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REVIEW

Molecular regulation of human skeletal muscle protein synthesis in response to exercise and nutrients: a compass for overcoming age-related anabolic resistance

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Hodson N, West DW, Philp A, Burd NA, Moore DR. Molecular regulation of human skeletal muscle protein synthesis in response to exercise and nutrients: a compass for overcoming age-related anabolic resistance. *Am J Physiol Cell Physiol* 317: C1061–C1078, 2019. First published August 28, 2019; doi:10.1152/ajpcell.00209.2019.—Skeletal muscle mass, a strong predictor of longevity and health in humans, is determined by the balance of two cellular processes, muscle protein synthesis (MPS) and muscle protein breakdown. MPS seems to be particularly sensitive to changes in mechanical load and/or nutritional status; therefore, much research has focused on understanding the molecular mechanisms that underpin this cellular process. Furthermore, older individuals display an attenuated MPS response to anabolic stimuli, termed anabolic resistance, which has a negative impact on muscle mass and function, as well as quality of life. Therefore, an understanding of which, if any, molecular mechanisms contribute to anabolic resistance of MPS is of vital importance in formulation of therapeutic interventions for such populations. This review summarizes the current knowledge of the mechanisms that underpin MPS, which are broadly divided into mechanistic target of rapamycin complex 1 (mTORC1)-dependent, mTORC1-independent, and ribosomal biogenesis-related, and describes the evidence that shows how they are regulated by anabolic stimuli (exercise and/or nutrition) in healthy human skeletal muscle. This review also summarizes evidence regarding which of these mechanisms may be implicated in age-related skeletal muscle anabolic resistance and provides recommendations for future avenues of research that can expand our knowledge of this area.

anabolic resistance; ERK1/2; mTORC1; muscle protein synthesis

INTRODUCTION

Skeletal muscle not only serves to aid locomotion, posture, and respiration in humans, it is also a major metabolic tissue contributing to glucose disposal, lipid oxidation, and basal metabolic rate (187). For this reason, it is well established that a greater amount of skeletal muscle mass is positively associated with longevity, health, and independence (163). Skeletal muscle mass is determined by the balance of muscle protein synthesis (MPS) and muscle protein breakdown (MPB). Therefore, when MPS exceeds MPB, such as after ingestion of an amino acid (AA)- and/or protein-containing meal (27), an individual will be in a state of net muscle protein accretion. When MPB exceeds MPS, for example, in the postabsorptive state, an individual will be in a state of net muscle protein loss. While both MPS and MPB are relevant to skeletal muscle net protein balance (or remodeling), MPS seems to be more sensitive to alterations in contractile activity and nutritional state, in the absence of an extreme catabolic situation such as burn injury or renal failure (17, 18, 127). Importantly, elevations of MPS are not always inextricably linked to positive net protein balance, nor do they exclusively contribute to muscle growth (hypertrophy), but they may represent enhanced non-hypertrophic remodeling (26), which would help maintain skeletal muscle proteostasis through removal and replacement of damaged and/or dysfunctional proteins.

Therefore, the aim of this review is to examine the cellular and molecular mechanisms that underpin MPS and then to explore the evidence for each mechanism in young healthy skeletal muscle. Finally, a discussion of whether these signaling events may contribute to age-related anabolic resistance is presented. The predominant focus will be

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on the effects of mechanical stimuli and protein ingestion on these mechanisms, as resistance exercise and essential AAs (EAAs) seem to be the predominant drivers of MPS in human skeletal muscle (27, 171). Although insulin is also able to elicit dose-dependent elevations of several of the signaling events that will be described (73), this review will not focus on this aspect, as insulin is permissive in the activation of MPS when AA availability is high (63) and can lead to a dissociation between anabolic signaling and MPS (73; see Ref. 1 for an in-depth review of the effects of insulin on MPS/anabolic signaling).

MUSCLE PROTEIN SYNTHESIS: WHAT IS IT?

MPS involves translation of a strand of messenger ribonucleic acid (mRNA) into a fully functioning protein by ribosomes within a muscle cell (Fig. 1). This process is initiated when mRNA is recognized by eukaryotic initiation factor (eIF) 4E (eIF4E), which binds to the 5′ end of the strand (78). This initiation factor can then recruit eIF4G, eIF4A, and eIF4B to this site to form the translation preinitiation (eIF4F) complex (67). This complex serves to "prime" the mRNA strand for translation through unwinding of the secondary structure (coordinated by eIF4A and eIF4B) (145) and recruitment of the small (40S) subunit of the ribosome to the strand (67). Also attached to the 40S subunit is a complex of eIF2, guanosine triphosphate (GTP), and a transfer RNA (tRNA) loaded with methionine (eIF2-GTP-Met-tRNA), which corresponds to the mRNA start codon (67). This group of proteins forms the 43S initiation complex, which is required to initiate translation at the start codon. The 43S complex moves along the mRNA strand until it encounters the start codon (AUG nucleotides in eukaryotes); at this point, the initiation factors are released to allow the large (60S) subunit of the ribosome to bind and form the full 80S ribosome needed for translation (67, 99).

Once the full ribosome has been constructed at the start codon, the process of translation elongation begins, with the 60S subunit recognizing each subsequent codon and recruiting the corresponding tRNA, which is loaded with the AA coded for by the codon (81). Once recruited, the catalytic activity of the ribosomal RNA (rRNA) produces a peptide bond between AAs, and the ribosome moves along the mRNA strand to the next codon and releases the now-empty tRNA back to the cytoplasm, where it can bind to another AA (14). Energy for

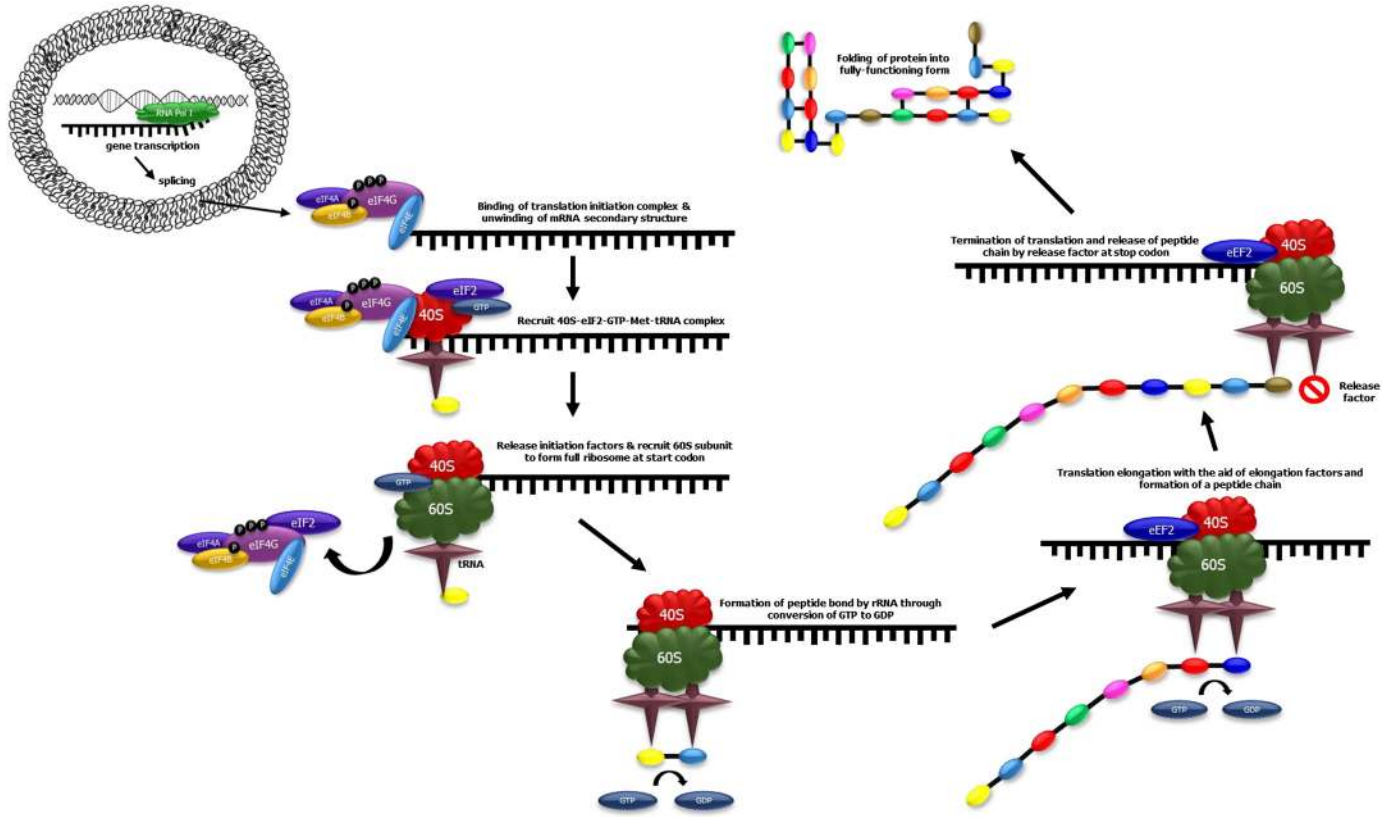

Fig. 1. Schematic illustration of mRNA translation into protein. After transcription of a new strand of mRNA in the nucleus, this mRNA strand will undergo splicing and then be transported to the cytosol, where eukaryotic initiation factor (eIF) 4E will bind to its 5′-end and allow formation of the preinitiation complex (eIF4G, eIF4A, and eIF4B). This complex will unwind the secondary structure of the mRNA strand, "priming" it for translation. Next, the 40S subunit, complexed with eIF2-guanosine triphosphate (GTP), and a transfer RNA (tRNA), loaded with methionine (eIF2-GTP-Met-tRNA), is recruited, forming the 43S initiation complex, which recognizes the start codon (AUG) on the mRNA strand. Upon arrival at the start codon, this complex of initiation factors is released, and the large (60S) ribosomal subunit binds with the 40S subunit to form the full 80S subunit needed for translation. The 60S subunit recognizes each codon and recruits the corresponding loaded tRNA. The catalytic activity of the rRNA then forms a peptide bond between 2 amino acids (AAs), and the ribosome moves to the next codon, while the now-empty tRNA is released to the cytoplasm to bind with another AA. This process, aided by translation elongation factors, continues to occur along the entire mRNA strand until the ribosome hits a stop codon. Here, a release factor, which aids release of the newly formed peptide chain from the ribosome, is recruited. This peptide chain then undergoes various folding steps to achieve full functionality.

this process is provided by hydrolysis of GTP to form guanosine diphosphate (14). Eukaryotic elongation factors (eEFs) aid this entire process by enhancing the recruitment of loaded tRNAs to the ribosome and accelerating the shift of the ribosome to the next codon once a peptide bond has been formed (4). Translation elongation continues along the whole mRNA strand, forming a polypeptide chain. Translation is terminated when the ribosome shifts to one of three stop codons, none of which is recognized by tRNA (14). Instead, these codons elicit the recruitment of release factors to the ribosome, which causes release of the polypeptide chain and mRNA strand from the ribosome and dissociation of the two ribosomal subunits (14).

The polypeptide chain then undergoes folding steps to produce the correct secondary or tertiary structure for the functionality of that protein (40). Some tertiary proteins also need to form multiprotein complexes, termed the quaternary structure, to carry out their functions. These folding mechanisms are not described here but are reviewed in detail elsewhere (40, 132).

MECHANISTIC TARGET OF RAPAMYCIN COMPLEX 1-DEPENDENT REGULATION OF MPS

Increases in rates of MPS are regulated at numerous molecular levels within the translation initiation and elongation processes to respond to anabolic (e.g., mechanical loading) and nutritional (e.g., EAA provision) stimuli. Until recently, these mechanisms were believed to be primarily, if not entirely, governed by the mechanistic target of rapamycin (mTOR), in particular mTOR complex (mTORC) 1 (mTORC1) (Fig. 2). mTOR is an evolutionarily conserved serine/threonine kinase belonging to the phosphatidylinositol 3-kinase (PI3K)-related kinase family (151). In muscle cells, mTOR resides as the core of two complexes, mTORC1 and mTORC2. As its downstream effects are known to elevate mRNA translation and repress autophagy (78), mTORC1 and its substrates have been the predominant focus of research aimed at understanding the molecular regulation of MPS. mTORC1 comprises mTOR and several regulatory proteins: regulatory-associated protein of mTOR (RAPTOR), proline-rich AKT substrate of 40 kDa (PRAS40), DEP domain-containing mTOR-interacting protein

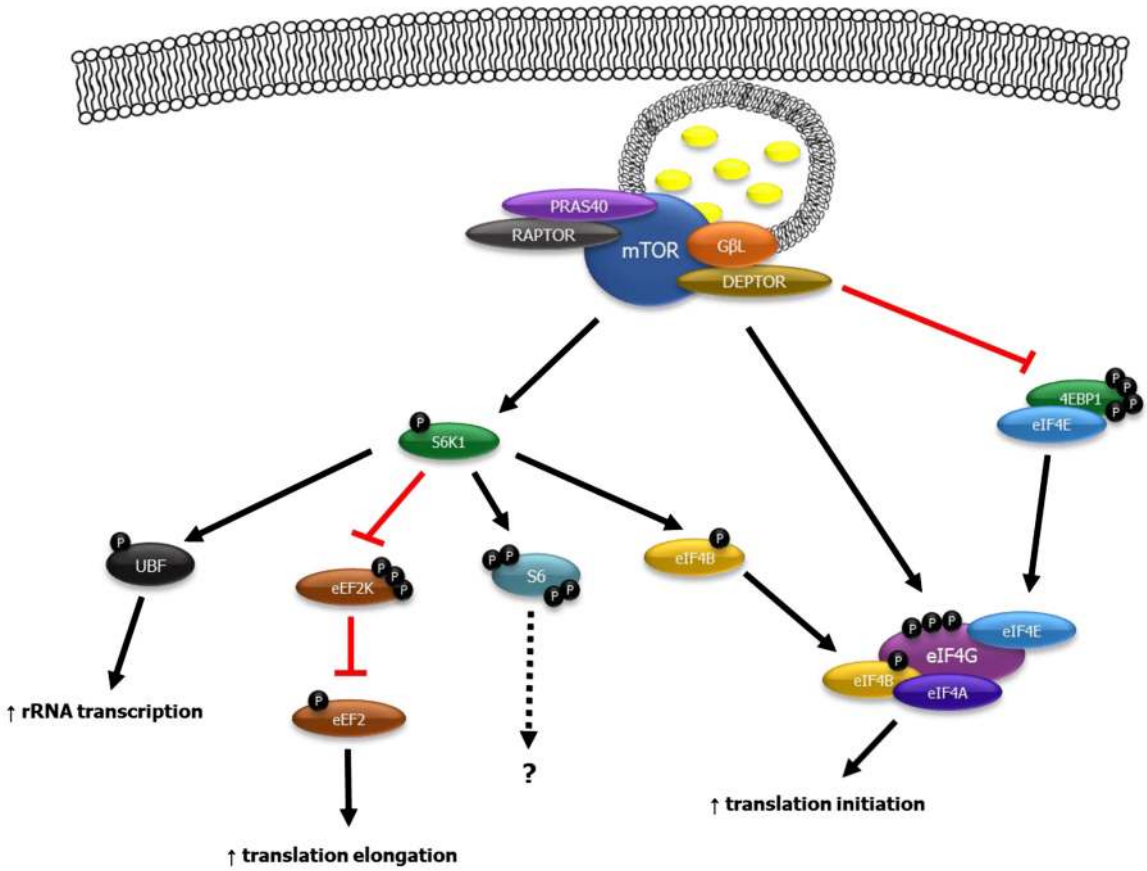

Fig. 2. Mechanistic target of rapamycin (mTOR) complex 1 (mTORC1)-dependent regulation of protein translation. Once activated, mTORC1 phosphorylates several downstream targets that regulate protein translation. The first is ribosomal protein S6 kinase 1 (S6K1), which is phosphorylated at Thr^{389} by mTORC1. This kinase phosphorylates its downstream targets, eukaryotic elongation factor (eEF) 2K (eEF2K), ribosomal protein S6 (rpS6), eukaryotic initiation factor (eIF) 4B (eIF4B), and upstream binding factor (UBF), which cause an elevation of translation initiation (eIF4B), translation elongation (eEF2K), and rRNA transcription (UBF). A second direct mTORC1 target is eukaryotic translation initiation factor 4E-binding protein 1 (4EBP1), which is phosphorylated and inhibited by the kinase complex. 4EBP1 is then removed from its association with eIF4E and allows the preinitiation complex to bind with the 5′ cap of the mRNA strand. mTORC1 also directly phosphorylates eIF4G on several residues in a further mechanism to enhance translation initiation. P, phosphorylation; RAPTOR, regulatory-associated protein of mTOR; PRAS40, proline-rich AKT substrate of 40 kDa; DEPTOR, DEP domain-containing mTOR-interacting protein; GβL, mammalian lethal with SEC13 protein 8/G protein β-subunit-like.

(DEPTOR), and mammalian lethal with SEC13 protein 8/G protein β-subunit-like (mLST8/GβL), each of which is essential for the proper functionality of the kinase complex (16, 78). The notion that mTORC1 signaling was vital for alterations in MPS was based on the finding that rapamycin treatment led to a 95% block in compensatory hypertrophy (19). Furthermore, use of the Rheb-specific inhibitor NR1, which elicits complete mTORC1 inhibition without affecting mTORC2 (110), significantly reduces protein synthesis in MCF-7 cells, implying that mTORC1 is an important regulator of protein synthesis in eukaryotic cells (110). Subsequent work in humans has shown that inhibition of mTORC1 through rapamycin ingestion prevents the increase in MPS after EAA ingestion (39) and muscle contraction (48), but not in the fasted state (38), highlighting its importance in regulation of muscle protein remodeling in response to acute anabolic stimuli. The key downstream targets of mTORC1 and how they regulate MPS are summarized below (see *Ribosomal Protein S6 Kinase 1 and Ribosomal Protein S6*). As recent reviews have addressed the activation of mTORC1 specifically (e.g., association with the lysosome and mTOR trafficking) (8, 83, 112), the current review will focus on the downstream effects of its activation in response to resistance exercise and or protein/EAA feeding.

Ribosomal Protein S6 Kinase and Ribosomal Protein S6

The most characterized substrate of mTORC1 is ribosomal protein S6 kinase 1 (S6K1). S6K1 is hyperphosphorylated at Thr^{389} in response to mTORC1 activation (30), which enhances the kinase activity of S6K1 and leads to phosphorylation of its downstream targets. S6K1 is known to phosphorylate ribosomal protein S6 (rpS6) at two key serine residues, $Ser^{235/236}$ and $Ser^{240/244}$ (78, 150). The specific function of this phosphorylation was initially believed to allow enhanced recruitment of 5′-terminal oligopyrimidine (TOP) mRNAs to ribosomes (170). More recently, however, this mechanism has been questioned (158, 166), as translation of 5′-TOP mRNAs has been reported to be regulated by PI3K signaling in a manner that is independent of rpS6 (166). Furthermore, although rpS6 phosphorylation can occur in the muscles of S6K1 knockout animals (111), deleterious effects of the absence of S6K1 are apparent, suggesting that rpS6 may not be the predominant mechanism of S6K1 activity in tissue. Nevertheless, rpS6 phosphorylation serves as an accurate and reliable readout of S6K1 activity and, therefore, has been extensively investigated in human skeletal muscle.

Insights from healthy human skeletal muscle. Many studies in healthy human skeletal muscle have shown that $S6K1^{Thr389}$ phosphorylation is potently elevated after mechanical loading and/or AA feeding (5, 48, 62, 96, 100, 101, 186). While either of these anabolic stimuli alone elevates $S6K1^{Thr389}$ phosphorylation (5, 62, 101), a combination of the two elicits a much greater effect (5, 100, 122, 124). Elevations of this phosphorylation event often occur in the first 6 h after anabolic stimuli, with peak phosphorylation at ~1–1.5 h (5); however, some reports note that $S6K1^{Thr389}$ phosphorylation remains above the basal level up to 24 h postexercise (24). A variety of dietary protein sources increase $S6K1^{Thr389}$ phosphorylation (20, 71, 87, 91, 96, 120, 143). However, most work has used isolated proteins, such as whey or soy, to assess protein phosphorylation events in human muscle and showed a particularly prolonged response to ingestion of whey compared with soy protein (120). Few studies have assessed the regulation of $S6K1^{Thr389}$ phosphorylation based on eating patterns more relevant to humans (e.g., whole foods or food combinations), but another factor that may impact this phosphorylation event is the leucine content of a meal. If leucine is removed from an EAA supplement, the extent of phosphorylation of $S6K1^{Thr389}$ is significantly reduced in the early postexercise recovery phase compared with the same supplement containing leucine (5, 122).

Mechanical loading variables also modulate $S6K1^{Thr389}$ phosphorylation. In work-matched resistance exercise bouts, high loads [~70% 1 repetition maximum (RM)] elicit greater elevations of $S6K1^{Thr389}$ phosphorylation (85). Conversely, if low loads (30% 1 RM) are lifted to failure and, therefore, a greater volume of weight is lifted, higher elevations of this phosphorylation event are elicited than when high loads (90% 1 RM) are lifted to failure (29). Our laboratories recently reported a significant positive relationship between $S6K1^{Thr389}$ phosphorylation and myofibrillar protein synthesis (myoMPS) after resistance exercise and ingestion of isonitrogenous amounts of egg whites or whole eggs (3). Other studies have reported qualitative associations between $S6K1^{Thr389}$ phosphorylation and myoMPS, in that interventions that elicit greater $S6K1^{Thr389}$ phosphorylation are generally associated with higher rates of myoMPS (21, 42, 91, 174). However, establishing a direct causal relationship between a particular phosphorylation event and MPS in human skeletal muscle from any population is challenging, as other reports have shown that alterations in $S6K1^{Thr389}$ phosphorylation are not paralleled by directionally or proportionally similar changes in synthetic rates of sarcoplasmic or mitochondrial fractions (21, 24, 28). This could highlight the relatively greater importance of $S6K1^{Thr389}$ phosphorylation for regulating changes in the more abundant myofibrillar proteins and/or the limitations of traditional Western blotting techniques that estimate kinase activity from changes in phosphorylation and may be prone to methodological variability (9). Moreover, this posttranslational modification at 5 h postexercise has been reported to positively correlate with skeletal muscle hypertrophy following 16 wk of resistance training (118), suggesting, under some circumstances, that this signaling event associates with both acute muscle turnover and chronic muscle growth.

A [γ-^{32}P]ATP kinase assay (116) may therefore provide a more valid and reliable measure of S6K1 activation, although one study suggests that this measure is positively correlated to $S6K1^{Thr389}$ phosphorylation (5). Nevertheless, with resistance exercise and AA feeding, S6K1 kinase activity is elevated (5, 82, 121), and to a greater extent when these stimuli are combined. Moreover, if more AAs are added to a supplement, S6K1 kinase activity increases in a stepwise fashion (121), as long as leucine content remains constant (5). Therefore, collectively, $S6K1^{Thr389}$ phosphorylation is generally considered an essential event to enhance the synthetic rates of the abundant myofibrillar protein fraction in human muscle.

Phosphorylation of rpS6 is frequently determined in studies of human skeletal muscle anabolism as a readout of mTORC1/S6K1 signaling. Phosphorylation of either $rpS6^{Ser235/236}$ or $rpS6^{Ser240/244}$ often mirrors the other (48, 114) and, after anabolic stimuli, follows a pattern similar to that of $S6K1^{Thr389}$ (100, 101, 126, 167, 169), its predominant upstream kinase. As

such, the effect of anabolic stimuli on these phosphorylation events is generally similar to that of S6K1^{Thr389}, peaking at ~60–90 min after exercise/feeding, and can remain elevated for ~24 h following exercise (25, 100, 101). Phosphorylation of rpS6 may be influenced by total exercise volume. Terzis et al. (169) showed that lifting the same load for 1, 3, or 5 sets elicited a stepwise rpS6$^{Ser235/236}$ phosphorylation response at 30 min after exercise. A similar finding was reported by Burd et al. (25) at 5 h postexercise, when rpS6$^{Ser240/244}$ phosphorylation was elevated to a greater extent upon completion of 3 sets than 1 set of resistance exercise. Thus, greater exercise volume and, therefore, mechanical stimulation may directly impact the degree of rpS6 phosphorylation.

Eukaryotic Initiation Factor 4B

A second target of S6K1 kinase activity is eIF4B, a component of the translation preinitiation complex. This protein is phosphorylated on Ser^{422} by S6K1 (140), which enables eIF4B to associate with the preinitiation complex and activate the helicase activity of eIF4A (67). eIF4A then unwinds the mRNA secondary structure, permitting efficient binding of the ribosome to mRNA (145). Therefore, this may be an additional mechanism by which mTORC1 elicits S6K1-dependent effects on mRNA translation.

Insights from healthy human skeletal muscle. To our knowledge, investigations of the posttranslational modification of the translation initiation factor eIF4B in healthy human skeletal muscle are limited to one study (186). Witard et al. (186) reported that acute lower-body resistance exercise did not alter eIF4B^{Ser422} phosphorylation either immediately or 6 h after exercise. Phosphorylation of S6K1^{Thr389}, the upstream kinase of eIF4B, was also not elevated at these time points, so the time course of any change in eIF4B^{Ser422} phosphorylation may have been missed. Therefore, as there is very little research regarding this phosphorylation event in human skeletal muscle, regardless of age or health, further investigations are required to fully understand its role in the initiation of MPS.

Eukaryotic Elongation Factor 2

When mTORC1 is activated, phosphorylation of eEF2 at Thr^{56}, an inhibitory site, is reduced (141). This relieved inhibition allows eEF2 to bind with ribosomes and accelerate their movement along mRNA strands (98, 141, 142). The phosphorylation status of eEF2 is primarily regulated by eEF2 kinase (eEF2K), which is phosphorylated and inhibited on Ser^{366} by S6K1 (23, 178). Therefore, mTORC1 regulates translation initiation as well as elongation.

Insights from healthy human skeletal muscle. Although not a direct target of mTORC1 or S6K1, eEF2^{Thr56} phosphorylation is often used as a readout of the activity of this pathway, as its upstream kinase eEF2K is phosphorylated and inhibited in what is believed to be a mTORC1-dependent manner (23). In young human skeletal muscle, the contraction-induced reductions in eEF2^{Thr56} phosphorylation are ablated by rapamycin, supporting the notion that this posttranslational modification is mTORC1-dependent (48). Many investigations in healthy human skeletal muscle report reduced eEF2 phosphorylation in response to AAs and/or resistance exercise (5, 42–44, 48, 62, 117, 121, 122), with several displaying a qualitative association to MPS; i.e., interventions/time points that show the largest reduction in eEF2^{Thr56} phosphorylation also elicit the greatest increases in MPS (43, 44, 48, 62, 117). A direct correlation between these two measures is yet to be reported; therefore, further research is required to understand the true relationship between them. Conversely, some reports suggest no impact of anabolic stimuli on eEF2^{Thr56} phosphorylation, despite elevated mTORC1/S6K1 activity (45, 54, 85).

Increased intracellular Ca^{2+} concentrations immediately postexercise may activate eEF2K and increase eEF2^{Thr56} phosphorylation (93, 136, 146), suggesting that translation elongation is repressed. This effect has also been associated with changes in energy status (i.e., increased ADP-to-ATP ratio) immediately postexercise in rodent skeletal muscle (185). Nevertheless, by 60 min following resistance exercise or protein ingestion, eEF2^{Thr56} phosphorylation is commonly reported to decrease below basal levels (5, 42, 62, 121, 122) and can remain attenuated for 3 h poststimulus (121), a response that is similar in men and women (44, 122). Reports suggest that this mechanism may be more sensitive than AA provision to alterations in mechanical loading, as addition of EAAs after resistance exercise did not further reduce eEF2^{Thr56} phosphorylation (5, 121), although feeding alone has been reported to reduce eEF2^{Thr56} phosphorylation in some (62), but not all (39), investigations. In agreement with this notion, eEF2^{Thr56} phosphorylation is not overly responsive to changes in protein source/composition after resistance exercise (5, 121, 122). This suggests that maximal inhibition of eEF2K may be achieved primarily by resistance exercise alone, with little additional role for the acute nutrient environment.

Changes in resistance exercise-rest interval/volume, however, do seem to influence eEF2^{Thr56} phosphorylation. In a cohort in which rest intervals were longer and, thereby, a greater volume was lifted, McKendry et al. (117) showed a greater reduction in eEF2^{Thr56} phosphorylation during postexercise recovery. Another report (25) of reduced eEF2 phosphorylation at 4 h postexercise when a greater overall volume of resistance exercise is performed concurs with this notion. Collectively, eEF2^{Thr56} phosphorylation appears to be regulated by anabolic stimuli, mechanical loading in particular, in healthy human skeletal muscle; however, more work is required to understand its full impact on regulation of human MPS.

Eukaryotic Translation Initiation Factor 4E-Binding Protein 1

A second major substrate of mTORC1 is eukaryotic translation initiation factor 4E-binding protein 1 (4EBP1) (66). mTORC1-dependent phosphorylation of 4EBP1 occurs at four different serine residues, which are phosphorylated in a hierarchical manner, and all are required to relieve the inhibition of 4EBP1 toward eIF4E (66, 78, 79). Initially, Thr^{37} and Thr^{46} are phosphorylated (66), acting to prime 4EBP1 for subsequent phosphorylation of Ser^{65} and Ser^{70} and removal of 4EBP1 from eIF4E (66, 76). Thereafter, the binding site for eIF4G is unblocked, and the preinitiation complex can be recruited to the mRNA strand before translation initiation (79).

Insights from healthy human skeletal muscle. 4EBP1 is phosphorylated on four different residues (Thr^{37}, Thr^{46}, Ser^{65}, and Ser^{70}) in a mTORC1-dependent manner, with the most common in human skeletal muscle being Thr$^{37/46}$. Results

surrounding the effect of anabolic stimuli on this phosphorylation site are equivocal, with some studies reporting elevations (5, 42, 62, 96, 121, 124) and others showing no change or even reductions (43, 49, 95, 96). These findings may be due to the hierarchical nature of 4EBP1 phosphorylation following mTORC1 activation (66), as this particular site may no longer be phosphorylated at certain time points. Interestingly, in one study, phosphorylation at this site 6 h after resistance exercise and AA feeding correlated to skeletal muscle growth, but not acute myoMPS (119). In contrast, 4EBP1$^{Thr37/46}$ phosphorylation at 24 h postexercise has been directly, and positively, correlated to myoMPS (29), potentially linking these two processes later in the postexercise recovery period. Additionally, phosphorylation at this site is qualitatively associated with elevations of MPS following AA feeding alone (62) and protein-carbohydrate ingestion following resistance exercise (42); however, further research across a variety of time courses/interventions is needed to fully determine if a direct association exists. Similar to S6K1^{Thr389}, 4EBP1$^{Thr37/46}$ phosphorylation, on this occasion measured 1 h postexercise, has been shown to associate with chronic resistance-training-mediated alterations in skeletal muscle volume (119). This seemingly supports the notion that mTORC1-dependent signaling pathways are implicated in the control of both acute skeletal muscle turnover and chronic muscle adaptation; however, it is important to note that 4EBP1$^{Thr37/46}$ phosphorylation was not significantly elevated above basal levels at this time point, even though it was significantly associated with muscle hypertrophy (119).

It is intriguing that both 4EBP1^{Thr46} and 4EBP1^{Ser65} phosphorylation were elevated following resistance exercise and intake of a variety of AAs (leucine, branched-chain AAs, and EAAs), which mimicked the response noted with S6K1 kinase activity, whereas the response of 4EBP1$^{Thr37/46}$ did not (121). This could suggest that relatively novel residues could be a target for future studies in human skeletal muscle. Alternatively, coimmunoprecipitation techniques, which, to our knowledge, have been used sparingly and in the absence of parallel measures of MPS (121) to measure the association between 4EBP1 and eIF4E, may provide additional insight into the molecular regulation of human MPS, especially considering that phosphorylation of 4EBP1 dissociates these proteins (67).

Eukaryotic Initiation Factor 4G

mTORC1 is also believed to phosphorylate a further component of the translation initiation machinery, eIF4G (78). This protein comprises three subunits that act as a scaffold for other translation initiation factors in the preinitiation complex at the 5′ end of mRNA (161). eIF4G is phosphorylated at three sites, Ser^{1108}, Ser^{1148}, and Ser^{1232}, when mTORC1 activity is high (139). While the precise implication of these phosphorylation events is unclear, they are purported to elicit a conformational change in these subunits, allowing for more efficient construction of the ribosomal preinitiation complex (78).

Insights from healthy human skeletal muscle. Similar to phosphorylation of eIF4B, phosphorylation of eIF4G has been relatively underinvestigated in healthy human skeletal muscle [to our knowledge, only 1 study (48)]. Drummond et al. (48) reported that acute lower-body resistance exercise elicited an elevation of eIF4G^{Ser1108} phosphorylation at 1 and 2 h postexercise and that this effect occurred despite the presence of rapamycin (although the authors acknowledge that the rapamycin dosage may not have been high enough to fully inhibit mTORC1 activity). Further research is required to fully understand the effects of anabolic stimuli on the phosphorylation event and its association to MPS.

mTORC1-INDEPENDENT REGULATION OF MPS

Although data from young, healthy human skeletal muscle suggest that mTORC1 activation is required to enhance MPS in response to anabolic stimuli (17, 21), it is important to acknowledge that these investigations measured MPS during a short (2-h) period. Therefore, the requirement of mTORC1 for persistent elevation of MPS in healthy human skeletal muscle is unknown. Several recent investigations in rodent skeletal muscle suggest that mTORC1 inhibition does not ablate the effects of muscle contraction on MPS during a more prolonged recovery period following isometric/eccentric contractions, mechanical overload, or endurance exercise (130, 131, 135, 181, 188). These observations are potentially important, as they imply that mTORC1-independent mechanisms may stimulate MPS after anabolic stimuli, especially later in the recovery period. Collectively, these studies (130, 131, 135, 181, 188) have begun to identify mechanisms that are rapamycin-sensitive, rapamycin-insensitive, and mTORC1-independent. In this section, the possible candidates for mTORC1-independent regulation of MPS (Fig. 3 and Table 1) are summarized, with a focus on the mitogen-activated protein kinase/extracellular signal-regulated kinases 1/2 (MAPK/ERK1/2) pathway.

MAPK/ERK1/2 Pathway

The MAPK/ERK1/2 pathway consists of several kinase-mediated steps that phosphorylate and initiate activation of MAPK/ERK1/2 (107). This cascade begins at the plasma membrane via binding of a ligand to a receptor or activation of focal adhesion kinase and integrins by mechanical stimuli (107, 152, 153). In response, guanine exchange factors become activated and recruit Ras proteins toward the plasma membrane (51). Ras proteins become GTP-loaded (active), elevating the kinase activity of rapidly accelerated fibrosarcoma proteins (104). These kinases then phosphorylate and activate MAPK and ERK kinases (MEKs), which serve to phosphorylate and activate ERK1/2 at Thr^{202} and Tyr^{204} (ERK1/2$^{Thr202/Tyr204}$) (147, 156). In most cases, this phosphorylation event is unaffected by rapamycin and, therefore, is believed to occur in a mTORC1-independent manner (31, 138, 181). Activated ERK1/2 can then phosphorylate several downstream targets, some of which are implicated in protein translation.

Insights from healthy human skeletal muscle. Phosphorylation of ERK1/2$^{Thr202/Tyr204}$ in human muscle seems to be mainly initiated by mechanical loading, as protein feeding alone has no effect on these residues (124) and addition of BCAA ingestion does not augment the exercise-induced elevation (97). Changes in ERK1/2$^{Thr202/Tyr204}$ phosphorylation following mechanical loading are commonly noted within 1 h of a resistance exercise bout (37, 88, 114, 124, 168, 184), although elevations up to 24 h postexercise have been reported (37, 114). Conversely, some studies report no change (69, 123) or a reduction (174) in ERK1/2$^{Thr202/Tyr204}$ phosphorylation in

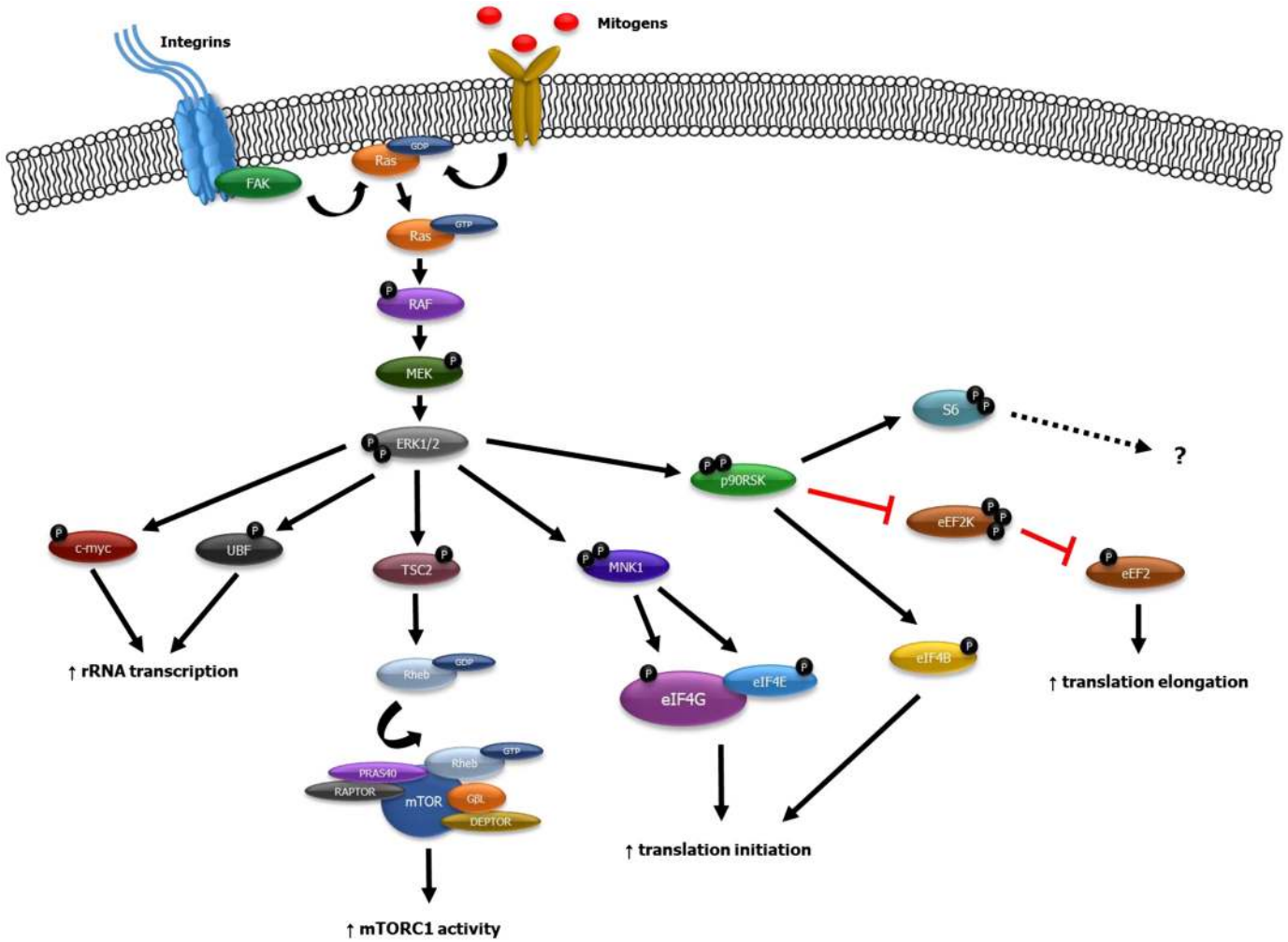

Fig. 3. Mechanistic target of rapamycin (mTOR) complex 1 (mTORC1)-independent regulation of protein translation. The majority of mTORC1-independent mechanisms governing protein translation occur via the MAPK/ERK1/2 pathway. This pathway begins at the cell membrane with activation of Ras kinases via conversion of GDP to GTP. These RAS proteins then initiate a signaling cascade, culminating in phosphorylation and activation of ERK1/2, which will phosphorylate several downstream targets, the most predominant of which is p90 ribosomal protein S6 kinase (p90RSK). This kinase phosphorylates a set of substrates similar to ribosomal protein S6 kinase 1 (S6K1). ERK1/2 will also phosphorylate MAP kinase-interacting kinase 1 (MNK1), which elevates translation initiation through phosphorylation of eukaryotic initiation factor (eIF) 4G and eIF4E. rRNA transcription is also elevated in an ERK1/2-dependent fashion via phosphorylation of c-myc and upstream binding factor (UBF). Finally, ERK1/2 signaling may affect mTORC1 activation through phosphorylation of tuberous sclerosis complex 2 (TSC2), which is removed from mTORC1's direct activator Rheb. eEF, eukaryotic elongation factor; FAK, focal adhesion kinase; P, phosphorylation; RAPTOR, regulatory-associated protein of mTOR; PRAS40, proline-rich AKT substrate of 40 kDa; DEPTOR, DEP domain-containing mTOR-interacting protein; GβL, mammalian lethal with SEC13 protein 8/G protein β-subunit-like.

postexercise recovery. Although this cellular mechanism is believed to be mTORC1-independent, investigations utilizing rapamycin in young, healthy human skeletal muscle have shown an ablation of the resistance exercise-induced elevations of ERK1/2$^{Thr202/Tyr204}$ when rapamycin is ingested (48). This, however, is in conflict with both in vitro and rodent skeletal muscle data, which show ERK1/2$^{Thr202/Tyr204}$ phosphorylation to be mTORC1-independent (31, 35, 135, 181), suggesting that further investigations are required for a full understanding of the regulation of this pathway in humans.

Resistance exercise volume may affect the magnitude of ERK1/2$^{Thr202/Tyr204}$ phosphorylation. Completion of an exercise bout consisting of 5 sets of 10 repetitions (at 10 RM) compared with 15 individual repetitions (at 1 RM) (~3-fold greater volume in the 10-RM condition) elicited greater changes in ERK1/2$^{Thr202/Tyr204}$ phosphorylation 30 min postexercise (88). These results mirror reports showing greater ERK1/2$^{Thr202/Tyr204}$ phosphorylation when 30% 1-RM loads are lifted to volitional failure than when 90% 1-RM loads are lifted to failure (29). Collectively, this suggests that the volume of mechanical loading is associated with the extent of ERK1/2$^{Thr202/Tyr204}$ phosphorylation, given full motor unit recruitment in both 30% and 90% 1-RM conditions (29).

Endurance exercise has also been reported to elevate ERK1/2$^{Thr202/Tyr204}$ phosphorylation, with elevations occurring immediately following a 60-min cycling bout at 70% of peak O_2 uptake (6, 13) and returning to basal levels by 3 h postexercise (13), although a more prolonged activation is reported after treadmill running (2). In addition, high-intensity sprints elevate phosphorylation of these residues (61), suggesting that varied mechanical stimuli activate the MAPK/ERK1/2 pathway. Despite elevations of ERK1/2$^{Thr202/Tyr204}$ phosphorylation and

Table 1. *Summary of evidence regarding phosphorylation events implicated in regulation of MPS*

Protein	Phosphorylation Site	AA Ingestion	Resistance Exercise	AA Ingestion + Resistance Exercise	Implicated in Age-Related Anabolic Resistance?	References
S6K1	Thr^{389}	↑ ↑	↑ ↑ ↑	↑ ↑ ↑ ↑ ↑	✓	5, 20, 22, 24, 29, 34, 48, 57, 59, 62, 71, 75, 87, 91, 96, 100, 101, 103, 117, 120, 123, 124, 143, 186.
rpS6	$Ser^{235/236}$	↑ ↑	↑ ↑ ↑	↑ ↑ ↑ ↑ ↑	✓	48, 59, 100, 114, 167, 169.
	$Ser^{240/244}$	↑ ↑	↑ ↑ ↑	↑ ↑ ↑ ↑ ↑	✗	22, 25, 48, 114.
eIF4B	Ser^{422}	N/A	N/A	↔	N/A	186
eEF2	Thr^{56}	↑ /↔/?	↓ ↓ ↓	↓ ↓ ↓	✗	5, 22, 25, 42, 43, 44, 47, 48, 62, 121, 122, 160.
4EBP1	$Thr^{37/46}$	↑ ↑	↑ / ↓ /↔/?	↑ ↑	✓	5, 33, 34, 42, 43, 49, 59, 62, 95, 96, 103, 119, 121, 124.
	Thr^{46}	N/A	↔	↑ ↑	N/A	121
	Ser^{65}	N/A	↔	↑ ↑	N/A	121
eIF4G	Ser^{1104}	N/A	↑	N/A	✗	52, 115.
ERK1/2	Thr^{202}/Tyr^{204}	↔	↑ ↑ ↑	↑ ↑ ↑	✓	29, 37, 47, 59, 88, 114, 124, 168, 184.
p90RSK	Thr^{573}	N/A	↑ ↑	N/A	✓	25, 33, 184.
	Ser^{380}	↔	N/A	↑ ↑	N/A	114, 124.
MNK1	$Thr^{197/202}$	N/A	↑ ↑	N/A	✓	47, 48, 55, 56, 60, 114, 124, 184.
eIF4E	Ser^{209}	N/A	↑ ↑ /↔ (Possible effect of trained state)	↑ ↑ (Only in trained state)	✗	5, 56, 114, 173, 183, 184.
UBF	Ser^{388}	N/A	↑ ↑	N/A	N/A	56, 65.
	Ser^{484}	N/A	↑ (Only in later stages of recovery)	N/A	✗ (Only measured in early stages)	22, 56.

MPS, muscle protein synthesis; S6K1, ribosomal protein S6 kinase 1; rpS6, ribosomal protein S6; eIF, eukaryotic initiation factor; eEF2, eukaryotic elongation factor 2; 4EBP1, eukaryotic translation initiation factor 4E-binding protein 1; p90RSK, p90 ribosomal protein S6 kinase; MNK1, MAP kinase-interacting kinase 1; UBF, upstream binding factor. Arrows indicate effect on phosphorylation event as follows: ↑ / ↓, small effect; ↑ ↑ / ↓ ↓, moderate effect; ↑ ↑ ↑ / ↓ ↓ ↓, large effect; ↔, no effect. Combined effect of amino acid (AA) ingestion and resistance exercise is shown by summation of arrow number from each individual stimulus. N/A, no data available for this scenario;?, lack of conclusive data.

MPS across exercise modalities, direct correlations between the two measures, as well as an understanding of the impact of $ERK1/2^{Thr202/Tyr204}$ phosphorylation on muscle subfraction (e.g., myofibrillar and mitochondrial pools) synthesis rates, are lacking. However, the mechanically sensitive nature of this posttranslational modification implies that it may be mainly related to turnover of the contractile portion of human skeletal muscle (myoMPS).

p90 Ribosomal Protein S6 Kinase

The first target of the ERK1/2 kinases to be linked to protein translation is p90 ribosomal protein S6 kinase (p90RSK), as phosphorylation of this kinase at Thr^{573} initiates a cascade that culminates in Ser^{221} phosphorylation and full activation of p90RSK (36). Similar to S6K1, p90RSK primarily coordinates phosphorylation of rpS6 at $Ser^{235/236}$ (148). As previously discussed, the full effects of $rpS6^{Ser235/236}$ phosphorylation are unclear but are purported to contribute to cap-dependent translation (149). Furthermore, in several studies in rodent skeletal muscle, rpS6 phosphorylation at these sites was inhibited by rapamycin/mTORC1 inhibition (135, 181, 188), suggesting that this may not be the mechanism whereby ERK1/2 signaling exerts its mTORC1-independent effects on translation. In addition to targeting rpS6, p90RSK has several other downstream targets that contribute to mRNA translation. p90RSK can phosphorylate eIF4B at Ser^{422}, the same site at which S6K1 phosphorylates this initiation factor (157); however, again, rapamycin treatment blocks Ser^{422} phosphorylation, suggesting that the mTORC1 pathway is dominant at this site (86, 140).

Insights from healthy human skeletal muscle. Similar to ERK1/2 phosphorylation, alterations in p90RSK phosphorylation (Ser^{380} and Thr^{573}) have been observed within 10 min of exercise cessation (33, 114), and p90RSK phosphorylation (Ser^{380} and Thr^{573}) can remain elevated for 5–24 h postexercise, depending on the phosphorylation site measured (25, 124). Another phosphorylation site on p90RSK, Thr^{359}/Ser^{363}, is shown to be elevated only following eccentric contractions (58). Completion of 3 sets vs. 1 set of resistance exercise did not further augment $p90RSK^{Thr573}$ phosphorylation (25), despite greater increases in rates of MPS after 3 sets. Feeding seems to have little to no effect on $p90RSK^{Thr380}$ phosphorylation (124); however, only one site (Thr^{380}) has been investigated in response to feeding alone, so further research is needed. In summary, existing evidence suggests that p90RSK phosphorylation occurs in response to mechanical loading and may contribute to mTORC1-independent increases in MPS after resistance exercise.

As p90RSK and S6K1 share a variety of downstream substrates, it is difficult to separate the activities of these two kinases following anabolic stimuli in human skeletal muscle. One potential way to separate p90RSK and S6K1 is by use of rapamycin, which in theory should specifically inhibit S6K1 (mTORC1-dependent), but not p90RSK. The Rasmussen laboratory (48) reported a rapamycin-induced ablation of postexercise elevations of MPS that was accompanied by elevated $rpS6^{Ser235/236}$ phosphorylation at 2 h postexercise. Although this could suggest that p90RSK was catalyzing this phosphorylation event, $S6K1^{Thr389}$ phosphorylation was also elevated,

so a mTORC1 effect cannot be ruled out. Therefore, further research is required to fully elucidate whether substrates such as rpS6 are phosphorylated in a mTORC1-independent manner in human skeletal muscle.

MAP Kinase-Interacting Kinase 1

A second target of ERK1/2 is MAP kinase-interacting kinase 1 (MNK1), which is phosphorylated at $Thr^{197/202}$ by both ERK1/2 and p38 mitogen-activated protein kinase in a mTORC1-independent manner (179). It is believed that once MNK1 becomes phosphorylated, it binds with eIF4G, the scaffolding of the translation preinitiation complex (137, 159). MNK1 then initiates phosphorylation of eIF4G at Ser^{1186} to stabilize this interaction while also phosphorylating a second component of the preinitiation complex, eIF4E, at Ser^{209} (41, 172). The interaction among MNK1, eIF4G, and eIF4E seems to be vital for MNK1-mediated kinase activity toward eIF4E (137). Given that 4EBP1 must be removed from eIF4E in a mTORC1-dependent process before eIF4G can bind to the 5′ cap of the mRNA strand, a synergism between mTORC1 and ERK1/2 pathways is possible (66). Thus, MNK1-dependent phosphorylation of eIF4E provides another mechanism by which anabolic stimuli may exert mTORC1-independent effects on protein translation; however, full functionality of this particular mechanism may also rely on mTORC1-dependent events.

Insights from healthy human skeletal muscle. MNK1 is a downstream substrate of ERK1/2 that is phosphorylated at $Thr^{197/202}$. $MNK1^{Thr197/202}$ phosphorylation has been relatively well characterized in healthy human skeletal muscle following anabolic stimuli, increasing immediately after acute exercise (114, 184) and remaining elevated for up to 3 h of recovery (47, 56, 114). Although the influence of protein ingestion alone on $MNK1^{Thr197/202}$ phosphorylation is yet to be studied, given that its upstream kinase ERK1/2 is activated by mechanical stimuli, it could be postulated that $MNK1^{Thr197/202}$ is similarly mechanically regulated (124).

$MNK1^{Thr197/202}$ phosphorylation is increased by low-load resistance exercise, but only in the blood flow-restricted state (60), implying the importance of increased metabolic load in the absence of mechanical stimuli. Training status does not seem to affect $MNK1^{Thr197/202}$ phosphorylation, as an identical bout of resistance exercise elicits similar elevations of $MNK1^{Thr197/202}$ phosphorylation before and after 8 wk of resistance exercise training (55). This suggests that the contraction-mediated regulation is conserved across both naïve and accustomed stimuli. Intriguingly, and similar to $ERK1/2^{Thr202/Tyr204}$ phosphorylation, rapamycin ingestion may attenuate the effects of contraction on $MNK1^{Thr197/202}$ phosphorylation in human muscle (48), which could suggest that, contrary to in vitro data (31, 35), it is a mTORC1-mediated event in humans. This suggests the need for further research in young, healthy human skeletal muscle to establish the true upstream signaling pathways that affect $MNK1^{Thr197/202}$ phosphorylation.

The two main substrates of MNK1 are eIF4G and eIF4E, which are phosphorylated on Ser^{1186} and Ser^{209}, respectively (137, 159, 172). In human skeletal muscle, $eIF4G^{Ser1186}$ has not been investigated, and the more commonly used readout of MNK1 activity is $eIF4E^{Ser209}$, most likely as it is more directly associated with translational capacity (64). $eIF4E^{Ser209}$ phosphorylation rises at 1–2 h postexercise in untrained individuals (48, 56) and, in some cases, can remain elevated 24 h following a bout of unaccustomed resistance exercise (115). Moreover, $eIF4E^{Ser209}$ phosphorylation in young, healthy human skeletal muscle does not seem to be affected by rapamycin ingestion, reinforcing the notion that it is a mTORC1-independent mechanism (48). However, in other reports, training status seems to affect $eIF4E^{Ser209}$ phosphorylation (183), in contrast to its upstream regulator, $MNK1^{Thr197/202}$, suggesting that training status may actually affect MNK1 kinase activity without alterations in its phosphorylation status or that other kinases are able to phosphorylate eIF4E on this residue. Wilkinson et al. (183) utilized a contralateral exercise model whereby one leg completed chronic endurance exercise training and the other completed chronic resistance exercise training. An acute bout of either exercise type in the untrained state did not affect $eIF4E^{Ser209}$ phosphorylation; however, when performed after 10 wk of training, both exercise types elicited an increase in $eIF4E^{Ser209}$ phosphorylation immediately postexercise (183). Moreover, resistance exercise elicited a further elevation of this phosphorylation event at 4 h postexercise, whereas endurance exercise did not. This suggests a divergent response between exercise types, but only in the trained state. This notion has been further reinforced by the absence of a change in $eIF4E^{Ser209}$ phosphorylation immediately postexercise in untrained individuals (184) and an elevation of phosphorylation at 5 h postexercise in untrained individuals only when resistance exercise was completed (173). How training status creates a molecular environment favoring $eIF4E^{Ser209}$ phosphorylation postexercise and whether this contributes to subsequent fraction-specific increases in rates of MPS postexercise (183) remain to be determined.

Potential Link to mTORC1 Activation via Tuberous Sclerosis Complex 2

It is intriguing that the MAPK/ERK pathway has also been implicated in the activation of mTORC1 itself. In the presence of an ERK-specific inhibitor, the normal elevations of tuberous sclerosis complex (TSC) 2 (TSC2) phosphorylation at Ser^{664} ($TSC2^{Ser664}$) following epidermal growth factor treatment in HEK-293 cells were significantly reduced (108). Moreover, these attenuations in ERK-mediated TSC2 phosphorylation caused a reduction in mTORC1 activity, suggesting an impact of the ERK pathway on mTORC1 activation following anabolic stimuli (108, 109). As phosphorylation of $TSC2^{Ser664}$ disrupts the TSC1-TSC2 heterodimer, it is thought that these phosphorylation events elicit removal of TSC2 from Rheb, a direct mTORC1 activator (190). At this point, Rheb is able to become GTP-loaded and bind with the catalytic domain of mTOR, enhancing its kinase activity (106), a cellular event we have observed on several occasions in young, healthy human skeletal muscle (2, 3, 162). These observations further suggest an overlap and/or synergism between these two pathways in the activation of translation machinery. Although this particular phosphorylation event is yet to be investigated in human skeletal muscle, data from rodent skeletal muscle suggest that $TSC2^{Ser664}$ phosphorylation is elevated following forced eccentric contractions (92), implying that this may be a promising avenue for future research in humans.

Eukaryotic Elongation Factor 2

As previously mentioned, the phosphorylation state of $eEF2^{Thr56}$ is governed by the activity of eEF2K, which is phosphorylated and inhibited by S6K1 at Ser^{366}. This phosphorylation of eEF2K renders its kinase ability inactive and, therefore, reduces $eEF2^{Thr56}$ phosphorylation and allows rates of translation elongation to be accelerated. These phosphorylation events were initially believed to be mTORC1-dependent through S6K1; however, there is recent evidence that reductions in $eEF2^{Thr56}$ phosphorylation in rodent skeletal muscle in response to muscle contraction still occur in the presence of rapamycin (181). Therefore, it seems that there are mTORC1-independent mechanisms that either inhibit eEF2K or catalyze the dephosphorylation of eEF2 itself. Similar to its effects on other substrates of S6K1, p90RSK can also phosphorylate $eEF2K^{Ser366}$, thereby inhibiting this kinase (177, 178). This finding was reinforced by the notion that this phosphorylation event can still occur in response to insulin-like growth factor-1, despite the presence of rapamycin, although to a lesser extent than in "normal" conditions (178). This suggests that S6K1 and p90RSK "merge" to inhibit eEF2K and activate eEF2 and, therefore, may explain, in part, the rapamycin-insensitive induction of eEF2 in skeletal muscle after eccentric exercise (181) (see above for insights from healthy human skeletal muscle for this mechanism).

RIBOSOMAL BIOGENESIS-RELATED SIGNALING

Ribosomal biogenesis appears to be regulated by both mTORC1-dependent and -independent mechanisms. mTORC1-mediated activation of S6K1 regulates ribosomal biogenesis by increasing rates of rRNA transcription (77). Rapamycin ablates the serum-refeeding-induced increase in rRNA transcription (90), and the knockdown of S6K1 has a similar effect (77), a finding that has been recently demonstrated in rodent skeletal muscle in vivo (111). Reduced levels of rRNA reduce the translational capacity of the cell (129). These serum-refeeding experiments (77, 90) demonstrated that S6K1 is necessary and sufficient for ribosomal DNA transcription and that these effects are mediated by phosphorylated upstream binding factor (UBF). In turn, phosphorylated UBF cooperates with RNA polymerase transcription factor I (SL1) to mediate human rRNA synthesis (11). More recent research has suggested that phosphorylation of UBF at Ser^{388} is required to enhance the transcription of ribosomal-related genes (175); however, early exercise-induced phosphorylation at this site appears to be independent of mTORC1 in rodent skeletal muscle (181). Conversely, phosphorylation of UBF at Ser^{637} was rapamycin-sensitive in early ($<$3 h), but not late (6–18 h), stages of recovery from muscular contractions, and rapamycin was also able to attenuate the postcontraction elevations of precursor rRNA (181). Future research should therefore aim to determine if the effects of UBF on rRNA transcription are exclusively mTORC1-dependent and, if so, to identify the underpinning levels of regulation in human skeletal muscle.

c-Myc upregulates the transcription of rRNA and genes encoding ribosomal proteins (72, 80, 154). Phosphorylation at Ser^{62} is thought to stabilize c-myc, allowing it to bind with certain promoter regions and enhance transcription (155). Inhibition of ERK1/2 expression with siRNA or a chemical inhibitor reduced phosphorylation at Ser^{62} and c-myc binding to promoter regions (12). Therefore, ERK-mediated c-myc phosphorylation and stabilization may be a further mechanism of enhanced mRNA translation through elevated translation capacity.

Insights from Healthy Human Skeletal Muscle

In young, healthy human skeletal muscle, UBF^{Ser484} phosphorylation, an event that primes UBF for phosphorylation at Ser^{388} and Ser^{637}, remained unchanged 1.5 h after resistance exercise in one study (22) but was elevated at 24 h postexercise in another (56). UBF^{Ser388} phosphorylation was also increased at 24 h postexercise and remained elevated at 48 h postexercise, when UBF^{Ser484} phosphorylation had returned to basal levels (56). This sustained elevation may provide a mechanism whereby myoMPS remains elevated for this time period, following unaccustomed resistance exercise, in an untrained population (28).

Elevations of UBF^{Ser388} phosphorylation have been reported at 1 and 3 h after resistance exercise (in the trained state) and are ablated if any form of endurance exercise is also completed (65). Despite this apparent concurrent exercise interference effect at the level of UBF^{Ser388} phosphorylation, rRNA content was greater following 8 wk of concurrent exercise than only resistance exercise training (65). This could suggest a potential negative-feedback mechanism whereby signaling related to rRNA transcription is reduced as rRNA content increases, the latter being a response that is positively associated to hypertrophy (55, 164). Conversely, in a separate study involving 8 wk of resistance training, significantly elevated basal levels of UBF^{Ser388} phosphorylation were reported after training (55). Therefore, as data regarding these phosphorylation events are equivocal, more research is needed to fully understand such mechanisms and how they impact basal and postexercise MPS. In addition, as research regarding the impact of $c\text{-}myc^{Ser62}$ phosphorylation in human skeletal muscle is lacking, future work should focus on understanding the impact of anabolic stimuli on this posttranslational modification and its ramifications for MPS.

ARE THESE MECHANISMS IMPLICATED IN AGE-RELATED SKELETAL MUSCLE ANABOLIC RESISTANCE?

Skeletal muscle "anabolic resistance" is defined as an attenuated MPS response to anabolic stimuli such as resistance exercise and/or AA ingestion (34, 128). As rates of MPS are underpinned by molecular mechanisms described above, it is likely that some of these signaling events would be compromised in populations exhibiting anabolic resistance, the most characterized of which is older populations. Data regarding these signaling pathways in this population and whether a relationship exists between them and MPS are summarized below.

Aging populations are one of the most commonly studied populations that experience anabolic resistance, which is believed to be a primary driver of sarcopenia, the age-related loss of muscle mass and function (15). Indeed, the dose of dietary protein required to maximize MPS in older individuals is approximately double that required by younger individuals (125). Moreover, MPS rates in response to a novel bout of acute resistance exercise are attenuated up to 24 h postexercise in older individuals compared with a younger cohort (59, 103),

which may contribute to some reports of an attenuated hypertrophic response to chronic resistance training (74, 102). However, the hypertrophic response to training is variable in older populations and may not be related to changes in strength (32, 180). Therefore, we argue that it is more important to consider the evidence of anabolic resistance to both nutrition and exercise as part of a larger metabolic dysregulation that could attenuate both hypertrophic and nonhypertrophic remodeling and contribute to suboptimal muscle quantity and/or quality over time.

Several studies directly compared the effect of resistance exercise and/or protein ingestion on anabolic signaling in both young and old skeletal muscle. Interestingly, higher basal levels of S6K1^{Thr389} phosphorylation have been reported in older than younger individuals (113). Ostensibly, this would suggest elevated mTORC1 activity and higher rates of protein translation; however, this was not apparent, as both older and young individuals displayed similar rates of MPS (113). It has been hypothesized that this elevated mTORC1 activity at baseline is required to maintain "normal" basal rates of MPS in older individuals (113), although this hypothesis requires further evidence, as other studies have failed to report elevations of basal mTORC1 activity (105).

There are numerous reports of reduced age-related anabolic signaling. Fry et al. (59) reported higher S6K1^{Thr389} and 4EBP1$^{Thr37/46}$ phosphorylation at 6 and 24 h of recovery from resistance exercise in young than older adults, suggesting elevated mTORC1 activation. Furthermore, elevations of ERK1/2$^{Thr202/Tyr204}$ phosphorylation at these time points occurred only in young individuals, suggesting that mTORC1-dependent and -independent pathways may be attenuated in older individuals. Importantly, these signaling deficits were congruent with lower rates of mixed MPS in older individuals (59). Others have reported similar findings in response to acute resistance exercise (22, 103). In addition, S6K1^{Thr389} phosphorylation has been shown to correlate to rates of MPS only in young individuals (103), implying that a mechanism other than reduced mTORC1 activity may be involved in age-related anabolic resistance.

mTORC1 signaling is also resistant to AA ingestion in older populations. Ingestion of 10 g of EAAs elicited a greater elevation of S6K1^{Thr389} and 4EBP1$^{Thr37/46}$ phosphorylation in young participants (34), and this response was mirrored by higher rates of MPS. A hyper-amino-acidemic/hyperinsulinemic infusion also induced divergent signaling responses (S6K1^{Thr389} phosphorylation) in young and older cohorts that were matched by reduced MPS rates in the myofibrillar and mitochondrial, but not sarcoplasmic, fractions (75). This notion is intriguing, as it suggests a fraction-specific anabolic resistance that may, in part, explain declines in muscle function and oxidative capacity that accompany aging (70, 94).

Age-related anabolic resistance is also evident after combined feeding and exercise. Francaux et al. (57) reported that resistance exercise combined with protein ingestion elicited a blunted S6K1^{Thr389} phosphorylation response in older subjects at 30 min postexercise/ingestion. Conversely, another report (53) showed no anabolic signaling attenuation in older individuals following a combination of these stimuli. Drummond et al. (47) found a delayed response, where MPS was elevated at 6 h postexercise/feeding in older individuals compared with the elevations at 3 h in the younger cohort. Anabolic signaling responses did not entirely align with MPS, as S6K1^{Thr389} and 4EBP1$^{Thr37/46}$ were elevated to a similar level in both cohorts at 3 and 6 h postexercise/feeding, and eEF2^{Thr56} was reduced similarly (47). MAPK/ERK1/2 signaling did, however, show a divergent response in the two cohorts, with ERK1/2$^{Thr202/Tyr204}$ and MNK1$^{Thr197/202}$ phosphorylation elevated only in the young cohort. This suggests that alterations in mTORC1-independent, rather than mTORC1-dependent, signaling may better explain the age-related divergence in MPS in this study (47) A further study reported an elevation of phosphorylation of many components of the MAPK/ERK1/2 pathway in basal conditions in older compared with young individuals (184). Moreover, immediately after resistance exercise, all these phosphorylation events declined in older individuals, whereas they were elevated in young skeletal muscle (184). These findings add further credence to the notion that mTORC1-independent signaling pathways are abnormally regulated in older individuals.

Although blunted signaling to feeding stimuli has been reported in older individuals (34), this is not always the case. Smeuninx et al. (160) reported no differences in S6K1^{Thr389}, 4EBP1$^{Thr37/46}$, or eEF2^{Thr56} phosphorylation between young and older individuals in response to ingestion of 15 g of milk protein, despite a greater change in myoMPS in the young cohort. Although this suggests discordance between signaling and protein turnover, phosphorylation and MPS were measured at the same time (4 h postfeeding), so preceding divergent signaling responses may have been missed. Nevertheless, similar results were reported by Mayhew et al., who found a discordance of signaling and MPS responses whereby some readouts of mTORC1 activity (i.e., rpS6$^{Ser240/244}$ and eIF4G^{Ser1108}) were elevated in older individuals, whereas MPS was elevated only in the young cohort (115). On the whole, however, older individuals often display divergent signaling responses to anabolic stimuli that are qualitatively related to an attenuated MPS response.

In comparison to the anabolic signaling pathways related to translation initiation, those associated with ribosomal biogenesis have been relatively understudied. Brook et al. (22), in their study of the effects of acute exercise, at varying stages of a training program, on UBF^{Ser484} phosphorylation in young and older individuals, reported no changes from baseline levels or differences between age groups. As UBF^{Ser484} was measured at only one time point (1.5 h) following each bout, it is possible that alterations in this signaling event were missed. Nevertheless, these data suggest that neither training nor age regulates this phosphorylation site. This study also reported that c-myc protein levels were elevated only postexercise in young, untrained muscle (22). In addition, basal rRNA content seems to be elevated in older skeletal muscle but is less responsive to an acute bout of resistance exercise than in younger muscle (165), suggesting possible resistance in this population. Collectively, these acute data suggest a potential divergence in ribosome-related gene transcription between young and older populations. However, chronic data in older humans demonstrated that the highest responders to a resistance-training program exhibit the greatest increases in rRNA content (164), suggesting that acute changes may not necessarily reflect chronic adaptations in magnitude and/or time course of change. Although data could suggest a role for ribosomal biogenesis in age-related anabolic resistance, human

data regarding related molecular signaling are limited. Future research should aim to elucidate whether these mechanisms impact skeletal muscle anabolic resistance.

FUTURE DIRECTIONS

mTORC1-dependent and -independent signaling pathways integrate to elevate rates of MPS after anabolic stimuli, predominantly regulating translation initiation and elongation, but also intersecting with ribosomal biogenesis-related signaling. In young, healthy individuals, the majority of these signaling events are well characterized; however, several mechanisms, such as S6K1 Aly/REF-like substrate (SKAR), a substrate of S6K1 (144), and protein phosphatase 2A (PP2A), an enzyme that dephosphorylates substrates of mTORC1 (134), are yet to be investigated in human skeletal muscle. eIF4G phosphorylation is also relatively understudied in human skeletal muscle, and more research should focus on this initiation factor. Furthermore, increasing amounts of evidence from in vitro and rodent models have begun to implicate other signaling pathways [e.g., activating transcription factor 4, Hippo, and TGF-β (50, 112, 176)] in the regulation of protein synthesis. As current evidence of these mechanisms in human skeletal muscle, in any population, is lacking, future research should also focus on the elucidation of their role in MPS regulation and anabolic resistance. In addition, recent evidence in young human skeletal muscle has suggested that the content of proteins involved in the signaling pathways described in this review differs between fiber types (52). As this suggests a potential for a differential regulation of anabolic signaling and MPS between fiber types, future research should also focus on understanding whether this is apparent in response to anabolic stimuli.

The cellular process of ribosomal biogenesis is attracting significant attention by virtue of its role in regulation of MPS and chronic alterations in muscle mass (65, 164, 165). Acutely, changes in phosphorylation of UBF and c-myc are important signaling nodes in the upregulation of ribosomal DNA transcription rates after anabolic stimuli (155, 175). Most of the research informing our knowledge, however, has been produced in vitro or in rodent skeletal muscle (181, 182); only a few investigations measured these posttranslational modifications in human skeletal muscle (22, 56, 65). As such, it is important that further investigations are conducted to study these anabolic mechanisms (e.g., UBF^{Ser637} phosphorylation) in human skeletal muscle and, specifically, to study whether age impacts such signaling events.

The contribution of the signaling pathways described here to aging-associated anabolic resistance is well characterized, with many studies displaying an inability to fully activate such signaling cascades in the skeletal muscle of older individuals (57, 59, 113). However, several investigations have failed to find congruence between these signaling events and MPS responses (115, 160, 189) or report that only select posttranslational modifications are affected (47). As a result, further investigation encompassing a wider array of signaling events, across a variety of postexercise/postfeeding time points, is most likely needed, so that the full extent of aging-associated signaling defects can be understood. Importantly, we recommend that muscle sampling time points should be chosen based on consideration for signaling, as well as protein turnover, as the time courses of these processes may differ. For example, a 4- to 6-h postprandial/exercise period for measurement MPS may not represent an optimal time frame for measurement of associated molecular signaling pathways that are commonly upregulated with 2 h of anabolic stimuli (7). Furthermore, additional investigations regarding the contribution of the signaling pathways described here to anabolic resistance associated with other factors, e.g., obesity and inactivity, are required, as our current knowledge is equivocal (10, 46, 68, 89). Importantly, the most well-researched signaling events contributing to MPS in populations exhibiting anabolic resistance are those associated with the mTORC1 pathway. As described in this review, many other signaling events (e.g., ERK1/2 and ribosomal biogenesis-related signaling) may regulate MPS, and these could garner greater attention in future studies aimed at advancing our understanding of anabolic resistance. It is also important to use an integrative research team that includes an expertise in exercise physiology and nutrition, as well as molecular biology, to address these research questions. While it is impressive to collect human biopsy samples and probe cutting-edge molecular signaling events, it is equally important to ensure that background dietary patterns and physical activity of research participants are well documented, given the potential for these factors (especially activity) to modulate the response to nutrient ingestion in traditionally "resistant" populations (160). Finally, the anabolic stimuli (e.g., exercise and nutrition) used within an acute experiment should be properly controlled, but also relevant to current physical activity and dietary guidelines, to facilitate more translational research. Finally, investigations regarding whether the posttranslational modifications described herein are associated with more chronic muscle adaptations (e.g., muscle hypertrophy) in both healthy and anabolic-resistant populations are lacking. Future research should also focus on these potential associations to help confirm the clinical significance of utilizing these molecular readouts as markers of skeletal muscle protein turnover/adaptations.

As a final note, recent developments in the field of "omics" (i.e., phosphoproteomics, transcriptomics, and metabolomics) analysis have begun to shed light on the intricate regulation of a multitude of signaling pathways in a variety of tissues and disease states (84, 92, 133). As MPS is most likely regulated by a combination of many signaling pathways/events, the integration of a variety of these analysis techniques may provide the most comprehensive assessment of the mechanisms underpinning MPS after anabolic stimuli. Insights from each of these analysis techniques could then be integrated to better understand whether particular signaling events are associated with alterations to the muscle transcriptome or metabolome (e.g., via weighted analyte correlation network analysis) (133). Not only would this aid in understanding the underlying mechanisms of MPS, it may also lead to identification of novel metabolites, posttranslational modifications, or transcriptional events that can be targeted by therapeutic interventions aimed at improving muscle mass/health. Importantly, these "cutting-edge" techniques should be accompanied by whole muscle/body measures, i.e., tracer-derived MPS rates, such that the true association of each signaling event to these processes can be established. Therefore, we recommend that future research should aim to utilize analysis of a variety of "omics," combined with stable isotopic tracer techniques, in an attempt to

more comprehensively answer the questions that remain in this field.

SUMMARY

A variety of molecular mechanisms that can be broadly divided into mTORC1-dependent and -independent categories regulate translation initiation, translation elongation, and ribosomal biogenesis. In young, healthy human skeletal muscle, many of these mechanisms have been investigated, are acutely regulated by anabolic stimuli, and broadly associate with elevated rates of MPS. Importantly, it is likely that some/all of these signaling events occur in combination to stimulate full alterations in MPS following anabolic stimuli. In older populations, many of these mechanisms are shown to be impaired after resistance exercise, nutritional intake, or a combination of these stimuli. More work is required to resolve equivocal signaling findings in this population and to fully understand if these signaling pathways are compromised in other populations that exhibit anabolic resistance of MPS (e.g., obese or inactive individuals).

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

N.H. prepared figures; N.H. drafted manuscript; N.H., D.W.D.W., A.P., N.A.B., and D.R.M. edited and revised manuscript; N.H., D.W.D.W., A.P., N.A.B., and D.R.M. approved final version of manuscript.

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