with the knowledge that MPS declines to postabsorptive levels 2 h after feeding at least in the fed state (Atherton et al., 2010), it is physiologically logical to assume that the consumption of 20 g of whey protein every 2−3 h following resistance exercise would be an effective means to enhance daily rates of MPS. This thesis was examined experimentally in a study where an equal amount of protein was distributed either as 8×10 g every 1.5 h, 4×20 g every 3 h, or 2×40 g every 6 h during a 12-h postresistance exercise recovery period (Areta et al., 2013). This approach resulted in differential profiles of postexercise aminoacidemia with the thesis that consumption of 4×20 g of protein 3h would maximize aggregate rates of MPS, which is exactly what this study showed. As such, these data confirm other reports (Moore et al., 2009; Witard et al., 2014) that the consumption of 20 g of high-quality protein postresistance exercise is sufficient to maximize MPS.

While it appears that the strategy to maximize the stimulation of MPS is consumption of 20 g of protein every 3 h (Areta et al., 2013) there are some important caveats to our understanding. Firstly, protein was administered as a liquid bolus and in the absence of other macronutrients. We remain ignorant as to the effect that coingestion of other macronutrients, particularly in the form of a mixed meal, would have on exercise-induced increases in MPS. Moreover, many of these studies employed either a unilateral or bilateral lower limb resistance exercise protocol which are not indicative of the whole-body resistance exercise regimens currently adopted by many physically active individuals, particularly athletes. The relevance of this point is that during whole-body resistance exercise there would be activation of a greater amount of skeletal muscle that would create a greater demand for amino acids to support muscle remodeling. Another important point is that the protein doses in these studies were administered as an absolute value (ie, 20 g) and were not adjusted for body mass. Recent retrospective analysis has showed that MPS is maximally saturated in young men following the consumption of 0.24 g/kg of high-quality protein (with a 90% confidence interval indicating this dose could be as high 0.30 g/kg) at rest (Moore et al., 2015) (Fig. 6.2), but whether this is true with resistance exercise is unknown. Thus, future work addressing these important questions may provide more information that will enable the refinement of current per meal recommendations for protein intake, especially for the athletic population.

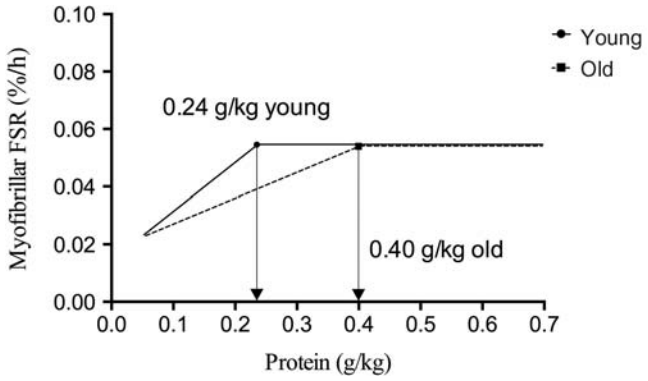

FIGURE 6.2 Biphase linear regression analyses of muscle protein synthesis in response to protein intake expressed as kilogram per body mass in young and older adults. *Source: Redrawn from Moore et al. (2015).*

6.7. THE INFLUENCE OF THE AGING PROCESS

Aging results in a progressive decline in muscle mass and function, collectively termed, sarcopenia. Sarcopenia commences in the fourth decade of life and proceeds on a population basis at a rate of 8% per decade until 70 years of age when the average loss is estimated to occur at 15% per decade (Mitchell et al., 2012). The etiology of sarcopenia is a topic of intense discussion and research. However, an important mechanism is likely related to the inability of skeletal muscle to mount an adequate MPS response to both resistance exercise (Kumar et al., 2009) and protein feeding (Katsanos et al., 2006; for extended discussion see Phillips, 2015). Given resistance exercise (loading) is one of the most potent nonpharmacological methods to enhance muscle mass and strength (Churchward-Venne et al., 2015), this "anabolic resistance" to resistance exercise and protein feeding represents mechanisms that would impair growth of skeletal muscle in aging persons. For instance, whereas 0.24 g/kg/body mass of whey protein is sufficient to maximize MPS following resistance exercise in young adults (Moore et al., 2015; Witard et al., 2014), older adults require 0.40 g/kg/body mass to elicit the same effect (Yang et al., 2012). The physiological mechanisms underlying the reduced sensitivity of older adult skeletal muscle to resistance exercise and protein feeding remain unknown. It has been suggested that older individuals have a reduced skeletal muscle translational capacity compared with their younger counterparts (Chaillou et al., 2014; Kirby et al., 2015) and thus lack the ability to mount a "youthful" response of MPS with the same aminoacidemia; however, this does not explain why consuming a greater amount of protein restores protein synthetic rates (Phillips, 2015). Another theory is that the impairment of insulin-induced increases in arterial blood flow and skeletal muscle microvascular perfusion associated with advancing age compromises the delivery of key amino acids to the translational machinery following exercise. In fact, it has been shown that a prior bout of aerobic exercise enhances microvascular perfusion, blood flow, and subsequent NPB in response to protein−carbohydrate coingestion (Timmerman et al., 2012). Additionally, it is well established that resistance exercise sensitizes skeletal muscle to the anabolic effects of amino acid ingestion. Thus, a combination of resistance exercise and aerobic training could be one means to improve amino acid utilization in skeletal muscle. In this regard, it has been shown that (HIIT) training stimulates increases in both myofibrillar and sarcoplasmic MPS. But whether chronic HIIT improves muscle mass and function to a greater degree than either resistance exercise training or endurance exercise training in the elderly is currently unknown.

Although exercise provides one effective means to counteract sarcopenia, there is some evidence that amino acids such as arginine may be important in determining the anabolic response to a meal. Arginine is an amino acid precursor to nitric oxide production (Stuehr, 2004). In response to feeding there is an increase in nitric oxide-mediated vasodilation that is impaired in older adults (Taddei et al., 2001). One suggestion is that arginine supplementation be used in conjunction with exercise in an attempt to reverse age-related impairments in vasodilation and improve amino acid delivery to skeletal muscle. Though, when ingested a significant proportion of arginine is first-pass cleared resulting in poor bioavailability (Wu et al., 2000a,b). Citrulline on the other hand, is a nonprotein amino acid that serves as the endogenous precursor for arginine that is not metabolized in the intestine or taken up by the liver (Curis et al., 2005). There is also evidence that citrulline of itself can target protein synthetic pathways (Cynober et al., 2010) and thus, could be an effective candidate to support exercise and feeding-induced increases in MPS. However, one study in elderly men who coingested 10 g of citrulline with either 45 g or 15 g of whey, showed no effect of citrulline on either blood flow, microvascular circulation, or postresistance exercise rates of MPS (Churchward-Venne et al., 2014b). Another study also failed to identify an impact of supplementation of 5 g of citrulline on either postprandial, postabsorbtive, or postresistance exercise rates of MPS before and after two weeks of muscle disuse (Devries and Phillips, 2015). The evidence regarding the impact of citrulline on muscle anabolism is, however, equivocal. In agreement with the aforementioned studies, one report has shown little benefit of citrulline on rates of MPS and MPB in the postabsorbtive state (Thibault et al., 2011), whereas others provide evidence that citrulline can improve nitrogen balance (Rouge et al., 2007). Moreover, in one study a high dose (11−24 g) of citrulline supplemented over a period of 8 h did increase rates of MPS compared to nonessential amino acids during a low protein diet (Jourdan et al., 2015), but this was conducted in healthy young adults. Thus, the relevance to this study for the elderly is unknown. Reconciling the differences in the literature is difficult due to differences in the dose, population, as well as exercise stimulus. Clearly more work is now needed to identify the efficacy of citrulline to enhance muscle anabolism, particularly following exercise training in any population.

6.8. THE ROLE OF THE ESSENTIAL AND BRANCHED-CHAIN AMINO ACIDS

Research has shown that the stimulatory impact of protein on MPS following resistance exercise is primarily due to the essential amino acids (Tipton et al., 1999a) with little-to-no role for the nonessential amino acids (Tipton et al., 1999b). This may explain why whey protein exerts such a potent impact on muscle anabolism following resistance exercise (Burd et al., 2012; Tang et al., 2009). In particular, the branched chain amino acid leucine has been shown to be a trigger for muscle anabolism (Averous et al., 2014; Breen and Churchward-Venne, 2012) playing a key role in the activation of the mechanistic/mammalian target of rapamycin complex 1 (mTORC1) and MPS (Jewell et al., 2013). However, while many studies have shown leucine to be stimulatory for MPS, others have failed to replicate the impact of leucine when administered in free form alongside essential amino acids (Glynn et al., 2010). It therefore appears that when other essential amino acids are provided in sufficient quantities, the addition of leucine to protein offers no further benefit. Indeed, the addition of leucine to a lower dose of whey protein (6.5 g), shown to be submaximal for stimulation of MPS, has been shown to be as effective at stimulating MPS at rest compared with a mixture of essential amino acids without leucine (Churchward-Venne et al., 2012).

As leucine is a branched chain amino acid there is a strong belief, especially amongst the sports nutrition and strength and conditioning community, that supplementation with branched chain amino acids may also be anabolic toward skeletal muscle. Indeed, branched chain amino acid supplementation has previously been shown to increase rates of MPS (Shimomura et al., 2006) as well as increase isometric grip strength (Candeloro et al., 1995). Moreover, intense exercise has been shown to result in a reduction in intracellular branched chain amino acid concentration, and thus supplementation with branched chain amino acids may serve to restore endogenous concentrations (Shimomura et al., 2006). Although in one of the most comprehensive studies in this area in which branched chain amino acids were supplemented for 8 weeks, supplementation failed to alter body composition, muscle strength, or muscle endurance (Spillane et al., 2012). Other placebo-controlled trials also have shown no effect of branched chain amino acids supplementation on body composition during resistance exercise training (Ispoglou et al., 2011). In fact, branched chain amino acids are known to compete for the same intestinal- and sarcolemmal-located amino acid transporters and may in fact be antagonistic in their actions with regards to uptake by the muscle (Churchward-Venne et al., 2014a).

6.9. THE MECHANISTIC TARGET OF RAPAMYCIN COMPLEX 1 (mTORC1)

The molecular regulation of MPS is highly integrated and complex. What many years of research have shown is that increases in MPS occur via two main biological processes, enhanced translational efficiency and/or translational capacity. Translational efficiency refers to an increase in rate of protein synthesis per unit of mRNA, presumably through an increased number of ribosomes bound to a single mRNA. Translational capacity refers to an increase in the overall content of ribosomal RNA as well as cellular mRNA and an increased abundance of the 40S and 60S ribosomal subunits. Both of these processes can be regulated by a protein kinase called mTORC1, which is an important signaling protein hub that serves to integrate the MPS response with various stimuli. Such stimuli would include resistance exercise and amino acid ingestion (Apró and Blomstrand, 2010; Areta et al., 2013; Churchward-Venne et al., 2014a; Guertin and Sabatini, 2007; McGlory et al., 2014). It is important to note that there are two mTOR complexes, mTORC1 and mechanistic target of rapamycin complex 2 (mTORC2). Both mTORC1 and mTORC2 impart different biological effects in muscle with mTORC1 being sensitive to the inhibitory effects of the bacterial macrolide rapamycin whereas mTORC2 is not. mTORC1 is the most studied of the complexes and will therefore be the focus of the continuing discussion.

Composed of multiple subunits, the interaction of which dictate its activity, mTORC1 receives and integrates multiple signals. These subunits include the catalytic subunit, mechanistic target of rapamycin (mTOR), regulatory-associated protein of mTOR (Raptor), Mammalian lethal with SEC13 protein 8 (mLST8), DEP domain-containing mTOR-interacting protein (Deptor), and Proline-rich Akt substrate of 40 kDa (PRAS40) (Foster and Fingar, 2010). For mTORC1 assembly to occur it requires interaction with the small guanosine triphosphatase (GTPase) Ras homolog enriched in brain (Rheb). Only when Rheb is in a guanosine triphosphate (GTP)-bound state, is it able to exert a stimulatory impact on mTORC1. In contrast, when in the guanosine diphosphate (GDP)-bound state, Rheb is unable to activate mTORC1, a process that is controlled by the upstream GTPase activating protein (GAP) tuberous sclerosis 2 (TSC2) (Aspuria and Tamanoi, 2004). In this

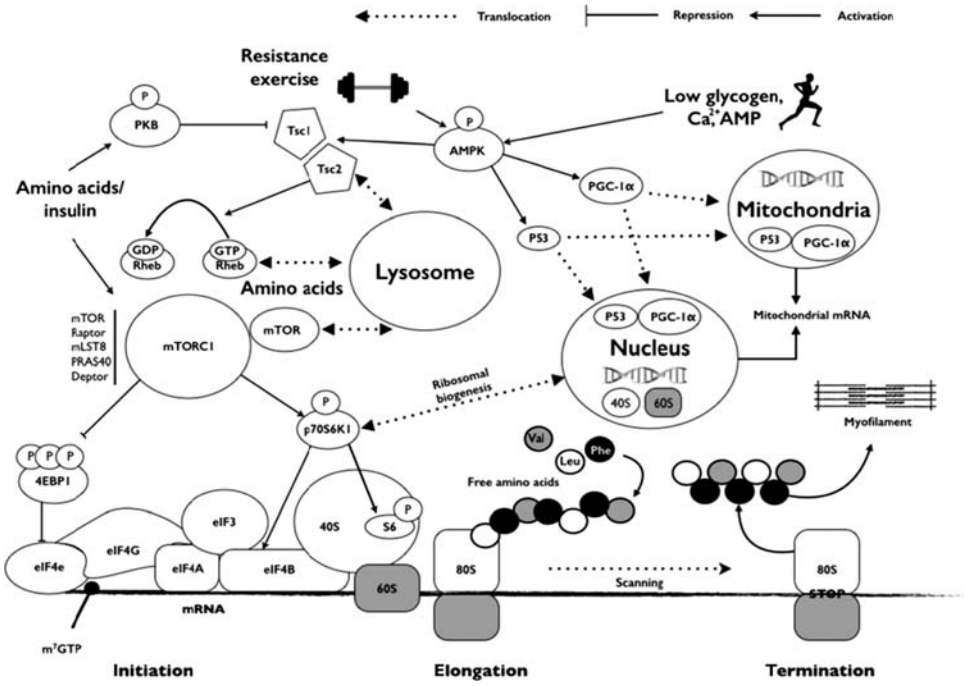

FIGURE 6.3 A schematic overview of the some of the cellular signaling responses regulating muscle protein synthesis and mitochondrial biogenesis in response to exercise. In response to both resistance exercise and endurance exercise there is a stimulation of AMPK that precipitates a series of posttranslational modifications, the intensity of which are highly related to the nature and magnitude of exercise bout. Prolonged, high frequency contractions stimulate an increase in AMPK activity that promotes mitochondrial protein synthesis and biogenesis via PCG-alpha and p53 translocation to the nucleus and mitochondria. Resistance exercise also stimulates AMPK activity and perhaps TSC2 movement away from the lysosomal surface. Following resistance exercise, amino acids promote mTOR and Rheb translocation to the lysosomal membrane as well as promoting PKB activity. The colocalization of Rheb and mTOR to the membrane and/or suppression of TSC2 by PKB promote mTORC1-dependent increases in translation initiation via 4E-BP1 and p70S6K1.

regard, when active, TSC2 activates Rheb by serving to drive its GTPase activity, and increasing its GDP/GTP-bound state, thus inhibiting mTORC1 activation (Inoki et al., 2003). In addition, the nutrient sensitive protein, protein kinase B (PKB) phosphorylates TSC2, preventing its ability to activate Rheb (Inoki et al., 2002). PKB also targets PRAS40, further driving mTORC1 complex assembly (Vander Haar et al., 2007). However, the negative influence of TSC2 on Rheb is enhanced by adenosine monophosphate activated protein kinase (AMPK), which also can inhibit mTORC1 via Raptor (Gwinn et al., 2008). TSC2-Rheb interactions therefore serve as a critical point of mTORC1 activity.

Following its activation, mTORC1 interacts with multiple downstream protein targets. These effectors include the ribosomal protein of 70 kDa S6 kinase 1 (p70S6K1) and 4E-binding protein-1 (4E-BP1) (Dickinson et al., 2011; Gingras et al., 1999; Pearson et al., 1995). Of particular note, p70S6K1 and 4E-BP1 are key signaling molecules that regulate the initiation of protein translation. The phosphorylation status of p70S6K1 and 4E-BP1 are often used as readouts of mTORC1 activity. Indeed, following stimulation mTORC1 phosphorylates p70S6K1 on Thr389 to upregulate translation initiation (Ma et al., 2008; Richardson et al., 2004). Phosphorylated p70S6K1 also serves to enhance translation elongation via eukaryotic elongation factor-2 kinase (eEF2K) (Wang et al., 2001) as well as up regulating ribosomal biogenesis (Chaillou et al., 2014; Hannan et al., 2003). In addition, 4E-BP1 is targeted by mTORC1 via phosphorylation on Thr37/46 reducing its affinity for eukaryotic initiation factor 4E (eIF4E), enabling eIF4E to interact with eukaryotic initiation factor 4G to commence translation initiation (Fig. 6.3).

6.10. RESISTANCE EXERCISE, AMINO ACIDS, AND MTORC1

There are now a significant number of studies that have characterized the impact of amino acid feeding on both MPS and mTORC1 activity. There are even studies to show that specific amino acids such as leucine, arginine and glycine can directly modulate mTORC1 activity (Jewell and Guan, 2013). Resistance exercise even in the

absence of protein feeding can result in the cellular uptake of amino acids (Biolo et al., 1995). One of the first studies to show a relationship between resistance exercise and mTORC1 signaling was conducted by Baar and Esser (1999) who demonstrated that the phosphorylation of p70S6K1 6 h following resistance exercise strongly correlated with the degree of muscle hypertrophy in rodents. Additional studies in humans also identified a correlation between resistance exercise training-induced gains in muscle mass and the degree of p70S6K1 phosphorylation (Terzis et al., 2008, 2010). But while many studies have shown correlations between skeletal muscle hypertrophy/MPS and signaling molecule phosphorylation, others have not (Areta et al., 2013; Mitchell et al., 2013). Potential reasons for the lack of congruence between exercise-induced signal phosphorylation and muscle hypertrophy/MPS include differences in the timing of muscle biopsies and or methods of analysis (immunoblotting vs direct measure of kinase activity). In reality, it also is likely due to differences in exercise intensity, as well as redundancy in signaling pathways supporting MPS following stimulation, and likely intersubject variability (Crozier et al., 2005). While informative, these studies (Areta et al., 2013; Terzis et al., 2008, 2010) provide only an association between human muscle hypertrophy and mTORC1 signaling rather than a direct cause and effect. Nonetheless, the publication of a paper in which the mTORC1 inhibitor, rapamycin, was injected into humans prior to a bout of resistance exercise (Drummond et al., 2009) provided unequivocal evidence of the importance of this signaling pathway in humans. In this seminal study the authors were able to show that treatment with ~12 mg of rapamycin reduced resistance exercise-induced increases in MPS as well as p70S6K1^{Thr389} phosphorylation 1 h postexercise. Furthermore, when the authors repeated this experiment to test whether or not rapamycin had any impact on amino acid-induced increases in MPS and mTORC1 activity they found very similar results (Dickinson et al., 2011). Indeed, in response to oral essential amino acid consumption rapamycin completely blocked feeding-induced increases in MPS as well as attenuating p70S6K1^{Thr389} phosphorylation. It should be acknowledged that in both studies rapamycin failed to inhibit 4E-BP1 phosphorylation suggesting that there are other, as yet undefined, mechanisms that act in concert with mTORC1 signaling to regulate MPS. Support for this contention arises from studies which show that rapamycin has no impact on either postabsorptive rates of MPS in humans (Dickinson et al., 2012) or endurance exercise-induced rates of mitochondrial and myofibrillar MPS in rodents (Philp et al., 2015). Although, in the latter study, there was no increase in mTOR^{Ser2448} or AMPK^{Thr172} phosphorylation in the control group either, suggesting that the exercise intensity was not sufficient enough to maximize rates of either myofibrillar or mitochondrial MPS. Nevertheless, the molecular network regulating changes in skeletal muscle morphology in response to endurance exercise is known to be somewhat distinct from that regulating resistance exercise-induced adaptations (for a comprehensive review see Egan and Zierath, 2013).

In addition to phosphorylation, the translocation of signaling proteins may play a key role in the adaptive response to exercise and nutrition. For example, in response to aerobic/endurance exercise the peroxisome proliferator-activated receptor-gamma coactivator (PGC-1) alpha and tumor suppressor protein p53 translocate to the nucleus and mitochondria to promote mitochondrial biogenesis (Safdar et al., 2011), an effect that may be modulated by carbohydrate availability (Bartlett et al., 2013, 2015). Similarly, others using cell models have shown that in response to amino acid provision, mTOR localizes to the Rheb positive lysosomal membrane via rags and the regulator complex (Sancak et al., 2008; Sancak and Sabatini, 2009) (for extensive review see Jewell et al., 2013). In response to amino acid withdrawal, mTOR disassociates from the lysosomal membrane where it is unable to interact with its coactivators to form the fully functional mTORC1 protein complex (Long et al., 2005). Interestingly, resistance exercise is also proposed to alter the trafficking of intracellular signaling molecules. One study in rodents has shown that lengthening contractions resulted not only in movement of mTOR to the lysosome but also disassociation of TSC2 from the lysosomal membrane (Jacobs et al., 2013). This dual effect of amino acid feeding and resistance exercise on mTOR and TSC2 trafficking could in some way be responsible for the potentiation of MPS observed when resistance exercise is performed prior to consuming amino acids. However, it is important to note that these studies did not assess rates of MPS nor were they conducted in human models of exercise thus more work is needed to experimentally corroborate these findings in humans with exercise.

6.11. FUTURE DIRECTIONS

To date, a significant amount of work has been conducted that has provided critical information for the field of exercise physiology and nutrition. With the introduction of the percutaneous muscle biopsy technique (Bergstrom, 1975), together with the application of isotopic tracer methodology in the form of isotopically-labeled

amino acids (Rennie et al., 1982), it has been possible to directly track rates of MPS in response to various nutritional and exercise interventions. However, due to the invasive nature of the stable isotope tracers the majority of these studies were limited to the laboratory setting and over a ~12 h period. Refinements in mass spectrometry have now enabled the use of deuterium as a tracer that can be orally consumed and does not require intravenous administration, and thus a laboratory (Wilkinson et al., 2015). The significance of this advance is that the rates of MPS that are measured are indicative of a free-living setting including longer-term periods of fasting, feeding, and sleep. This method also allows participants to engage in everyday tasks while also being under the constraints of the experimental paradigms which would enhance the practical applicability of any findings. Contemporary studies using this method have yielded interesting results including characterization of the adaptations of skeletal muscle in the initial stages of resistance training (Brook et al., 2015), as well as the response of the protein synthetic responses of different muscle fractions to different exercise modes (Bell et al., 2015). However, a common criticism of many studies that assess MPS is that they fail to concomitantly measure MPB and thus are unable to provide a complete picture of muscle protein turnover. There has been, however, one paper that has detailed a method to measure changes in MPB with deuterium that warrants further investigation (Holm et al., 2013). By concurrently measuring both MPS and MPB over a period of days it will be possible to gauge the relative contribution of both MPS and MPB to any given changes in muscle size. Such advances in the measurement of muscle protein turnover, have been accompanied by developments in methods to assess changes in the activity (McGlory et al., 2014) and localization (Jacobs et al., 2013) of protein and protein-kinases that are responsible for regulating MPS at the molecular level. When married together with methods that enable the direct determination of MPS and MPB, these new developments will provide greater insight as to how periods of exercise and feeding as well as inactivity, impact skeletal muscle morphology.

6.12. CONCLUSION

Skeletal muscle is a critical organ, the loss of which is associated with numerous clinical pathologies. Currently, pharmaceutical interventions to mimic the pleiotropic health benefits of exercise, particularly resistance exercise, are nonexistent. Thus, exercise and nutrition remain the key tools to promote muscle mass and improve human health on a population basis. However, there is a significant amount of information that has yet to be discovered, particularly with respect to the molecular processes by which exercise and amino acid ingestion confer an anabolic influence toward skeletal muscle. With the application of deuterium to directly track muscle protein turnover alongside methods to examine changes in the cellular location of anabolic signaling proteins, it is hoped that future work will provide exciting new data for the field.

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