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Exercise-specific adaptations in human skeletal muscle: Molecular mechanisms of making muscles fit and mighty[☆]

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ABSTRACT

The mechanisms leading to a predominantly hypertrophied phenotype versus a predominantly oxidative phenotype, the hallmarks of resistance training (RT) or aerobic training (AT), respectively, are being unraveled. In humans, exposure of naïve persons to either AT or RT results in their skeletal muscle exhibiting generic ‘exercise stress-related’ signaling, transcription, and translation responses. However, with increasing engagement in AT or RT, the responses become refined, and the phenotype typically associated with each form of exercise emerges. Here, we review some of the mechanisms underpinning the adaptations of how muscles become, through AT, ‘fit’ and RT, ‘mighty.’ Much of our understanding of molecular exercise physiology has arisen from targeted analysis of post-translational modifications and measures of protein synthesis. Phosphorylation of specific residue sites has been a dominant focus, with canonical signaling pathways (AMPK and mTOR) studied extensively in the context of AT and RT, respectively. These alone, along with protein synthesis, have only begun to elucidate key differences in AT and RT signaling. Still, key yet uncharacterized differences exist in signaling and regulation of protein synthesis that drive unique adaptation to AT and RT. Omic studies are required to better understand the divergent relationship between exercise and phenotypic outcomes of training.

1. Introduction

In healthy adults, skeletal muscle comprises approximately ~40 % of total body mass and is essential to functions of daily life, including locomotion, nutrient storage, and metabolic regulation [1,2]. Skeletal muscle is fundamental to athletic performance, and a certain amount of functional muscle is vital for good health. The preventative effects of exercise against non-communicable diseases such as cardiovascular disease and type 2 diabetes are, in part, attributed to skeletal muscle responses to exercise training [3]. In addition, regular exercise helps maintain muscle mass in aging, with older individuals who are in the upper tertile of mass exhibiting lower all-cause mortality and a lower incidence of cancer-related deaths [4]. The ability of exercise to improve physical performance and health outcomes is indisputable; however, substantial molecular details are missing from our knowledge of the mechanisms that underpin the beneficial processes of muscle adaptation to exercise. Muscle adaptation is largely an intrinsic process; nevertheless, the intracellular signals that occur transiently during and in

recovery following exercise cannot yet be accurately linked to the phenotypic changes in muscle content and function that are associated with chronic, repeated bouts of the exercise stimulus. Further mechanistic understanding of how exercise leads to improvements in muscle mass and metabolic function is needed to fully ‘unlock’ the potential of skeletal muscle and optimize health and athletic performance.

Training-induced improvements in exercise performance are a direct reflection of the specificity of the perturbations elicited by the stimulus. Therefore, research on muscle responses to exercise is largely dichotomized into either aerobic or resistance-type stimuli, which lead to distinct muscle adaptations. Aerobic exercise (AE) includes activities involving relatively low forces over prolonged periods (minutes to hours), requiring drastic elevations in ATP usage and resynthesis via oxidative phosphorylation to sustain work output. The majority of ATP generated during aerobic exercise is derived from carbohydrate (i.e., glycogen) and fatty acid (i.e., intramuscular triglyceride and circulating free fatty acids) oxidation by elevations in mitochondrial oxidative phosphorylation [5]. The power output of the exercise session, often measured in W, estimates the rate of ATP consumption, and relative

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Abbreviations

1-RM	–	single repetition maximum (maximal strength)
AE	–	aerobic exercise
AMPK	–	adenosine monophosphate kinase
AKT	–	Akt serine/threonine kinase
AT	–	aerobic training
CAMK	–	Ca ²⁺ /calmodulin-dependent protein kinase
eIF	–	eucaryotic initiation factor
eEF	–	eucaryotic elongation factor
FAK	–	focal adhesion kinase
ERK	–	extracellular signal-regulated kinase
FSR	–	fractional synthesis rate
HIIT	–	high-intensity interval-type training
JNK	–	Jun N-terminal kinase
LKB1	–	liver kinase B1
MAPK	–	mitogen-activated protein kinase
MPS	–	muscle protein synthesis
MPB	–	muscle protein breakdown

mTORC	–	mammalian target of rapamycin complex
PGC-1 α	–	peroxisome proliferator-activated receptor-gamma coactivator
PIP3	–	phosphatidylinositol-3,4,5-trisphosphate
PIP3K	–	phosphoinositide 3-kinase
PRAS	–	proline-rich substrate of AKT
PTM	–	post-translational modification
RE	–	resistance exercise
Rheb	–	Ras homolog enriched in brain
RT	–	resistance training
SH2	–	Src homology region 2
SIRT1	–	sirtuin-1
STRAD α	–	Ste20-related adaptor protein- α
TAZ	–	transcriptional activator with PDZ-binding motif
TSC	–	tuberous sclerosis complex
UBF	–	upstream binding factor
ULK	–	Unc-51-like autophagy-activating kinase
VO ₂ peak	–	peak aerobic capacity
YAP	–	yes-associated protein

exercise intensity (as a percent of VO₂ peak) dictates the contribution of energy derived from carbohydrates and fatty acids available [2,5,6]. Regular aerobic training (AT) remodels skeletal muscle into a more oxidative, fatigue-resistant phenotype underpinned by changes in substrate utilization (i.e., altered % carbohydrate and fatty acid oxidation at relative work intensities), mitochondrial content, efficiency and capillarization [7,8]. Just 14 consecutive days of AT can increase VO₂ peak by 17 % in sedentary males [9], which suggests an early rapid phase of adaptation in response to unfamiliar exercise stimuli. The improvements in whole-body aerobic performance (i.e., VO₂ peak) are underpinned by muscle adaptations, including mitochondrial biogenesis, which enhances oxidative metabolism and alters substrate utilization [10,11].

Resistance exercise (RE) is characterized by movements against higher loads for a shorter duration of time relative to those associated with AT [7]. RE primarily relies on anaerobic metabolism to meet the immediate and high-energy demands of the mechanical loading of the target muscle. During very intense and brief intermittent RE, the primary source of energy is derived from the breakdown of phosphocreatine and glycogen with a high contribution, at least initially, from non-oxidative glycolytic flux, resulting in lactate production [5]. Phosphocreatine and glycogen metabolism rapidly regenerate ATP, typically able to sustain high output for ~10–30s and 30–120s, respectively, before recovery is necessary [12,13]. Although anaerobic energy pathways are the primary contributor to ATP production with RE if the effort is prolonged or repetitive, then oxidative metabolism of glucose and fatty acids occurs, and recovery from all forms of AE or RE is always an oxidative process [14–17]. Regular resistance training (RT) facilitates muscle remodeling toward growth and increased force output [18]. We have previously observed an 8.1 % increase in *vastus lateralis* muscle cross-sectional area, assessed by MRI, following 10 weeks of RT that targeted the quadriceps muscle [19]. Although there are many variables associated with RE (i.e., load, sets, frequency, type of contraction), a recent systematic review from our lab of 192 articles emphasizes that most RT prescriptions are effective for increasing muscle strength, range of standardized mean difference vs. control (0.75–1.60) and hypertrophy, range of standardized mean difference vs. control (0.10–0.66) when compared to non-exercise [14]. The evidence is overwhelming for the efficacy of RT to consistently improve measures of muscle mass, function, and strength [20].

Despite the divergent outcomes of the different modes of training, relatively little is known about the signaling mechanisms that are specific to the transition to an aerobic or resistance-trained muscle phenotype. Chronic responses of muscle to exercise occur over a time

frame of weeks-months and cannot, as yet, be readily predicted from the transient molecular responses of muscle to each exercise session. A greater understanding of the processes that link the acute responses of muscle during or soon after exercise to the longer-term changes in hypertrophy and metabolic phenotype are prerequisite for efficiently exploiting programmed training or lifestyle recommendations that reliably benefit performance and health. Molecular markers that are predictive of desirable changes in muscle phenotype could be used to optimize exercise prescription but there are issues to be resolved before this becomes a reality. Interindividual differences exist at baseline (untrained state), such that health and disease risk profile and responsiveness to environmental stimuli such as exercise training, acute pathological insults, or chronic responses to unhealthy environments give rise to different acute physiological responses to exercise. Muscles of individuals that are normal weight, overweight, or diagnosed with type 2 diabetes start from different baselines at the beginning of a training intervention, and the demonstrable benefits of exercise (e.g. relative change in insulin sensitivity) will differ in each case. Likewise, it is unclear which beneficial components of exercise occur irrespective of age or exhibit sexual dimorphism because the full repertoire of muscle molecular responses to exercise is unknown.

High-intensity interval-type training (HIIT) is a popular form of training that encompasses higher energy demand aerobic exercise [21] interspersed with rest and requires higher force than traditional lower aerobic power activity [22,23]. While there have been some studies examining the impact of intensity on post-exercise MPS [24–26] and signalling [23], the conclusion is less than clear as to whether HIIT resembles AE or RE in terms of the phenotypic changes it induces. Most evidence suggests HIIT definitely promotes mitochondrial biogenesis [27] and can lead to hypertrophy [22], although this appears to be less than that achieved with RE.

Recognizing that the cellular molecular events that transpire after exercise are complex, we hone in on some of the most commonly studied intracellular processes associated with the conspicuous adaptations to AE, namely mitochondrial expansion, or RE, increased myofibrillar protein accretion. Our review initially focuses on literature from targeted studies on protein phosphorylation because this is amongst the most extensively studied post-translational modification (PTM). We first review literature arising from targeted analysis of key proteins associated with signal transduction in muscle adaptations to exercise. Mechanical loading is a key differentiator between muscle responses to resistance versus aerobic training, and we, secondly, consider some leading candidate proteins in mechano-transduction and also the

concept that some degree of muscle ‘damage’ may be necessary or involved in distinguishing muscle responses to exercise training. The third aspect of our review addresses limitations of the existing literature that currently constrain our understanding of the mechanisms underpinning muscle adaptation. We highlight issues and new avenues of research brought forth by more contemporary phospho-proteomic analyses, which have expanded the number of proteins of interest and highlighted that complex phosphorylation networks, rather than linear pathways, may underpin muscle adaptation. A lack of knowledge on the detailed time-course of molecular responses to exercise and on protein-specific dynamics in muscle are further constraints to our current understanding of muscle adaptations to exercise training. Our review concludes by highlighting the fundamental but underappreciated role of protein turnover in muscle adaptation to AE and as well as RE, and we identify ideas for future experiments that clarify the processes that distinguish between muscle adaptations to AT versus RT.

2. Exercise-responsive phosphorylation of skeletal muscle proteins

Each session of exercise is detected in muscle as stimuli in the form of mechanical stress and metabolic perturbation. These stimuli are largely transduced through transient changes in the PTM of signaling proteins and transcription factors, which in turn alter the abundance, location, or activity of muscle proteins. The accumulated effect of repeated exercise bouts shifts the balance between the synthesis and degradation of specific proteins and elicits changes in muscle protein content and function that can lead to improvements in exercise performance. Covalent attachment by kinases or removal by phosphatases of phosphate can regulate the cellular location and activity of target proteins. Reversible phosphorylation of specific serine (S), threonine (T), and tyrosine (Y) residues is a prominent and extensively studied mechanism of intracellular signaling. Phosphorylation can be detected using antibodies specific to a phosphorylation site on a particular protein, and mechanistic studies can be conducted using phospho-mimetic or refractive proteins wherein the S/T/Y residue of interest is substituted with alanine (A) (inert/non-phosphorylatable) or aspartic acid (D) (active/phosphomimetic). We, however, acknowledge other PTM, including acetylation, neddylation, and ubiquitination are also transiently regulated by exercise and likely contribute to muscle adaptation. Nevertheless, phosphorylation has also been a dominant focus, and several canonical signaling pathways have been elucidated that collectively contribute to the adaptation of muscle, including mammalian target of rapamycin complex (mTORC), mitogen-activated protein kinase (MAPK) and AMP-activated protein kinase (AMPK) activation/modification. In addition, we briefly discuss the role of reactive oxygen species (ROS), which can modulate some phosphorylation pathways.

2.1. Mammalian target of rapamycin (mTOR)

The mTOR complex is a serine/threonine kinase that has two distinct complexes, rapamycin-sensitive mTORC1 and rapamycin-insensitive mTORC2 [28]. Each mTOR complex is defined by distinct binding partners that specify its function. Unique to mTORC1 is the subunit regulator associated protein of mTOR (raptor) and proline-rich substrate of AKT of 40 kDa (PRAS40) [29]. mTORC2 includes similar components to mTORC1 (mLST8 and DEPTOR) but lacks raptor and instead contains the rapamycin-insensitive companion of mTOR [30]. Raptor has multiple points of potential interaction with mTOR, is essential for regulating mTORC1 activity, and contains the binding sites for downstream signaling targets (i.e. p70S6K and 4EBP1) [31]. Exercise primarily regulates mTORC1 activity via upstream Ras homolog enriched in brain (Rheb), which in its GTP-bound state enhances the recruitment of substrates to mTORC1 [32]. The activation of mTOR kinase activity is denoted by the phosphorylation of specific serine residues (S²⁴⁴⁸, S²⁴⁸¹, and S²⁴⁴⁶) in addition to the phosphorylation of well-characterized

downstream targets [33–35]. The phosphorylation activation of mTOR has long been associated with muscle growth and is thought to initiate signaling for protein translation [36]. However, mTOR S²⁴⁴⁸ phosphorylation also occurs following acute AE [37], indicating mTOR S²⁴⁴⁸ phosphorylation is not specific to RE. It is, therefore, necessary to pair investigations on mTORC1 activation with RE- or AE-specific downstream targets to link signaling mechanisms with training outcomes. Well-defined downstream targets of mTORC1 kinase activity include proteins associated with ribosomal biogenesis and translation, including T³⁸⁹ of ribosomal protein S6 kinase beta-1(70S6K), T³⁷/T⁴⁶ of eukaryotic translation initiation factor 4E (eIF4E)-binding protein 1 (4E-BP1), and T⁵⁶ of eukaryotic elongation factor-2 kinase (eEF2K; a.k.a. CaMKIII). Further, mTORC1 is known to phosphorylate master regulators of the autophagy-lysosomal pathway, such as S⁷⁵⁷ of Unc-51-like autophagy-activating kinase 1 (ULK1) and S²¹¹ of transcription factor EB (TFEB) [38,39]. Together, this implicates mTOR signaling in the regulation of both synthetic and degradative pathways with acute exercise. Although mTOR S²⁴⁴⁸ has been extensively used as a marker of protein synthesis/anabolic response, it may not be a direct indicator of mTOR kinase activity, and analysis of known downstream targets is recommended to infer the presence of the active forms of mTOR complexes 1 and 2 (Fig. 1) [40].

RE stimulates mTOR activity and is the most investigated mechanism linked to skeletal muscle hypertrophy. RE-induced mTORC1 S²⁴⁴⁸ phosphorylation results in increases in mRNA translation initiation via downstream phosphorylation of 4E-BP1 T^{37/46}, resulting in disassociation of the binding protein from eIF4E allowing interaction with eIF4G and cap-dependent translation of mRNA to proceed [41]. mTORC1 phosphorylation of p70S6K1 T³⁸⁹ induces downstream C-terminal phosphorylation of rpS6 S^{235/236} and S^{240/244}, whose modification potentiates 40S ribosomal subunit binding the 5' mRNA cap, promoting assembly of the 43S pre-initiation complex [42]. Regulation of exercise-induced signaling through mTORC1 is one mechanism that contributes to shifting the balance between anabolism and catabolism in skeletal muscle, and differences in mTOR regulation may, therefore, be a point of delineation between AE and RE signaling outcomes in skeletal muscle [43].

Upstream of mTOR, phosphoinositide 3-kinase (PI3K) phosphorylates the inositol ring of phosphatidylinositol-4,5-bisphosphate (PI-4,5-P2), converting to phosphatidylinositol-3,4,5-trisphosphate (PIP3) at the plasma membrane [44]. PIP3 binds to AKT and 3-phosphoinositide-dependent kinase 1 (PDK1), increasing the phosphorylation and activation of AKT T³⁰⁸/S⁴⁷⁸ by PDK1 [45]. Phosphorylation of AKT S⁴⁷⁸ alone is not sufficient to increase AKT activity; instead, it induces a conformational change, stabilizing the active structure and promoting phosphorylation of AKT T³⁰⁸ for full activation [45]. In human muscle, following RE, AKT S⁴⁷⁸ and T³⁰⁸ are often robustly phosphorylated rapidly (immediately post-exercise) and remain elevated for ~2–3hrs post-exercise [46,47]. AKT activity can also be regulated by mTORC2 at AKT S⁴⁷⁷/T⁴⁷⁹ and AKT S⁴⁷³, hypothesized to stabilize active AKT [48]. Active AKT directly phosphorylates TSC2 T¹⁴⁶², a GTPase activating protein for Rheb, preventing signal transduction through mTOR [49]. At the same time, Rheb bound to GTP increases mTOR S²⁴⁴⁸ phosphorylation [50]. In non-muscle, HEK293 cells, phosphorylation of mTOR S²¹⁵⁹/T²¹⁶⁴ has also been demonstrated to alter interaction with raptor and PRAS40, found to be required for mTORC1 associated mTOR autophosphorylation at S²⁴⁸¹, promoting downstream signaling and growth [51]. However, phosphorylation of mTOR S²¹⁵⁹/T²¹⁶⁴ has not been demonstrated in human muscle to our knowledge.

Baar and Esser (1999) [52] first identified p70S6K phosphorylation in response to high resistance lengthening contractions and found there to be a correlation with the chronic (6-week) change in muscle mass. Work by Bodine et al. (2001) [36] characterized the AKT/mTORC1 pathway as an upstream regulator of p70S6K, with its ability to increase muscle mass and build upon the anabolic signaling mechanism of loading-induced hypertrophy. Bodine et al. (2001) [36] manipulated the

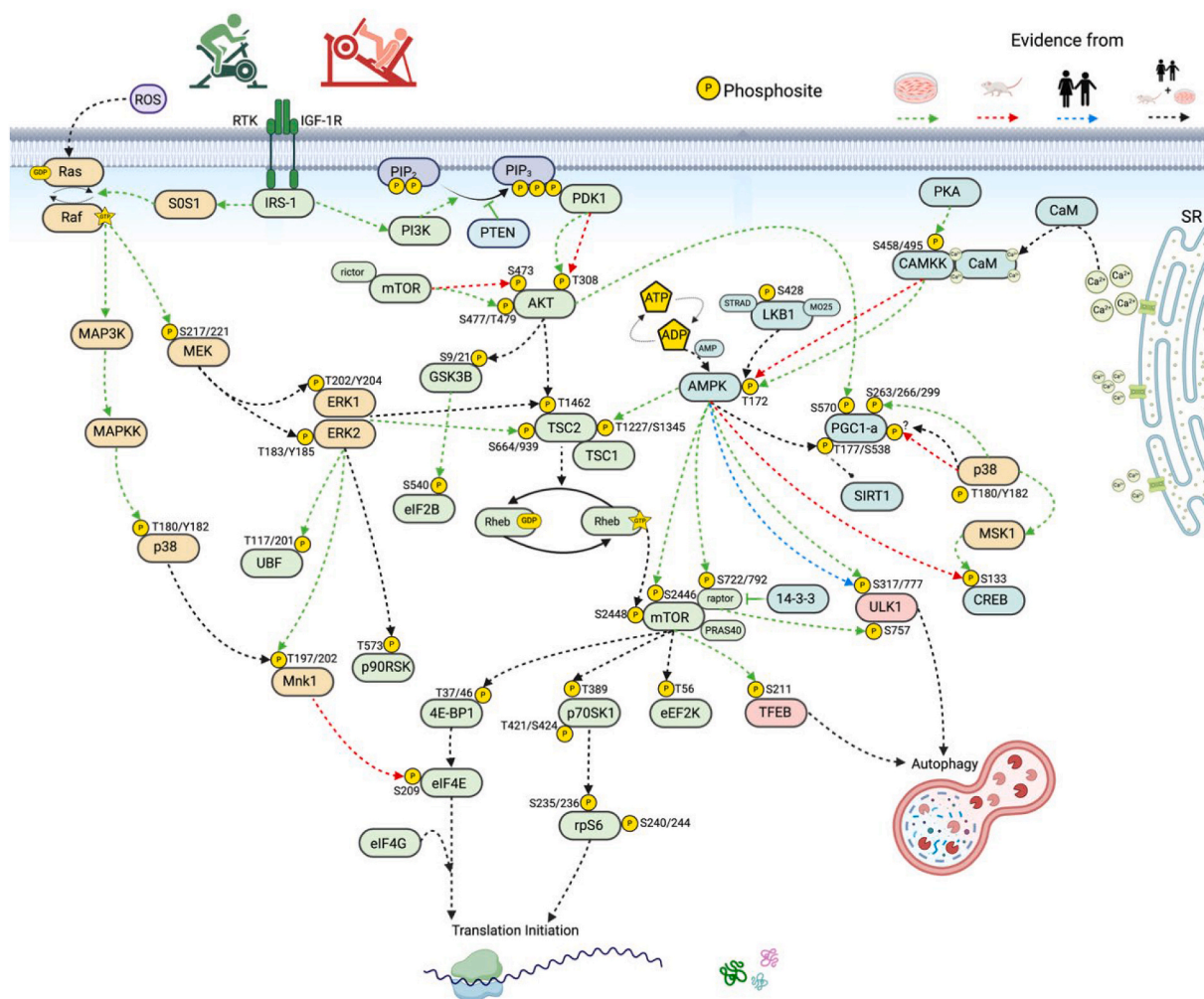


Fig. 1. Simplified signaling network of the key proteins, kinases, and transcription factors involved in the regulation of translation initiation and autophagy following acute exercise. Targeted PTM studies following exercise have identified AKT-mTOR, MAPK, and AMPK cascades as primary regulators of translation initiation and autophagy. The color of the arrow denotes the model of evidence used to demonstrate the link between signaling proteins.

AKT/mTOR signaling pathway *in vivo* to demonstrate that signaling through this pathway is a critical regulator of muscle growth, and activation is sufficient to induce hypertrophy. These data [36] led to the hypothesis that the prolonged significant activation (6 h) of p70 with a transient elevation of AKT may be resistance exercise specific (high vs. low-frequency stimulation) and potentially a point of divergent signaling adaptation between AE and RE [53]. Since the work of Bodine and colleagues [36], mTOR and its relationship to the regulation of muscle mass have been extensively studied [54]. Regulation of mTORC1 is sensitive to multiple factors, including amino acids, glucose, growth factors (IGF1), and mechanical stress [54,55]. Mechanistic work in humans demonstrates that rapamycin, a potent inhibitor of mTORC1, treatment was sufficient to ablate the immediate (1–2 h) post-exercise elevation of muscle protein synthesis (MPS) and blunt downstream phosphorylation of S6K1 T⁴²¹/S⁴²⁴ and eEF2 T⁵⁶ [17]. Early work that established an interaction between mTORC1 with increases in protein synthesis and muscle mass has led others to focus their investigations on the canonical pathways of mTORC1 signaling in relation to myriad models of acute and chronic RT [56].

The ability of mechanically induced mTORC1 S²⁴⁴⁸ phosphorylation to regulate phosphorylation of p70 T³⁸⁹ via a completely rapamycin-sensitive mechanism has been confirmed [57], and mTOR signaling is detectable yet attenuated in a trained state. In trained (18 sessions) mice, acute phosphorylation of mTORC1 downstream targets p70S6K

T³⁸⁹, rpS6 S^{235/236}, and rpS6 S^{240/244} were blunted in comparison to a single (naive) session of RE [58]. However, both total and phosphorylated proteins of the targets p70S6K T³⁸⁹ and rpS6 S^{235/236} were significantly greater than the control (non-exercising), demonstrating that mTOR signaling in trained muscle is still sensitive to mechanical stimuli [58]. Furthermore, in human skeletal muscle, AKT S⁴⁷³ and mTOR S²⁴⁴⁸ are still significantly phosphorylated following RE after 10 weeks of RT [25]. Additionally, in 8 chronically resistance-trained men, the phosphorylation of p70S6K T³⁸⁹ was found to be related ($r = 0.34$, $p = 0.03$) to myofibrillar FSR, suggesting conservation of mTORC1 signaling even in a trained state [59]. However, transient phosphorylation of AKT S⁴⁷³ is not RE-specific and was found to be elevated (50%) immediately post-exercise in response to AE (70% VO₂ peak for 60 min) in endurance-trained individuals [60]. Interestingly, the same study observed no change in AKT S⁴⁷³ or AKT T³⁰⁸ following resistance exercise (8 sets \times 5 reps maximal intensity contraction) in previously trained individuals, further suggesting attenuation of the exercise signaling response in a trained state [60].

In addition to temporal differences in signaling activity following exercise, mTORC1 activation is spatially distinct and regulated by changes in subcellular localization [61]. The presence of mTORC1 at the muscle periphery and mTOR translocation to the lysosome may be necessary for the elevated activity [62]; nonetheless, this has primarily been observed in addition to amino acid provision [63,64], but one

study has demonstrated a rapid (<30 min) increase (20 %) in mTOR translocation following resistance exercise alone [65]. The same group [66] reports mTOR translocation to the lysosomal membrane and colocalization with Rheb increased 60 and 300 min after AE (60 min run at 70 % $\text{VO}_{2\text{max}}$), with a mixed-macronutrient meal (18g protein), observed post-exercise, concomitant phosphorylation of downstream mTORC1 targets rpS6 S^{240/244} and 4E-BP1 T^{37/46}. In sum, RE and AE (albeit with amino acids) appear to be sufficient to induce mTORC1 spatial regulation and subsequent activation.

2.2. Mitogen-active protein kinases (MAPK)

MAPK are a family of highly conserved serine/threonine kinases that can influence a variety of cellular processes in response to growth stimuli, playing a key role in skeletal muscle signaling for growth, repair, and adaptation [67]. In humans, ERK, JNK, and p38 are the primary MAPK involved in the regulation of muscle adaptation to contraction-type stimuli. In addition to mTOR, MAPK activation can influence protein translation via signaling through mitogen-activated protein kinase signal-integrating kinase 1 (Mnk1). The phosphorylation of Mnk1 on T^{197/202} by ERK1/2 and p38MAPK has been shown to increase the phosphorylation of eIF4E on S²⁰⁹, influencing the affinity for the 5' -mRNA cap and decreasing protein synthesis [68,69]. The independent activity of MAPK signaling networks is partly dependent on exercise variables such as modality (AE vs. RE), duration, and intensity [70]. For example, the extent of contraction-induced ERK1/2 T^{202/Y204} phosphorylation is well correlated to the intensity of the exercise stimulus [71], which has been demonstrated for both ERK1/2 T^{202/Y204} and ERK2 T^{183/Y185} phosphorylation in an exercise intensity-dependent manner, following AE [72].

The MAPK are activated in an array of phosphorylation events facilitated via upstream MAPKKK and upstream Ras GTP signaling with Raf induces the phosphorylation of MEK (MAPKK1) at S²¹⁷ and S²²¹, a known activator of ERK [73]. MAPK signaling was first identified to be exercise-regulated by notable muscle-specific increases in MAPK phosphorylation and upstream MAPKK activity following exercise [74,75]. Subsequently, p38, JNK, and ERK signaling were hypothesized to regulate muscle growth and remodeling in response to exercise stimuli [76,77]. ERK1/2 has since been identified as an important component of the signaling pathway for the regulation of MPS [78,79].

In human skeletal muscle, p38a is known to be phosphorylated at T^{180/Y182}, increasing its kinase activity and signaling [80]. However, there appear to be distinct mechanisms for the activation and subsequent signaling of p38aMAPK as compared to ERK or JNK. AE has been shown to stimulate robust phosphorylation signaling through p38aMAPK. In contrast, the effect of RE on p38aMAPK signaling is controversial, with RE-induced activation being dependent on timing, contraction type, or volume of exercise [81]. It is hypothesized that the unique ROS produced from AE vs. RE stimulates p38MAPK signaling and subsequently increases the activity of PGC-1 α by interacting with upstream transcription factors ATF2 and MEF2 [82]. Early work in cardiac myocytes demonstrated that p38 activation is responsive to hypoxia, inducing rapid phosphorylation of Y¹⁸², thought to be regulated via the Src family of tyrosine kinases and Ras [83]. In human muscle, p38a T^{180/Y182} phosphorylation is also demonstrated to occur following high-intensity (4 bouts of all-out 30s) cycling [84]. MAPK signaling is further associated with AT outcomes, as both ERK and p38MAPK activation can lead to the phosphorylation of CREB S¹³³ via MSK1, initiating the transcription of genes involved in energy metabolism and mitochondrial biogenesis [85,86].

In human muscle, ERK1/2 T^{202/Y204} and p38MAPK T^{180/Y182} are robustly (~7 and ~4.5-fold change, respectively) and rapidly (immediately post-exercise) phosphorylated in response to AE [87]. However, it should be acknowledged that ERK1/2 T^{202/Y204} and p38MAPK T^{180/Y182} phosphorylation is not AE-specific and has also been observed following resistance exercise [88]. Interestingly, AE and RE result in a

similar magnitude of ERK1/2 T^{202/Y204} phosphorylation [87,89]; however, significant phosphorylation following RE has been detected at later times post-exercise [46], which may influence the downstream outcomes. Additionally, RE in human skeletal muscle that demonstrated MAPK (ERK1/2 T^{202/Y204}, p38MAPK T^{180/Y182}, and JNK T^{183/Y185}) phosphorylation immediately post-exercise with significant Mnk1 T^{197/202} phosphorylation but no significant increase in eIF4E S²⁰⁹ phosphorylation [90]. The lack of increase of eIF4E S²⁰⁹ phosphorylation in this study could be attributed to biopsy timing (immediate post-exercise) or the magnitude of MAPK activation or other unknown signalling regulation (Fig. 1).

A mechanism for ERK-dependent regulation of protein synthesis is thought to be mediated upstream of mTOR via TSC1-2. Specifically, ERK 1/2 phosphorylation of TSC2 S⁶⁶⁴ leads to TSC1-TSC2 dissociation, impairing TSC2s ability to inhibit mTORC1 signaling [91]. In C2C12 myoblasts, MAPK (ERK) activation has also been demonstrated to induce TSC2 phosphorylation at multiple residues (S^{664/939} and T¹⁴⁶²) and shown to regulate mTOR activity as assessed by downstream S6K1 phosphorylation at T³⁸⁹ and T^{421/S424} [92]. ERK1/2 can also modulate TSC2's GAP (GTPase-activating protein) activity towards Rheb, a negative regulator of mTORC1 activity [93]. Further, ERK1/2 T^{202/Y204} is significantly phosphorylated above rest in human skeletal muscle 1 h after RE, coinciding with increased phosphorylation of p70S6K T³⁸⁹ [89]. ERK is, therefore, responsive to both AE and RE, likely in an intensity-dependent manner, regulating protein synthesis through site-specific phosphorylation of TSC2-mTOR (Fig. 1).

Although some MAPK signaling does appear to converge on mTOR, it has been commonly reported as a mTORC1-independent mechanism of mechanically regulating muscle growth [94]. ERK2 signaling can alter protein synthesis by phosphorylation of T¹¹⁷ and T²⁰¹ of UBF (RNA polymerase factor 1), which is essential for transcription enhancement and regulating ribosomal gene expression [95]. ERK1/2 can also target the substrate p90RSK (p90 ribosomal S6 kinase) T⁵⁷³, leading to the downstream activation of transcription factors, demonstrated in human skeletal muscle following RE [90]. In addition to contraction, ERK1/2 activation and signaling are sensitive to insulin [74,96]. IGF1 knock-down has been shown to reduce ERK1/2 T^{202/Y204} phosphorylation in myotubes [97]. Fluckey et al. [98] demonstrated that insulin-mediated elevation in muscle protein synthesis following RE is dependent on ERK1/2 T^{202/Y204} and ERK T^{183/Y185} phosphorylation signaling, and inhibition of ERK has been demonstrated to prevent hypertrophy induced by IGF1 [99]. Further investigation into the exercise-induced changes in ERK1/2 MAPK signaling is necessary to determine if signaling timing and magnitude play roles in the divergent regulation of protein pool specific (i.e., myofibrillar vs. mitochondrial) synthesis associated with AT and RT.

In well-trained individuals, p38MAPK T^{180/Y182} phosphorylation still occurs in response to AE (1.6-fold increase); nonetheless, the magnitude of the response may be attenuated in comparison to naive controls (2.1-fold increase) when muscle is working at the same relative intensity [87]. This observation has also been made in rat skeletal muscle, where oxidative stress-induced activation of p38MAPK T^{180/Y182} is reduced by 59 % in trained muscle as compared to control [100]. MAPK signaling, therefore, contributes to the contraction-induced mechanisms of adaptation that occur in skeletal muscle with AE and RE, possibly altering timing and magnitude with training status. Distinct modalities (AE vs. RE), volume, and intensity of exercise may differentially regulate MAPK activation and potentially influence the divergent exercise-specific adaptations observed with training.

2.3. Adenosine monophosphate-activated protein kinase (AMPK)

AMPK is a serine/threonine protein kinase that becomes active in response to cellular stress (i.e., low nutrient availability or prolonged exercise). AMPK acts as a central regulator of cellular metabolism and is

sensitive to intracellular AMP/ADP levels (i.e., activated when the ATP:AMP ratio is lowered) and aids its function as a coordinator of growth, differentiation, and metabolism [101,102]. The most prevalent AMPKs in skeletal muscle are AMPK α 1 and AMPK α 2, and activity is primarily exercise-regulated via phosphorylation at sites T¹⁸³ and T¹⁷², respectively [103,104]. AMPK α 1 and α 2 become activated during prolonged activity and appear to stay elevated for ~3 h post-cessation of exercise [105]. However, there is some evidence to suggest exercise can differentially regulate the amplitude and timing of AMPK α 1 and α 2 activity [106]. AMPK α 2 T¹⁷² activation occurs during exercise/contraction, functioning to both generate and conserve ATP via activation of catabolic pathways as well as suppression of protein and fatty acid synthesis [107,108]. In untrained males, cycling (60 min at 70 % VO_{2peak}) elevated AMPK T¹⁷² phosphorylation 16-fold immediately post-exercise [37]. Additionally, phosphorylation of AMPK(α 1 and α 2) T¹⁷² has been observed following only 4 bouts of 30s all-out sprint cycling, demonstrating that even relatively low volume (but high intensity) aerobic-type training can cause significant cellular energy stress to stimulate altered metabolism through downstream targets of AMPK signaling (i.e. PGC-1 α) [84]. A potent upstream regulator of AMPK T¹⁷² phosphorylation in skeletal muscle is LKB1 [109–111]. Pseudokinase STRAD α promotes the active conformation of LKB1, which is stabilized by MO25 α interacting with the LKB1 activation loop [112]. LKB1 S⁴²⁸ phosphorylation alters cellular location and affinity for AMPK activation [113]. When LKB1 expression in muscle is knocked down by ~90 %, AMPK T¹⁷² demonstrates significantly reduced phosphorylation following muscle contraction [114]. Downstream, the acute increase in AMPK T¹⁷² activity regulates SIRT1 affinity for PGC-1 α by phosphorylating PGC-1 α T¹⁷⁷/S⁵³⁸ in addition to elevating the intracellular NAD⁺ concentration, which in turn modulates mitochondrial protein synthesis [115–118]. Furthermore, chronic (repeated) activation of AMPK T¹⁷², via exercise or pharmacological intervention results in increased mitochondrial content [109,119–121] and has also been postulated to alter metabolism and improve muscle insulin sensitivity due to its ability to regulate the expression of proteins (i.e., GLUT4) involved with insulin signal transduction [122–124].

During exercise, muscle protein synthesis is attenuated, presumably due to it being an energy-consuming process, and this is thought to be regulated via AMPK activity [108]. With RE, AMPK α 2 activity is significantly increased during and immediately following (1–2 h) exercise [108]. AMPK phosphorylates TSC2 T¹²²⁷/S¹³⁴⁵ and heightens its ability to suppress translation regulation via the mTORC1 pathway [125]. Additionally, work performed in TSC2-depleted cells identified raptor S⁷²²/S⁷⁹² as alternative phosphorylation targets of AMPK inducing 14-3-3 binding to raptor and subsequent suppression of mTORC1 activity [126]. AMPK can further regulate mTOR via phosphorylation of T²⁴⁴⁶, which has been demonstrated to reduce activity via attenuation of phosphorylation at mTOR S²⁴⁴⁸ [34]. Evidence of AMPK attenuating anabolic signaling has been demonstrated in human skeletal muscle, where individuals who performed high-intensity AE prior to RE had no significant elevation of phosphorylation at downstream targets of mTORC1, including p70S6 T³⁸⁹ and p70S6 T⁴²¹/S⁴²⁴ [127]. However, although mixed MPS is repressed during RE while AMPK α 2 is known to be active, there is a robust increase (75 %) in MPS following RE (1–2 h) despite AMPK α 2 remaining significantly elevated from basal levels [108]. Further, an elevation in protein synthesis has been shown to occur concomitantly with an increase in mTOR S²⁴⁴⁸ phosphorylation (significantly elevated 1- and 2-h post-resistance exercise) despite elevated AMPK [108]. Elevation of protein synthesis in spite of elevated AMPK activity suggests that the anabolic signaling from RE is able to overcome some level of AMPK-induced suppression. Interestingly, AMPK T¹⁷² phosphorylation has also been observed 24 h following RE, suggesting alternative signaling rolls beyond immediate energy stress [128], such as the promotion of autophagy by phosphorylating ULK1 at S³¹⁷ and S⁷⁷⁷ [38]. Indeed, AMPK activation with RE can serve to more than regulate immediate energy metabolism and may also signal for

alteration of mitochondrial protein synthesis, as demonstrated by increased respiration following 12 weeks of RT [129]. Furthermore, AMPK activation is also implicated in the modulation of glycolytic pathways via phosphorylation of PFK2 S⁴⁶⁶, which may alter energy metabolism adaptation with chronic RT [130].

Although AMPK activity is regulated by both AE and RE, the differences in isoform, magnitude, and timing of AMPK T¹⁷² phosphorylation may be a potential mechanism for divergent functional and phenotypic adaptation with chronic AT and RT. Although much is known regarding the regulation and interplay of AMPK and mTOR with exercise, they alone do not appear to dictate/distinguish between the divergent phenotypes observed in skeletal muscle with chronic training, which raises the question of what other signaling mechanisms occur that have not been previously considered or recognized as specific to an AT or RT phenotype.

2.4. Reactive oxygen species (ROS)

Acute exercise induces disturbances to cellular homeostasis. The metabolic perturbation from exercise induces the production of ROS thought to be derived from the mitochondria and other cell compartments in response to muscle contraction and strenuous exercise [131, 132]. Initially, ROS production with exercise was thought to have negative consequences on health and performance; however, ROS production is now recognized as an important mediator of signaling pathways in the adaptation of skeletal muscle [133]. Skeletal muscle produces superoxide and hydrogen peroxide at rest, and exercise elicits a drastic increase in the rate of production [134]. During contraction and prolonged exercise, skeletal muscle has three main contributing sources of ROS, including the mitochondria [135], xanthine oxidase [136], and NAD(P)H oxidase enzymes [133,137]. However, there is experimental evidence that challenges mitochondria as a primary source of ROS in skeletal muscle, demonstrated by ablation of activity-induced ROS production in the presence of an NADPH oxidase inhibitor [138, 139].

A likely mechanism by which ROS signals for exercise-induced adaptation in skeletal muscle is through its regulation of AMPK α indirectly and directly (redox changes to cysteine residues) [140,141] and via activation of p38MAPK (thought to be responsive to hydrogen peroxide concentrations) [142], both of which have been previously shown to increase activation in response to elevated ROS production. The attenuation of ROS (via xanthine oxidase inhibition) prevents the increase in signaling through redox-sensitive p38MAPK T¹⁸⁰/Y¹⁸² and ERK1/2 T²⁰²/Y²⁰⁴ phosphorylation, which is associated with a reduction of gene expression of mitochondrial transcription factor A (mtFA) but does not alter PGC-1 α mRNA transcription or protein content with AT [143]. ROS production also triggers an elevation in cytosolic calcium via ROS-mediated opening of calcium-release activated channels and activation of ryanodine receptors of the T-tubules, increasing phosphorylation of AMPK α T¹⁷² through a CAMKK β -dependent mechanism [144,145].

In addition to ROS, nitric oxide production is increased during muscle contraction [146]. Nitric oxide signaling can regulate AMPK α T¹⁷² and CAMK T²⁸⁶ phosphorylation [147,148] and, therefore, may influence downstream GLUT4, PGC-1 α , and mitochondrial gene expression. The sustained presence of nitric oxide can also stimulate mitochondrial biogenesis via guanylate cyclase and the generation of cGMP [148]. However, evidence for these mechanisms is lacking in human muscle, and less is known regarding how distinct ROS production with AE and RE could elicit divergent phenotypic adaptation with chronic training.

RE and AE primarily rely on different energy systems (i.e., glycolytic vs. oxidative, respectively), causing a purported distinct generation of ROS and reactive nitrogen species, which may contribute towards the distinct skeletal muscle adaptation with RT and AT [149,150]. Although ATP generation is still significantly elevated in RE, leading to the

stimulation of ROS-producing mechanisms (i.e., NOX and XO) the concentration of ROS may differ from AE and elicit unique RE-specific signaling [151]. Further evidence in human muscle is necessary to elucidate whether contraction-induced ROS plays a significant role in the determination of exercise-specific phenotype adaptation to training.

3. Mechanosensing and mechanotransduction

Mechanical loading of muscle regulates mass. Several proteins have been identified as potential mechanosensors that play a key role in mediating muscle mass [152–154]. Yet, the mechanistic link determining how load is sensed and then propagated as an acute biochemical signal to alter muscle protein synthesis is not fully understood [152, 155]. In addition to metabolic perturbation, the detection of mechanical load intrinsically in muscle may determine the downstream molecular signaling that affects a change in protein synthesis. It is important to understand which mechanosensors play prominent roles in the signaling regulation of muscle mass and how they respond under different loading/unloading conditions. This section of the review will briefly highlight some of the prominent mechanosensors, transducers, and mediators of mechanical signaling mechanisms involved in the regulation of PTM signaling pathways and potentially initiating/regulating protein synthesis for skeletal muscle adaptation.

3.1. Yes-associated protein (YAP)

Yes-associated protein (YAP) and transcriptional activator with PDZ-binding motif (TAZ) have been identified as candidate mediators of mechanical signal transduction in skeletal muscle, regulating cell size and growth through a variety of mechanisms [156]. Mechanically activated YAP mediates protein synthesis by regulating crosstalk between the HIPPO pathway and mTORC1 [157,158]. The large tumor suppressor kinases coordinate the primary mediation of YAP activity 1 and 2 (LAMP1/2) of the HIPPO pathway [159]. A secondary upstream regulator of YAP is phosphatidic acid, which is a product of phosphatidylinositol 4,5 biphosphate conversion by phospholipase C γ 1 in response to mechanical stimuli [160]. Also upstream of YAP is Bag cochaperone 3 (Bag3), a chaperone protein that relays matrix stiffness by redistributing YAP and TAZ in muscle progenitor cells [161]. The knockout of Bag3 reduces the nuclear localization of YAP and TAZ, effectively inhibiting mechanically induced signaling in myoblasts [161]. YAP in its active form induces miR-29, which downregulates the translation of phosphatase and tensin homolog deleted on chromosome 10 (PTEN), an upstream inhibitor of mTOR signaling that acts via a reduction in PDK1 ability to phosphorylate AKT1 T³⁰⁸ [158]. The expression of YAP has been demonstrated to increase as a response to acute muscle loading, with a strong correlation between the change in YAP expression and AKT T³⁰⁸ phosphorylation [159]. Watt et al. (2015) [162] demonstrated that inhibition of YAP by mutation of S⁷⁹ within the C-terminus (blocking interaction with TEAD transcription factors) was sufficient to reverse hypertrophy observed from YAP overexpression. TEAD transcription factors are known to interact with miR-29; however, this result was associated with no increase in mTOR S²⁴⁴⁸ activity [162]. Therefore, other mechanisms for YAP to mechanically influence muscle size may exist. TEAD transcription factors can also induce the expression of high-affinity leucine transporter LAT1, which may aid muscle in amino-acid-induced mTORC1 activation and elevating protein synthesis [163].

Additionally, YAP activity may also dictate muscle growth through its role in the proliferation and differentiation of satellite cells. Elevation of YAP stimulates the proliferation of activated Pax7+/MyoD + progenitor cells in muscle, whereas YAP inactivation appears necessary for terminal differentiation [164,165]. Altogether, the mechanically induced activation of YAP expression may have myriad pathways for influencing the acute signaling of MPS; however, little is known regarding the differential activity of YAP in response to AE vs. RE

loading stimuli. YAP activity has primarily been studied in the context of muscle growth and it is unclear whether YAP regulation of downstream targets may also mediate adaptation towards an aerobic phenotype.

3.2. Integrin – focal adhesion kinase (FAK)

Integrins are a family of transmembrane proteins that connect the extracellular matrix to intracellular actin, a necessary component of lateral force transduction [166]. Despite their critical role in regulation and force transduction, integrins have no known kinase activity in human skeletal muscle [167] and, therefore, must interact with other proteins to propagate mechanically induced signals. Current evidence of how integrin protein signal for elevated protein synthesis *in vivo* is lacking, and most studies have utilized FAK and ILK phosphorylation as indirect markers of integrin signaling [168]. FAK is a non-receptor tyrosine kinase that is known to coordinate signaling through the costamere-associated protein complex [152,167]. Activation of FAK kinase domain propagates mechanically induced signals and is known to regulate cytoskeletal remodeling [166,169]. There is no paucity of evidence that mechanical signals are capable of being transduced via integrin – FAK [94]. FAK Y³⁹⁷ phosphorylation is thought to regulate protein synthesis via the PI3K/AKT/mTORC1 signal pathway, as phosphorylation of FAK allows for binding to 85 kDa subunit of PI3K through the SH2 domains of p85, leading to an elevation in its activity [170]. Phosphorylation of FAK Y³⁹⁷ may also regulate mTORC1 activity through direct phosphorylation inhibition of TSC2 T¹⁴⁶² [171,172]. However, another mechanism has been identified for FAK Y³⁹⁷ as an upstream mechanosensitive regulator of p70S6K activity, coordinating protein synthesis in an AKT-independent mechanism [173]. FAK overexpression in transfected mouse muscle demonstrated significantly greater phosphorylation of p70S6K S⁴¹¹ and p70S6K T⁴²¹/S⁴²⁴ at 6 and 24 h following reloading, respectively, in comparison to control [173]. In human muscle, RE significantly increased phosphorylated FAK Y^{576/577} to a greater extent at 4 h following exercise in an untrained state, and 10 weeks of exercise training (AT and RT) was sufficient for increasing FAK Y^{576/577} phosphorylation at baseline (rest) [25]. Integrin-FAK mechanosensing may, therefore, be a key player in the signal transduction pathway coordinating transient changes in PTM signaling following exercise.

3.3. Filamin C – Bag cochaperone 3 (Bag3)

Filamin C is a Z-disc-associated protein with a “V” shaped structure that deforms when force is applied, opening to reveal binding sites for downstream signaling [174]. Filamin C has 90+ known binding partners, highlighting its involvement and significance in skeletal muscle signaling regulation [175]. The growing list of binding partners for Filamin C includes ERK1/2, titin, IGFN1, actin, and Bag3 [175]. Phosphoproteomic analysis has confirmed that both Filamin C and Bag3 phosphorylation status is altered in response to high-intensity exercise [176]. Filamin C and its interaction with Bag3 has been identified as a mechanosensitive pathway with the ability to regulate downstream mTORC1 and YAP activity [152]. As noted previously, Filamin C - Bag3 also engages in YAP/TAZ signaling in response to acute mechanical stress [174]. Additionally, Bag3 can act as a filamin signaling mediator for tension-induced increases in transcription and degradation [174]. Furthermore, cochaperone Bag3 has been demonstrated to interact and sequester the mTORC1 inhibitor TSC1 [177]. It has also been observed that mTORC1 inhibition attenuates Bag3-mediated extracellular matrix autophagy, providing further evidence for Bag3 as a central mediator of transcription in response to mechanical stress [177]. Furthermore, the phosphoproteomic analysis identified AKT and PKCa as dual kinases of Filamin C S²²³⁴/S²²³⁷, propagating PI3K/AKT hypertrophic signaling [178].

3.4. Titin

Titin is a large protein that extends from the Z-disc to the M-band with several domains that are considered to be functionally active, providing binding sites for diverse proteins, enzymes, and kinases [179]. Titin has also been implicated as a sensor of mechanical stimuli, with phosphorylation activity within the kinase domain of titin predicting hypertrophic changes in muscle following exercise in a load-dependent mechanism [180]. The serine/threonine kinase domain of titin is associated with the M-band portion of the sarcomere, belonging to the myosin light-chain kinase (MLCK S¹⁴³ and S^{586/587/588}) family of kinases, and is known to be regulated by CAMK [80,179,181]. Specifically, it has been demonstrated that CAMKKII phosphorylates titin at several sites, including Titin S²⁶, S³⁴, S¹⁷⁰, S⁴⁹⁶, T⁷⁰, T⁸⁰, T¹¹⁷ [182]. The kinase domain of titin is structurally and positionally optimal for sensing tension during muscle contraction, opening the protein, and exposing phosphorylation sites that may be upstream of mechanosensitive signaling pathways [180]. In response to mechanical stress, titin signaling has been shown to regulate gene expression and protein turnover [183]. The work by Iбата et al. (2021) [180] and van der Pijl et al. (2018) [184] demonstrate that titin may be a key mechanosensor able to propagate signaling cascades that vary in response to the proportion of mechanical stress. Titin can acutely increase protein synthesis via interaction with muscle LIM protein, which binds calcineurin and dephosphorylated nuclear factor of activated T cells (NFAT), an upstream regulator of transcription and cellular remodeling [185].

Titin is just one example of several structural proteins that can be considered key regulators in the sensing and transduction of mechanical stimuli. Although these key sensors have been proven to be essential for the signaling regulation of protein synthesis, it is not well understood how varying factors such as the intensity, load, volume, and frequency of mechanical stimuli can be sensed and elicit divergent transient signaling responses. The application of load to skeletal muscle results in a coordinated signaling network response that produces the phenotypes commonly associated with RT and AT, yet relatively little is understood about the mechanosensory mechanisms initiating and regulating this phenomenon. The interplay of several mechanosensors may be responsible for interpreting the modality and relative intensity of load on skeletal muscle. Therefore, it is critical to continue to examine these and other mechanosensitive signaling mechanisms in the context of muscle mode-specific adaptation.

4. Mechanisms for adaptation

4.1. Muscle damage as a driver of adaptation

Mechanical loading (such as RE) of muscle unaccustomed to exercise can induce muscle ‘damage.’ Damage is especially prevalent following heavy RE in exercise naïve muscle and after eccentric contractions [186, 187]. Skeletal muscle has incredible potential for repair and remodeling, adapting to handle future stress, and impeding future damage induced by bouts of loading through altered cytoskeletal structure and contractile components [188]. RE-induced damage can occur at several sites in muscle and is typically localized to the sarcomere, costamere, sarcolemma, basal lamina, contractile structures, and connective tissue [187]. Muscle damage is associated with a transient decrease in force production, muscle swelling/soreness, and metabolic perturbation due to disrupted signaling and mitochondrial damage. Unfortunately, most experimental methods for assessing contraction-induced muscle damage [189] are based on proxies of damage and do not directly assess structural disruption to the cytoskeletal or muscle architecture. Examples of such methods are creatine kinase assays, soreness (DOMS), inflammatory markers (i.e., interleukin-6, -10, macrophages), as well as satellite cell proliferation and activation. Currently, the most direct method of assessing muscle damage is with electron microscopy, quantifying focal disruption of the sarcomere thought to occur primarily at the z-disc

(z-disc smearing/streaming) or complete dissolution of the entire sarcomere [190]. Z-disc disruption is often interpreted as structural muscle damage following heavy resistance exercise [191,192]. However, this notion has been challenged by Yu et al. (2004) [193], who observed greater Z-disc disruption on days 2–3 and 7–8 post-exercise in comparison to 1-h post-exercise. Greater occurrence of Z-disc streaming and smearing in the days following exercise may, therefore, be indicative of myofibrillar remodeling and sarcomerogenesis [193]. Therefore, significant elevation of skeletal muscle proteolysis that is stimulated following exercise could present as ‘damage’ but instead may be remodeling and repair necessary for adaptation [194,195]. While damage is a plausible mechanism, we propose that what may appear as mechanical disruption of the sarcomere under electron microscopy could be an early stage of robust focal elevations in protein turnover in response to heavy, eccentric, or unaccustomed exercise [196].

Contrary to the protein turnover thesis is the idea of sarcomere ‘popping,’ which has been proposed to occur in response to heavy or eccentric contractions [197]. Briefly, it is hypothesized that marginal differences in contraction force along the length of the myofibril may cause disparate stretch in sarcomeres with less filament overlap. Under strenuous stimuli (eccentric contraction), contractile myofibrils no longer overlap and ‘pop,’ causing physical disruption to the sarcomere [197]. Remodeling to adapt to such damage is focused on structural maintenance of sarcomere stability, which could occur rapidly without a drastic increase in muscle fiber cross-sectional area [198]. This observation may explain why some markers of ‘damage’ are attenuated rapidly following only a few bouts of unaccustomed exercise without measurable hypertrophy or other (known) significant adaptations [198]. Exercise-induced muscle damage is attenuated following as little as a single session of exercise and tends to decrease even more with subsequent bouts; however, significant hypertrophic adaptation is typically not reliably measurable until 6 weeks (12–15 bouts) of RT or later [199]. Notably, protein synthesis following resistance exercise correlates with hypertrophy only after muscle damage is attenuated, as assessed by microscopy and indirect markers (i.e., reduced contraction force and CK assay) [200]. However, muscle hypertrophy occurs independent of damage, examined in naïve and trained individuals using markers of CK and soreness to assess damage [201]. Similarly, mice that performed eccentric exercise experienced myofiber hypertrophy and an increase in Pax7+ cell content without indication of disruption (damage) in myofibers [202]. Furthermore, there is evidence that satellite cells can contribute to skeletal muscle remodeling in the absence of hypertrophy [203]. We propose that the initial (early training) stimulation of protein synthesis is, at least in untrained persons, more directly related to the repair and/or remodeling of skeletal muscle. At the same time, myofibrillar hypertrophy may occur with progressive attenuation of damage after subsequent bouts with chronic RT [198]. Taken together, although damage is commonly associated with an elevation in protein synthesis and breakdown, it does not appear to be an essential factor for anabolic signaling or hypertrophy to occur in skeletal muscle. It is, therefore, unlikely a key regulator determining the different outcomes of aerobic vs. resistance exercise training.

4.2. Protein turnover in muscle adaptation

The functional attributes of skeletal muscle are underpinned by the function of its constituent proteins (i.e., the proteome), which exist in a dynamic equilibrium of protein turnover (encompassing the balance of synthesis and degradation). MPS is the metabolic process of incorporating amino acids into new muscle proteins, and MPB is the antagonistic function whereby proteins are broken down into amino acids. Rates of MPS and MPB are dynamic and can change in response to exercise and protein ingestion. Loading and aerobic exercise induce robust, transient rises in MPS, whereas periods of disuse reduce MPS and transiently stimulate MPB [204]. Unlike measures of PTM, mRNA expression, or protein content, the dynamic processes of MPS and MPB

cannot be assessed solely from ‘snapshots’ taken at isolated time points. Measurements of MPS and MPB require the use of tracer methodologies and measure the rates of incorporation of a tracer (e.g., stable isotope labeled amino acid) into muscle protein to calculate a mixed-protein fractional synthesis rate (FSR)

Typically, MPS is measured from whole muscle (bulk) or muscle sub-fractions, e.g., myofibrillar and sarcoplasmic fractions. Although bulk MPS (measured as fractional synthesis rate, typically %/h in humans) provides information on the overall efficacy of an intervention, fraction-specific data offers deeper perspectives on exercise mode-specific responses to exercise. In response to RE, Mitchell et al. (2014) [205] observed an increase in myofibrillar FSR above resting values in the recovery timeframe at both 60–180 min ($235 \pm 38\%$) and 180–360 min ($184 \pm 28\%$). Whereas, after a single session of AE, bulk (mixed muscle protein) MPS is elevated by $\sim 58\%$ between 2 and 6 h following exercise [206]. The lower MPS response to AE is not typically associated with hypertrophy, but the relative intensity of an AE stimulus may be positively associated with MPS and myofibrillar FSR [24,26]. For example, a higher intensity AE has been demonstrated to stimulate myofibrillar protein synthesis ($\sim 60\%$) over a longer time (24–28 h) as compared to a lower intensity group [26].

Studies such as those by Fry et al. (2011) [46] and Moore et al. (2011) [89] demonstrate significant regulation of kinases in the mTOR pathway (4E-BP1 and p70S6K1) at periods of 5–6 h after resistance exercise concomitant a significant increase in protein synthesis. A few groups have examined the mTOR signaling axis (AKT/mTOR/p70S6K1) along with protein synthesis over longer periods (up to 24 h) after RE; however, the results are currently difficult to interpret because the data are sparsely distributed (i.e. large gaps in-between timepoints in the 24 h period) and the exercise intervention varied in loads as well as

amino-acid provision [46,59,88,207–209]. Mixed results of later (6 < hours) mTOR signaling denotes that mTORC1-dependent signaling may play a more significant role in the early (~ 1 –6 h) rise of protein synthesis following RE, but later time points (~ 18 –36 h) may be regulated by rapamycin-insensitive or mTORC1 independent pathways [56,210]. Phosphorylation of mTORC1 S²⁴⁴⁸ is associated with a general elevation in MPS, but heightened MPS is sustained during knockout of raptor (i.e., mTORC1 inhibition), which points to an mTORC1-independent mechanism [57]. We have attempted to summarize these time courses in Fig. 2.

The exercise-induced regulation of translation initiation has been primarily studied in the context of RE and protein accretion [79]. In contrast, AE has been primarily examined in relation to its regulation of transcription factors and mitochondrial genes [211]. The effectors downstream of AE signaling-induced increases in MPS are relatively less well characterized in contrast to RE due to the context of previous literature.

In untrained individuals, translation initiation with AE may be in part mediated by mTORC1 S²⁴⁴⁸ phosphorylation and its downstream target 4EBP1 T^{37/46} [212]. However, the magnitude of mTORC1 S²⁴⁴⁸ signaling downstream targets S6K1 T³⁸⁹ and 4EBP1 T^{37/46} at 1–4 h post-exercise is less ($\sim 50\%$ lower) following AE vs. RE, which may serve as a mechanism delineating the divergent adaptation with different exercise modalities or signaling events may follow a different time course after AE vs RE. Although AE and RE significantly elevated mTOR S²⁴⁴⁸ phosphorylation 1 h after exercise, there were differences at a later time point (4 h post-exercise) following RE [212].

Wilkinson et al. (2008) [25] hypothesized that the divergent phenotypes observed with chronic AT and RT are derived by the differential repeated stimulation of predominantly myofibrillar and mitochondrial protein synthesis elicited by RE and AE, respectively. In their seminal

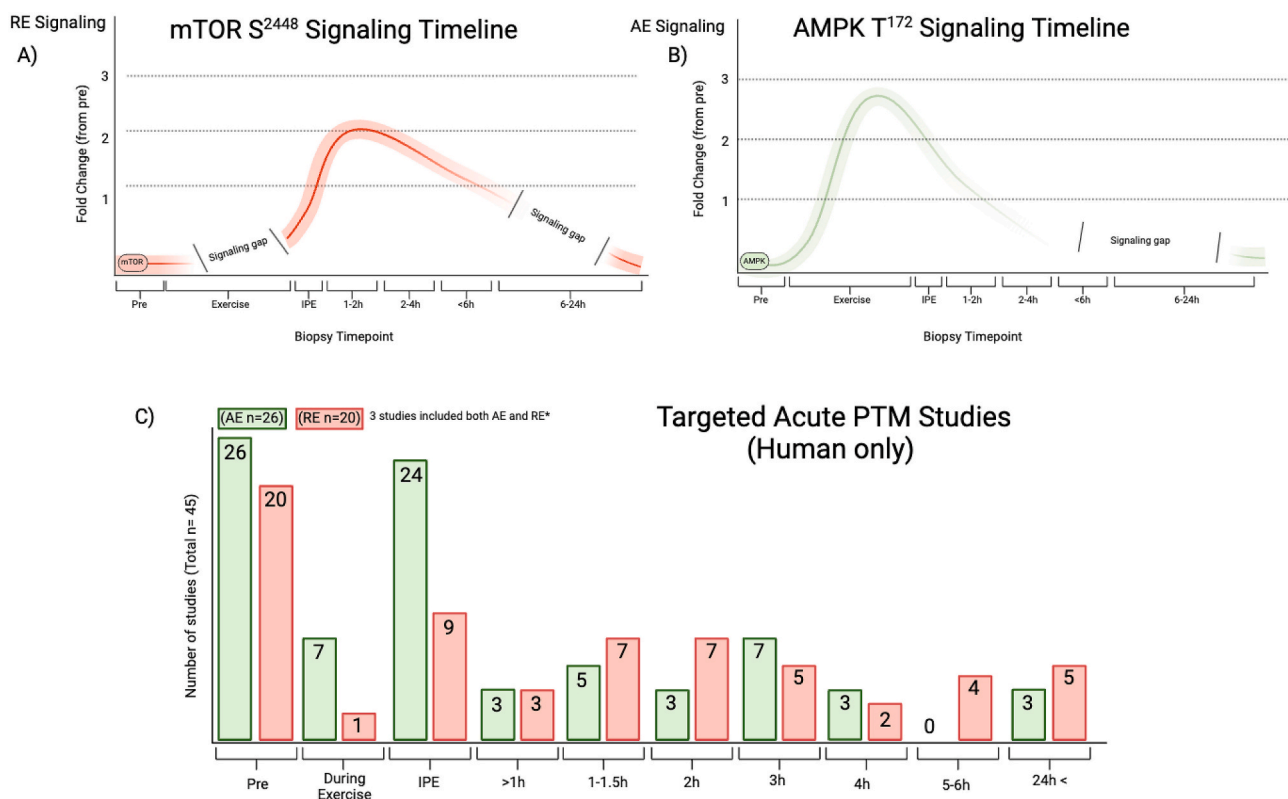


Fig. 2. Acute exercise PTM signaling timelines for representative proteins of resistance (mTOR) and aerobic (AMPK). Sixteen studies each for resistance and aerobic exercise were identified from a systematic search of the literature to have assessed (A) mTOR S2448 and (B) AMPK T172 phosphorylation following a single session of exercise. The data was used to graph the approximate average signaling response of each signaling factor following the respective exercise. (C) Forty-five studies that included data of targeted PTMs following exercise (AE and RE) were identified from a systematic search of the literature and assessed for time points of signaling (biopsies) to compare and contrast the strength of signaling data in the minutes and hours following exercise. The mean number of time points per study was 3.

study, acute myofibrillar and mitochondrial protein synthesis were measured after exercise in untrained and trained muscle (following 10 weeks of training in the same participants). In the untrained state, RE stimulated similar (~70 %) increases in myofibrillar and mitochondrial protein synthesis above resting values. Whereas AE stimulated an increase in mitochondrial protein synthesis in the untrained (by 154 %) and trained (by 104 %) state but failed to significantly alter myofibrillar FSR [25]. Additionally, after 10 weeks of training, resting myofibrillar protein synthesis was greater (~40 %) in the RT condition in comparison to AT, and the same relative acute RE stimulus elevated myofibrillar protein synthesis a further 37 % above resting values [25]. Skeletal muscle is, therefore, able to resolve different exercise modes and elicit exercise-specific stimulation of protein synthesis and subsequent adaptation.

Developments in the application of deuterium oxide as a biosynthetic label to trace dynamic processes combined with proteomic techniques offer new possibilities to gain higher resolution information on muscle responses (i.e., changes in protein synthesis, degradation, and turnover rate) that occur in response to specific stimuli (i.e., AT compared to RT). Mixed muscle and fraction-specific measurements of protein synthesis represent an average of thousands of proteins, which may respond differently to a particular exercise intervention. Deuterium oxide labeling with proteomic analyses of muscle samples can resolve the synthesis of individual proteins within the muscle [213] and has highlighted that proteins with different functions have different synthesis rates in human muscle [214]. The first dynamic proteomic analysis of human skeletal muscle responses to RT included parallel measurement of protein-specific synthesis and abundance data for almost 100 muscle proteins [215], adding new detail on the selectivity of protein turnover responses to RT. The patterns of protein synthesis and abundance changes in skeletal muscle included subsets of proteins that i) increased in turnover but exhibited no change in abundance, ii) increased in abundance without a parallel rise in synthesis rate, and iii) decreased in abundance even though the synthesis rate was increased. In comparison, profiling of individual protein synthetic rates in response to aerobic-type exercise showed a significant increase in the turnover of 22 proteins (from 409 measured) associated with energy metabolism, proteasome, and cell stress in endurance-trained muscle [216].

5. Future directions

5.1. Important considerations for acute post-exercise signalling

To date, much of our understanding of molecular exercise physiology has arisen from hypothesis-led studies targeting small numbers of proteins that have well-defined modification sites. Necessarily, the current literature includes a mix of studies at different levels (cell, animal, or human) and using different interventions (gain-loss- function, pharma, or exercise). The diversity of experimental designs is a strength but also should not be overlooked when attempting to summarise the mechanisms of exercise-induced muscle adaptation. Some mechanistic data (e.g., gain-/loss-of function in cell models) may not apply in the context of exercise. For example, data collected in non-muscle cells or experiments that did not include exercise should be treated with caution until validated specifically in the context of exercised muscle. Changes in PTM (e.g., phosphorylation of S, T, or Y) must be interpreted against the context of cooccurring changes across the networks in which protein residue is involved. Intracellular signaling represents a computational system that reads various inputs (e.g., changes in energy status, metabolism, redox state, mechanical stress, etc.) and generates a particular output (e.g., modulation of gene expression, protein synthesis, degradation, and abundance). The processing algorithm may be different in the muscles of trained athletes versus untrained individuals, or the input may be sensed differently depending on the cellular context (e.g., redox state), leading to concepts of hormesis and bi- or multi-phasic responses to exercise.

More recently, non-targeted ‘omics’ studies have brought forth new

hypotheses and may reveal new candidates that distinguish between AE and RE mode-specific adaptation. Højlund et al. (2009) [217] reported the first non-targeted data on the human muscle phosphoproteome and identified phosphorylation of muscle-specific proteins, confirming the role of phosphorylation in the regulation of skeletal muscle function. Hoffman et al. (2015) [176] reported phosphoproteomic profiling of muscle collected from young men before and immediately after ~10 min of high-intensity aerobic exercise and highlighted 1004 exercise-regulated phosphosites, of which 92 % were yet to be associated with upstream kinases [176]. Surprisingly, just 5 exercise-responsive phosphorylation sites were known substrates of AMPK, which questions the narrow focus of targeted studies on ‘master regulators’ of exercise adaptation.

Potts et al. (2017) [218] reported phosphoproteomic profiling of mouse tibialis anterior collected 1 h after a bout of maximal intensity contractions (MIC) induced by electrical stimulation. Contractile activity resulted in significant differences in phosphorylation of 621 sites on 313 proteins, with the majority (531 sites) increasing in phosphorylation after exercise. Most exercise-responsive phospho-sites had not previously been detected and only 12 phosphosites had known upstream kinases, which were mapped to ERK1/2 and CAMKII. In a subsequent study [155], rapamycin was used alongside the MIC protocol to investigate mTORC1-dependent and independent phosphorylation. Over 2000 unique phosphorylation sites were significantly regulated, but just 38 sites were rapamycin-sensitive, and most MIC-induced phosphorylation was rapamycin-insensitive anabolic pathways [155].

Exercise mode-specific phosphorylation of muscle proteins was reported by Blazev et al. (2022) [219], who identified 5486 phosphosites (on 1573 proteins) that were significantly regulated 1 h and 3 h after at least one of either endurance (90 min, 60 % of VO₂ max), sprint (3 × 30 s all-out cycling), or resistance exercise (6 sets of 10 rep max knee extensions) in 8 healthy, untrained men [219]. Phosphorylation of 430 sites were common across the different training modes, including phosphorylation of the novel gene product C18ORF25, which was validated as an AMPK substrate [219]. In contrast, the phosphorylation of rapamycin-sensitive mTORC1 substrates was specific to different training modes [219]. Phosphoproteomic analyses can reveal unique signaling networks in response to different training modes that may accumulate to result in differences in the adaptive responses to training.

Non-targeted analyses add significant power to discover new mechanisms and, simultaneously, have exposed how little is known about signaling networks and the paucity of information on which kinases regulate which phosphorylation sites. Despite their comprehensive nature, non-targeted PTM studies so far have raised more questions than answers, and it is still unclear which key signaling events might be mechanistically linked to phenotypic muscle adaptations. As yet, few omic studies have considered more than one type of PTM. This review focuses on phosphorylation, which currently has the largest body of evidence in the context of skeletal muscle and exercise adaptation. However, crosstalk exists between different PTM (e.g., ubiquitination) on the same protein [220]. Multi-PTM omic studies are required to bring a complete understanding of intracellular signaling networks.

Currently, the full repertoire of mechanisms of exercise is unknown, and many questions remain unanswered regarding how different modes of exercise result in distinct muscle responses. Even when constrained to protein phosphorylation, higher levels of complexity exist than are currently considered. The archetypal exercise substrate, AMPKα1, has 50 known sites of post-translational modification, including 36 phospho-sites, 12 ubiquitylation sites, 1 acetylation, and 1 SUMOylation site (Phosphosite, AMPKα1 human site table). Just 20 of these sites have been investigated using targeted (low throughput; LTP) methods, and >80 % (227/280) of those studies focus on a single phosphorylation site (T¹⁸³). Similarly, 34 modification sites are known on the human isoform of AMPKα2, and only 12 sites have been studied by LTP targeted analyses, including the exercise responsive T¹⁷² mapped with 222 targeted studies from a total of 249 mapped to the entirety of AMPKα2. From a

total of 109 PTM sites mapped to mTOR (Phosphosite, mTOR human site table) from discovery and targeted analyses (60 phosphorylation, 47 ubiquitylation, 2 acetylation), only 11 sites have been followed up with targeted investigation and only 2 sites (i.e. the exercise responsive S²⁴⁴⁸ and S²⁴⁸¹ phosphorylation sites) mapped with >10 targeted analyses (217 and 68, respectively from a total of 304 citations providing low throughput analysis of the human isoform of mTOR).

6. Summary

In the future, it will be necessary to understand the interactions between different types of PTM across and within different proteins. In such studies, changes in gene expression, ribosomal activity, cellular location of proteins, and degradation rate are the immediate outcome measures that, in turn, need to be related to changes to the muscle proteome (i.e., protein abundance profile) and phenotype/functional characteristics of the muscle. Adaptation is a time-dependent process that, so far, has been investigated by time-series studies of samples collected at discrete time points. Time-series studies attempt to describe the continuous dynamic process of adaptation using snapshots that are an incremental sequence of isolated points. Emerging new dynamic proteomic methods that combine proteomics with stable isotope labeling offer new opportunities to investigate the process that occurs between each sampling point. If a protein becomes more abundant from one time to the next, it is not clear whether more of that protein was synthesized or less degraded. Protein turnover is the mechanism of adaptation; therefore, readouts of synthesis and degradation should be the most relevant outcome or end point for studies on intracellular signaling.

Mechanistic studies cannot be conducted in humans but the analysis of molecular responses to exercise in human muscle is essential to validate data from cell and non-human animal models. Studies in humans provide the only substrate for within-subject analysis of molecular processes, which is a key advantage that is currently under-exploited. Both cell and animal experiments necessarily use independent groups at different time points, which introduces an additional source of error because different animals or cell cultures are used at different time points. Although the number of biopsies is limited, there are examples of multi-time point studies, and in these cases, extensive omic analyses should be employed to make the most of the limited samples. Currently, the focus on limited numbers of molecular targets at too few time points limits our understanding of the crosstalk between different molecular mechanisms involved in the adaptive process with exercise.

Despite divergent outcomes of chronic training of one form of exercise or the other, relatively little is known about the exercise mode-specific acute signaling mechanisms that are proposed to result in the transition to an aerobic or resistance-trained muscle phenotype. Undoubtedly, exercise of either type (AE or RE) results in profound transcriptional responses, with some common genes being transcribed and some that are unique [221]. The general thesis is that with each exercise session, there are transcribed genes that are subsequently translated, and the resultant proteins accumulate to change, over time, the phenotype of the muscle [2]. Studies on AE have often focused on linking signaling to transcription factors and changes in gene expression [222]. Studies on RE often try to link signaling to translation initiation and subsequent protein synthesis [223]. What we know far less about is what occurs when concurrent exercise is performed, combining both AE and RE, which were generally thought to oppose each other [224]. We propose that while the ultimate phenotype of the muscles of aerobic and resistance-trained persons are different, there may be more in common than there are distinct, at least initially, until training progresses to a point where the persistent practice of one type of exercise refines the response leading to a more distinct phenotype (Fig. 3).

The molecular responses to acute exercise in an untrained state may largely consist of a ‘generic stress’ response to the unaccustomed

Hypothesis for exercise specific PTM signaling perturbation

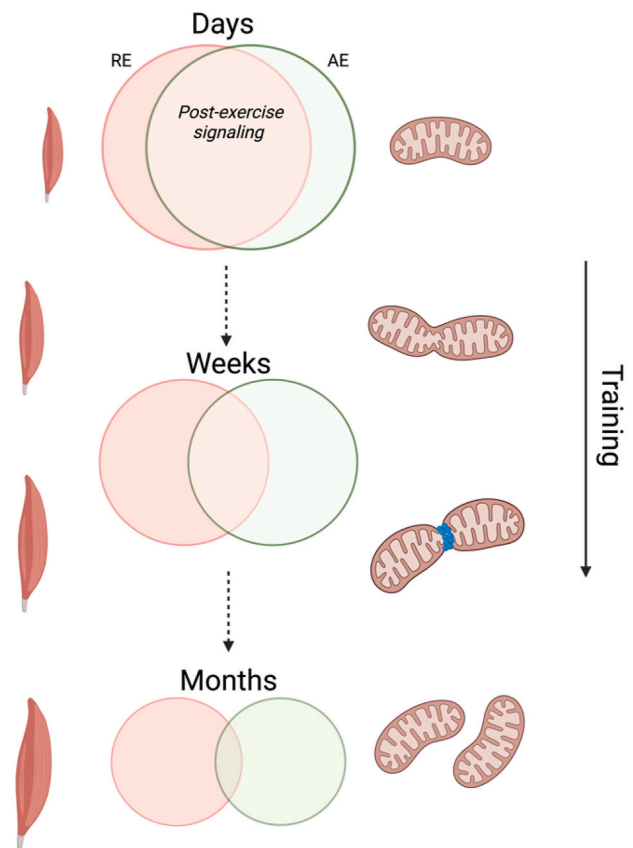


Fig. 3. Exercise perturbation in unaccustomed muscle elicits a less specific signaling (PTM, transcription, and translation) response that becomes more refined with chronic training. With repeated exercise of a specific modality, the molecular response to exercise has a lesser but more efficient PTM cascade to induce exercise-specific changes in muscle protein content and phenotype.

perturbation. Hence, only over time, with repeated exercise bouts, is muscle able to adapt to the stimuli, whereby the extent of the perturbation is refined and potentially targeted towards efficient adaptation for improved muscle function and substrate utilization specific to the exercise stimulus [225]. Therefore, a preparatory shift in the muscle proteome and phosphoproteome should be apparent in the early phase of training.

Differences should exist between the proteomes of untrained, trained, and highly trained individuals that could aid the interpretation of differences in muscle signaling in these states. In line with these hypotheses, Damas et al. (2016) [200] demonstrated that the acute exercise-induced increase in protein synthesis only correlated with hypertrophy after an initial attenuation of damage had occurred [200]. We propose that a significant overlap of exercise-induced signaling patterns exists in response to the initial exposures to exercise, regardless of modality. With chronic exposure (i.e., training), skeletal muscle transcriptional and translational programs are refined and initiate signaling pathways that drive phenotypic changes [25,226]. Acute signaling can, therefore, change as an individual becomes more accustomed to the stress of exercise training. For example, in untrained individuals, the acute signaling response of canonical signaling pathways, including AMPK, ERK1/2, and p38, is attenuated after only 10 days of intensified aerobic exercise, such that acute exercise no longer elicits a significant elevation in phosphorylation [37].

The degree of post-exercise signaling and transcription does not

necessarily equate to a proportional change in protein content or function. There exist some relationships between mRNA and protein expression that have been established [227]; still, the acute expression does not correlate well with protein translation/content or accurately predict phenotypic outcomes of training in humans [2,228–230]. More recent omic studies have substantially expanded on the discordant relationship between genes and proteins [231]. The discordance between mRNA expression and protein content may be related to (i) post-transcriptional processing and transcript stability, (ii) translational capacity and efficiency, and (iii) protein degradation. Discordant responses between PTM exercise signaling, mRNA abundance, protein synthesis, and long-term adaptation (typically assessed as change in muscle protein content) are not uncommon in exercise literature [200, 205,225,232–234]. Therefore, more omic studies that encompass transcriptomic, phosphoproteomic, proteomic, and proteome dynamics are required to unravel the relationship between acute stimuli and phenotypic outcomes.

Lastly, changes in signal transduction following AE or RE elicit divergent elevation of protein synthesis despite commonly regulated signaling pathways. For example, AE and RE have demonstrated similar phosphorylation status of AMPK T¹⁷² and FAK Y^{576/577} and eIF4E S²⁰⁹ but elicited differential responses in synthesis rates when assessing myofibrillar and mitochondrial protein fractions [25]. Therefore, studies that better relate the acute (transient) and chronic (adaptation) changes in signaling to protein-specific dynamics and muscle function are required to improve our understanding of the mechanisms of adaptation with exercise training. We hypothesize that there are key yet uncharacterized differences in the signaling and subsequent regulation of protein-specific synthesis and degradation that drive unique adaptation to AT and RT. These differences in molecular responses can only be detected with multi-timepoint/omic methods that consider both key differences and similarities in the transient PTM responses to acute exercise, as well as changes in proteome dynamics over longer periods of training.

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CRedit authorship contribution statement

Aaron C.Q. Thomas: Writing – review & editing, Writing – original draft, Conceptualization. **Connor A. Stead:** Writing – review & editing, Writing – original draft. **Jatin G. Burniston:** Writing – review & editing. **Stuart M. Phillips:** Writing – review & editing, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

- [1] W.R. Frontera, J. Ochala, Skeletal muscle: a brief review of structure and function, *Calcif. Tissue Int.* 96 (3) (2015) 183–195.
- [2] B. Egan, J.R. Zierath, Exercise metabolism and the molecular regulation of skeletal muscle adaptation, *Cell Metabol.* 17 (2) (2013) 162–184.
- [3] Z.A. Saqib, et al., Physical activity is a medicine for non-communicable diseases: a survey study regarding the perception of physical activity impact on health wellbeing, *Risk Manag. Healthc. Pol.* 13 (2020) 2949–2962.
- [4] J.R. Ruiz, et al., Association between muscular strength and mortality in men: prospective cohort study, *BMJ* 337 (7661) (2008) a439.
- [5] M. Hargreaves, L.L. Spriet, Skeletal muscle energy metabolism during exercise, *Nat. Metab.* 2 (9) (2020) 817–828.
- [6] J.A. Romijn, et al., Regulation of endogenous fat and carbohydrate metabolism in relation to exercise intensity and duration, *Am. J. Physiol.* 265 (3 Pt 1) (1993) E380–E391.
- [7] D.C. Hughes, S. Ellefsen, K. Baar, Adaptations to endurance and strength training, *Cold Spring Harb Perspect Med* 8 (6) (2018).
- [8] S.M. Phillips, et al., Effects of training duration on substrate turnover and oxidation during exercise, *J. Appl. Physiol.* 81 (5) (1985) 2182–2191, 1996.
- [9] B. Egan, et al., Time course analysis reveals gene-specific transcript and protein kinetics of adaptation to short-term aerobic exercise training in human skeletal muscle, *PLoS One* 8 (9) (2013) e74098.
- [10] Z. Gerhart-Hines, et al., Metabolic control of muscle mitochondrial function and fatty acid oxidation through SIRT1/PGC-1alpha, *EMBO J.* 26 (7) (2007) 1913–1923.
- [11] P.D. Chilibeck, et al., The effect of aerobic exercise training on the distribution of succinate dehydrogenase activity throughout muscle fibres, *Can. J. Appl. Physiol.* 23 (1) (1998) 74–86.
- [12] W. Chidnok, et al., Muscle metabolic responses during high-intensity intermittent exercise measured by ³¹P-MRS: relationship to the critical power concept, *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 305 (9) (2013) R1085–R1092.
- [13] M.L. Parolin, et al., Regulation of skeletal muscle glycogen phosphorylase and PDH during maximal intermittent exercise, *Am. J. Physiol.* 277 (5) (1999) E890–E900.
- [14] B.S. Currier, et al., Resistance training prescription for muscle strength and hypertrophy in healthy adults: a systematic review and Bayesian network meta-analysis, *Br. J. Sports Med.* 57 (18) (2023) 1211–1220.
- [15] R.W. Morton, et al., A systematic review, meta-analysis and meta-regression of the effect of protein supplementation on resistance training-induced gains in muscle mass and strength in healthy adults, *Br. J. Sports Med.* 52 (6) (2018) 376–384.
- [16] T.A. Hornberger, K.A. Esser, Mechanotransduction and the regulation of protein synthesis in skeletal muscle, *Proc. Nutr. Soc.* 63 (2) (2004) 331–335.
- [17] M.J. Drummond, et al., Rapamycin administration in humans blocks the contraction-induced increase in skeletal muscle protein synthesis, *J. Physiol.* 587 (Pt 7) (2009) 1535–1546.
- [18] C. Goreham, et al., High-resistance training and muscle metabolism during prolonged exercise, *Am. J. Physiol.* 276 (3) (1999) E489–E496.
- [19] T. Stokes, et al., Molecular transducers of human skeletal muscle remodeling under different loading states, *Cell Rep.* 32 (5) (2020) 107980.
- [20] J.C. McLeod, et al., The influence of resistance exercise training prescription variables on skeletal muscle mass, strength, and physical function in healthy adults: an umbrella review, *J. Sport Health Sci.* 13 (1) (2023) 47–60.
- [21] J.B. Gillen, M.J. Gibala, Is high-intensity interval training a time-efficient exercise strategy to improve health and fitness? *Appl. Physiol. Nutr. Metabol.* 39 (3) (2014) 409–412.
- [22] C. Caparrós-Manosalva, et al., Effects of high-intensity interval training on lean mass, strength, and power of the lower limbs in healthy old and young people, *Front. Physiol.* 14 (2023) 1223069.
- [23] M.J. Callahan, et al., Can high-intensity interval training promote skeletal muscle anabolism? *Sports Med.* 51 (3) (2021) 405–421.
- [24] R. Bagheri, et al., Muscle protein synthesis responses following aerobic-based exercise or high-intensity interval training with or without protein ingestion: a systematic review, *Sports Med.* 52 (11) (2022) 2713–2732.
- [25] S.B. Wilkinson, et al., Differential effects of resistance and endurance exercise in the fed state on signalling molecule phosphorylation and protein synthesis in human muscle, *J. Physiol.* 586 (15) (2008) 3701–3717.
- [26] D.M. Di Donato, et al., Influence of aerobic exercise intensity on myofibrillar and mitochondrial protein synthesis in young men during early and late postexercise recovery, *Am. J. Physiol. Endocrinol. Metab.* 306 (9) (2014) E1025–E1032.
- [27] D.J. Bishop, et al., High-intensity exercise and mitochondrial biogenesis: current controversies and future research directions, *Physiology* 34 (1) (2019) 56–70.
- [28] C.A. Goodman, et al., The role of skeletal muscle mTOR in the regulation of mechanical load-induced growth, *J. Physiol.* 589 (Pt 22) (2011) 5485–5501.
- [29] M. Jhanwar-Uniyal, et al., Diverse signaling mechanisms of mTOR complexes: mTORC1 and mTORC2 in forming a formidable relationship, *Adv. Biol. Regul.* 72 (2019) 51–62.
- [30] D.D. Sarbassov, et al., Rictor, a novel binding partner of mTOR, defines a rapamycin-insensitive and raptor-independent pathway that regulates the cytoskeleton, *Curr. Biol.* 14 (14) (2004) 1296–1302.
- [31] P.T. Bhaskar, N. Hay, The two TORCs and Akt, *Dev. Cell* 12 (4) (2007) 487–502.
- [32] N. Parmar, F. Tamanoi, *Rheb G-Proteins And the Activation Of mTORC1*, *Enzymes*, 27, 2010, pp. 39–56.
- [33] B.T. Navé, et al., Mammalian target of rapamycin is a direct target for protein kinase B: identification of a convergence point for opposing effects of insulin and amino-acid deficiency on protein translation, *Biochem. J.* 344 (Pt 2) (1999) 427–431. Pt 2.
- [34] S.W. Cheng, et al., Thr2446 is a novel mammalian target of rapamycin (mTOR) phosphorylation site regulated by nutrient status, *J. Biol. Chem.* 279 (16) (2004) 15719–15722.
- [35] J. Copp, G. Manning, T. Hunter, TORC-specific phosphorylation of mammalian target of rapamycin (mTOR): phospho-Ser2481 is a marker for intact mTOR signaling complex 2, *Cancer Res.* 69 (5) (2009) 1821–1827.
- [36] S.C. Bodine, et al., Akt/mTOR pathway is a crucial regulator of skeletal muscle hypertrophy and can prevent muscle atrophy in vivo, *Nat. Cell Biol.* 3 (11) (2001) 1014–1019.

- [37] B. Benziane, et al., Divergent cell signaling after short-term intensified endurance training in human skeletal muscle, *Am. J. Physiol. Endocrinol. Metab.* 295 (6) (2008) E1427–E1438.
- [38] J. Kim, et al., AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1, *Nat. Cell Biol.* 13 (2) (2011) 132–141.
- [39] J.A. Martina, et al., mTORC1 functions as a transcriptional regulator of autophagy by preventing nuclear transport of TFEB, *Autophagy* 8 (6) (2012) 903–914.
- [40] V.C. Figueiredo, J.F. Markworth, D. Cameron-Smith, Considerations on mTOR regulation at serine 2448: implications for muscle metabolism studies, *Cell. Mol. Life Sci.* 74 (14) (2017) 2537–2545.
- [41] A.C. Gingras, et al., Regulation of 4E-BP1 phosphorylation: a novel two-step mechanism, *Genes Dev.* 13 (11) (1999) 1422–1437.
- [42] J.A. Hutchinson, et al., Regulation of ribosomal protein S6 phosphorylation by casein kinase 1 and protein phosphatase 1, *J. Biol. Chem.* 286 (10) (2011) 8688–8696.
- [43] R.A. Saxton, D.M. Sabatini, mTOR signaling in growth, metabolism, and disease, *Cell* 169 (2) (2017) 361–371.
- [44] C.C. Dibble, L.C. Cantley, Regulation of mTORC1 by PI3K signaling, *Trends Cell Biol.* 25 (9) (2015) 545–555.
- [45] L.R. Pearce, D. Komander, D.R. Alessi, The nuts and bolts of AGC protein kinases, *Nat. Rev. Mol. Cell Biol.* 11 (1) (2010) 9–22.
- [46] C.S. Fry, et al., Aging impairs contraction-induced human skeletal muscle mTORC1 signaling and protein synthesis, *Skeletal Muscle* 1 (1) (2011) 11.
- [47] H. Roschel, et al., Effect of eccentric exercise velocity on akt/mTOR/p70s6k signaling in human skeletal muscle, *Appl. Physiol. Nutr. Metabol.* 36 (2) (2011) 283–290.
- [48] P. Liu, et al., Cell-cycle-regulated activation of Akt kinase by phosphorylation at its carboxyl terminus, *Nature* 508 (7497) (2014) 541–545.
- [49] X. Long, et al., Rheb binds and regulates the mTOR kinase, *Curr. Biol.* 15 (8) (2005) 702–713.
- [50] K. Inoki, et al., Rheb GTPase is a direct target of TSC2 GAP activity and regulates mTOR signaling, *Genes Dev.* 17 (15) (2003) 1829–1834.
- [51] B. Ekim, et al., mTOR kinase domain phosphorylation promotes mTORC1 signaling, cell growth, and cell cycle progression, *Mol. Cell Biol.* 31 (14) (2011) 2787–2801.
- [52] K. Baar, K. Esser, Phosphorylation of p70(S6k) correlates with increased skeletal muscle mass following resistance exercise, *Am. J. Physiol.* 276 (1) (1999) C120–C127.
- [53] G.A. Nader, K.A. Esser, Intracellular signaling specificity in skeletal muscle in response to different modes of exercise, *J. Appl. Physiol.* 90 (5) (1985) 1936–1942, 2001.
- [54] S.C. Bodine, The role of mTORC1 in the regulation of skeletal muscle mass, *Fac Rev.* 11 (2022) 32.
- [55] A. Szwed, E. Kim, E. Jacinto, Regulation and metabolic functions of mTORC1 and mTORC2, *Physiol. Rev.* 101 (3) (2021) 1371–1426.
- [56] C.A. Goodman, Role of mTORC1 in mechanically induced increases in translation and skeletal muscle mass, *J. Appl. Physiol.* 127 (2) (1985) 581–590, 2019.
- [57] J.S. You, et al., The role of raptor in the mechanical load-induced regulation of mTOR signaling, protein synthesis, and skeletal muscle hypertrophy, *Faseb. J.* 33 (3) (2019) 4021–4034.
- [58] R. Ogasawara, et al., mTOR signaling response to resistance exercise is altered by chronic resistance training and detraining in skeletal muscle, *J. Appl. Physiol.* 114 (7) (1985) 934–940, 2013.
- [59] N.A. Burd, et al., Resistance exercise volume affects myofibrillar protein synthesis and anabolic signalling molecule phosphorylation in young men, *J. Physiol.* 588 (Pt 16) (2010) 3119–3130.
- [60] V.G. Coffey, et al., Early signaling responses to divergent exercise stimuli in skeletal muscle from well-trained humans, *Faseb. J.* 20 (1) (2006) 190–192.
- [61] C.T. Tinlin-Goodfellow, M.J. Lees, N. Hodson, The skeletal muscle fiber periphery: a nexus of mTOR-related anabolism, *Sports Med. Health Sci.* 5 (1) (2023) 10–19.
- [62] Y. Sancak, et al., Ragulator-Rag complex targets mTORC1 to the lysosomal surface and is necessary for its activation by amino acids, *Cell* 141 (2) (2010) 290–303.
- [63] S. Abou Sawan, et al., Whole egg, but not egg white, ingestion induces mTOR colocalization with the lysosome after resistance exercise, *Am. J. Physiol. Cell Physiol.* 315 (4) (2018) C537–C543.
- [64] N. Hodson, et al., Differential localization and anabolic responsiveness of mTOR complexes in human skeletal muscle in response to feeding and exercise, *Am. J. Physiol. Cell Physiol.* 313 (6) (2017) C604–C611.
- [65] Z. Song, et al., Resistance exercise initiates mechanistic target of rapamycin (mTOR) translocation and protein complex co-localisation in human skeletal muscle, *Sci. Rep.* 7 (1) (2017) 5028.
- [66] S. Abou Sawan, et al., Translocation and protein complex co-localization of mTOR is associated with postprandial myofibrillar protein synthesis at rest and after endurance exercise, *Phys. Rep.* 6 (5) (2018).
- [67] C.M. Brennan, et al., p38 MAPKs - roles in skeletal muscle physiology, disease mechanisms, and as potential therapeutic targets, *JCI Insight* 6 (12) (2021).
- [68] A.J. Waskiewicz, et al., Mitogen-activated protein kinases activate the serine/threonine kinases Mnk1 and Mnk2, *EMBO J.* 16 (8) (1997) 1909–1920.
- [69] J.S. Shenberger, et al., Roles of mitogen-activated protein kinase signal-integrating kinases 1 and 2 in oxidant-mediated eIF4E phosphorylation, *Int. J. Biochem. Cell Biol.* 39 (10) (2007) 1828–1842.
- [70] H.F. Kramer, L.J. Goodyear, Exercise, MAPK, and NF-kappaB signaling in skeletal muscle, *J. Appl. Physiol.* 103 (1) (1985) 388–395, 2007.
- [71] U. Widegren, et al., Influence of exercise intensity on ERK/MAP kinase signalling in human skeletal muscle, *Pflügers Archiv* 441 (2–3) (2000) 317–322.
- [72] E.A. Richter, et al., Differential effect of bicycling exercise intensity on activity and phosphorylation of atypical protein kinase C and extracellular signal-regulated protein kinase in skeletal muscle, *J. Physiol.* 560 (Pt 3) (2004) 909–918.
- [73] D.R. Alessi, et al., Identification of the sites in MAP kinase kinase-1 phosphorylated by p74raf-1, *EMBO J.* 13 (7) (1994) 1610–1619.
- [74] L.J. Goodyear, et al., Effects of exercise and insulin on mitogen-activated protein kinase signaling pathways in rat skeletal muscle, *Am. J. Physiol.* 271 (2 Pt 1) (1996) E403–E408.
- [75] D. Aronson, et al., Exercise stimulates the mitogen-activated protein kinase pathway in human skeletal muscle, *J. Clin. Invest.* 99 (6) (1997) 1251–1257.
- [76] A. Zetser, E. Gredinger, E. Bengal, p38 mitogen-activated protein kinase pathway promotes skeletal muscle differentiation. Participation of the Mef2c transcription factor, *J. Biol. Chem.* 274 (8) (1999) 5193–5200.
- [77] S.R. Kimball, R.L. Horetsky, L.S. Jefferson, Signal transduction pathways involved in the regulation of protein synthesis by insulin in L6 myoblasts, *Am. J. Physiol.* 274 (1) (1998) C221–C228.
- [78] U. Widegren, J.W. Ryder, J.R. Zierath, Mitogen-activated protein kinase signal transduction in skeletal muscle: effects of exercise and muscle contraction, *Acta Physiol. Scand.* 172 (3) (2001) 227–238.
- [79] M.J. Drummond, et al., Nutritional and contractile regulation of human skeletal muscle protein synthesis and mTORC1 signaling, *J. Appl. Physiol.* 106 (4) (1985) 1374–1384, 2009.
- [80] K. Hojlund, et al., In vivo phosphoproteome of human skeletal muscle revealed by phosphopeptide enrichment and HPLC-ESI-MS/MS, *J. Proteome Res.* 8 (11) (2009) 4954–4965.
- [81] L.C. Martineau, P.F. Gardiner, Insight into skeletal muscle mechanotransduction: MAPK activation is quantitatively related to tension, *J. Appl. Physiol.* 91 (2) (1985) 693–702, 2001.
- [82] T. Akimoto, et al., Exercise stimulates Pgc-1alpha transcription in skeletal muscle through activation of the p38 MAPK pathway, *J. Biol. Chem.* 280 (20) (2005) 19587–19593.
- [83] Y. Seko, et al., Hypoxia and hypoxia/reoxygenation activate p65PAK, p38 mitogen-activated protein kinase (MAPK), and stress-activated protein kinase (SAPK) in cultured rat cardiac myocytes, *Biochem. Biophys. Res. Commun.* 239 (3) (1997) 840–844.
- [84] M.J. Gibala, et al., Brief intense interval exercise activates AMPK and p38 MAPK signaling and increases the expression of PGC-1alpha in human skeletal muscle, *J. Appl. Physiol.* 106 (3) (1985) 929–934, 2009.
- [85] E. Bengal, S. Aviram, T. Hayek, p38 MAPK in glucose metabolism of skeletal muscle: beneficial or harmful? *Int. J. Mol. Sci.* 21 (18) (2020).
- [86] M. Deak, et al., Mitogen- and stress-activated protein kinase-1 (MSK1) is directly activated by MAPK and SAPK2/p38, and may mediate activation of CREB, *EMBO J.* 17 (15) (1998) 4426–4441.
- [87] M. Yu, et al., Metabolic and mitogenic signal transduction in human skeletal muscle after intense cycling exercise, *J. Physiol.* 546 (Pt 2) (2003) 327–335.
- [88] L. Deldicque, et al., Decrease in Akt/PKB signalling in human skeletal muscle by resistance exercise, *Eur. J. Appl. Physiol.* 104 (1) (2008) 57–65.
- [89] D.R. Moore, et al., Resistance exercise enhances mTOR and MAPK signalling in human muscle over that seen at rest after bolus protein ingestion, *Acta Physiol.* 201 (3) (2011) 365–372.
- [90] D. Williamson, et al., Mitogen-activated protein kinase (MAPK) pathway activation: effects of age and acute exercise on human skeletal muscle, *J. Physiol.* 547 (Pt 3) (2003) 977–987.
- [91] L. Ma, et al., Phosphorylation and functional inactivation of TSC2 by Erk implications for tuberous sclerosis and cancer pathogenesis, *Cell* 121 (2) (2005) 179–193.
- [92] M. Miyazaki, T. Takemasa, TSC2/Rheb signaling mediates ERK-dependent regulation of mTORC1 activity in C2C12 myoblasts, *FEBS Open Bio.* 7 (3) (2017) 424–433.
- [93] J. Huang, B.D. Manning, The TSC1-TSC2 complex: a molecular switchboard controlling cell growth, *Biochem. J.* 412 (2) (2008) 179–190.
- [94] M.D. Roberts, et al., Mechanisms of mechanical overload-induced skeletal muscle hypertrophy: current understanding and future directions, *Physiol. Rev.* 103 (4) (2023) 2679–2757.
- [95] V. Stefanovsky, et al., Growth factor signaling regulates elongation of RNA polymerase I transcription in mammals via UBF phosphorylation and r-chromatin remodeling, *Mol Cell* 21 (5) (2006) 629–639.
- [96] J.F. Wojtaszewski, et al., Differential regulation of MAP kinase by contraction and insulin in skeletal muscle: metabolic implications, *Am. J. Physiol.* 277 (4) (1999) E724–E732.
- [97] T. Saneyasu, et al., IGF-1 knockdown inhibits phosphorylation of Akt and ERK in chicken embryonic myotubes, *Growth Hormone IGF Res.* 65 (2022) 101478.
- [98] J.D. Fluckey, et al., Insulin-facilitated increase of muscle protein synthesis after resistance exercise involves a MAP kinase pathway, *Am. J. Physiol. Endocrinol. Metab.* 290 (6) (2006) E1205–E1211.
- [99] F. Haddad, G.R. Adams, Inhibition of MAP/ERK kinase prevents IGF-I-induced hypertrophy in rat muscles, *J. Appl. Physiol.* 96 (1) (1985) 203–210, 2004.
- [100] K. Vichaiwong, et al., Attenuation of oxidant-induced muscle insulin resistance and p38 MAPK by exercise training, *Free Radic. Biol. Med.* 47 (5) (2009) 593–599.
- [101] S.H. Park, et al., Phosphorylation-activity relationships of AMPK and acetyl-CoA carboxylase in muscle, *J. Appl. Physiol.* 92 (6) (1985) 2475–2482, 2002.

- [102] M.M. Mihaylova, R.J. Shaw, The AMPK signalling pathway coordinates cell growth, autophagy and metabolism, *Nat. Cell Biol.* 13 (9) (2011) 1016–1023.
- [103] J.T. Treebak, et al., Acute exercise and physiological insulin induce distinct phosphorylation signatures on TBC1D1 and TBC1D4 proteins in human skeletal muscle, *J. Physiol.* 592 (2) (2014) 351–375.
- [104] S.L. McGee, et al., Exercise-induced histone modifications in human skeletal muscle, *J. Physiol.* 587 (Pt 24) (2009) 5951–5958.
- [105] J.F. Wojtaszewski, et al., Isoform-specific and exercise intensity-dependent activation of 5'-AMP-activated protein kinase in human skeletal muscle, *J. Physiol.* 528 (Pt 1) (2000) 221–226. Pt 1.
- [106] M.J. Abbott, A.M. Edelman, L.P. Turcotte, CaMKK is an upstream signal of AMP-activated protein kinase in regulation of substrate metabolism in contracting skeletal muscle, *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 297 (6) (2009) R1724–R1732.
- [107] D.G. Hardie, AMP-activated/SNF1 protein kinases: conserved guardians of cellular energy, *Nat. Rev. Mol. Cell Biol.* 8 (10) (2007) 774–785.
- [108] H.C. Dreyer, et al., Resistance exercise increases AMPK activity and reduces 4E-BP1 phosphorylation and protein synthesis in human skeletal muscle, *J. Physiol.* 576 (Pt 2) (2006) 613–624.
- [109] H.J. Koh, J. Brandauer, L.J. Goodyear, LKB1 and AMPK and the regulation of skeletal muscle metabolism, *Curr. Opin. Clin. Nutr. Metab. Care* 11 (3) (2008) 227–232.
- [110] D.B. Shackelford, R.J. Shaw, The LKB1-AMPK pathway: metabolism and growth control in tumour suppression, *Nat. Rev. Cancer* 9 (8) (2009) 563–575.
- [111] C.B. Tanner, et al., Mitochondrial and performance adaptations to exercise training in mice lacking skeletal muscle LKB1, *Am. J. Physiol. Endocrinol. Metab.* 305 (8) (2013) E1018–E1029.
- [112] E. Zeqiraj, et al., Structure of the LKB1-STRAD-MO25 complex reveals an allosteric mechanism of kinase activation, *Science* 326 (5960) (2009) 1707–1711.
- [113] Z. Xie, et al., Phosphorylation of LKB1 at serine 428 by protein kinase C-zeta is required for metformin-enhanced activation of the AMP-activated protein kinase in endothelial cells, *Circulation* 117 (7) (2008) 952–962.
- [114] K. Sakamoto, et al., Deficiency of LKB1 in skeletal muscle prevents AMPK activation and glucose uptake during contraction, *EMBO J.* 24 (10) (2005) 1810–1820.
- [115] C. Canto, et al., AMPK regulates energy expenditure by modulating NAD⁺ metabolism and SIRT1 activity, *Nature* 458 (7241) (2009) 1056–1060.
- [116] S.R. Costford, et al., Skeletal muscle NAMPT is induced by exercise in humans, *Am. J. Physiol. Endocrinol. Metab.* 298 (1) (2010) E117–E126.
- [117] C. Canto, et al., Interdependence of AMPK and SIRT1 for metabolic adaptation to fasting and exercise in skeletal muscle, *Cell Metabol.* 11 (3) (2010) 213–219.
- [118] S. Jäger, et al., AMP-activated protein kinase (AMPK) action in skeletal muscle via direct phosphorylation of PGC-1 α , *Proc. Natl. Acad. Sci. U. S. A.* 104 (29) (2007) 12017–12022.
- [119] W.W. Winder, et al., Activation of AMP-activated protein kinase increases mitochondrial enzymes in skeletal muscle, *J. Appl. Physiol.* 88 (6) (1985) 2219–2226, 2000.
- [120] D.G. Hardie, AMP-activated protein kinase: a key system mediating metabolic responses to exercise, *Med. Sci. Sports Exerc.* 36 (1) (2004) 28–34.
- [121] H. Zong, et al., AMP kinase is required for mitochondrial biogenesis in skeletal muscle in response to chronic energy deprivation, *Proc. Natl. Acad. Sci. U. S. A.* 99 (25) (2002) 15983–15987.
- [122] J.A. Hawley, S.J. Lessard, Exercise training-induced improvements in insulin action, *Acta Physiol.* 192 (1) (2008) 127–135.
- [123] N. Jessen, et al., Effects of AICAR and exercise on insulin-stimulated glucose uptake, signaling, and GLUT-4 content in rat muscles, *J. Appl. Physiol.* 2003 (94) (1985) 1373–1379, 4.
- [124] E.J. Kurth-Kraczek, et al., 5' AMP-activated protein kinase activation causes GLUT4 translocation in skeletal muscle, *Diabetes* 48 (8) (1999) 1667–1671.
- [125] K. Inoki, T. Zhu, K.L. Guan, TSC2 mediates cellular energy response to control cell growth and survival, *Cell* 115 (5) (2003) 577–590.
- [126] D.M. Gwinn, et al., AMPK phosphorylation of raptor mediates a metabolic checkpoint, *Mol Cell* 30 (2) (2008) 214–226.
- [127] V.G. Coffey, et al., Effect of consecutive repeated sprint and resistance exercise bouts on acute adaptive responses in human skeletal muscle, *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 297 (5) (2009) R1441–R1451.
- [128] T. Moro, et al., Exercise intensity and rest intervals effects on intracellular signals and anabolic response of skeletal muscle to resistance training, *J. Strength Condit Res.* (2022). https://journals.lww.com/nsca-jscr/abstract/9000/exercise_intensity_and_rest_intervals_effects_on.93822.aspx.
- [129] C. Porter, et al., Resistance exercise training alters mitochondrial function in human skeletal muscle, *Med. Sci. Sports Exerc.* 47 (9) (2015) 1922–1931.
- [130] A. Almeida, S. Moncada, J.P. Bolanos, Nitric oxide switches on glycolysis through the AMP protein kinase and 6-phosphofructo-2-kinase pathway, *Nat. Cell Biol.* 6 (1) (2004) 45–51.
- [131] S.K. Powers, M.J. Jackson, Exercise-induced oxidative stress: cellular mechanisms and impact on muscle force production, *Physiol. Rev.* 88 (4) (2008) 1243–1276.
- [132] F. McArdle, et al., Intracellular generation of reactive oxygen species by contracting skeletal muscle cells, *Free Radic. Biol. Med.* 39 (5) (2005) 651–657.
- [133] J. Bouviere, et al., Exercise-stimulated ROS sensitive signaling pathways in skeletal muscle, *Antioxidants* 10 (4) (2021).
- [134] A. Vasilaki, et al., Free radical generation by skeletal muscle of adult and old mice: effect of contractile activity, *Aging Cell* 5 (2) (2006) 109–117.
- [135] K. Sahlin, et al., Ultraendurance exercise increases the production of reactive oxygen species in isolated mitochondria from human skeletal muscle, *J. Appl. Physiol.* 108 (4) (1985) 780–787, 2010.
- [136] Y. Hellsten, et al., Xanthine oxidase in human skeletal muscle following eccentric exercise: a role in inflammation, *J. Physiol.* 498 (Pt 1) (1997) 239–248. Pt 1.
- [137] C. Henriquez-Olguin, et al., Cytosolic ROS production by NADPH oxidase 2 regulates muscle glucose uptake during exercise, *Nat. Commun.* 10 (1) (2019) 4623.
- [138] L.P. Michaelson, et al., Mitochondrial redox potential during contraction in single intact muscle fibers, *Muscle Nerve* 42 (4) (2010) 522–529.
- [139] G.K. Sakellariou, et al., Studies of mitochondrial and nonmitochondrial sources implicate nicotinamide adenine dinucleotide phosphate oxidase(s) in the increased skeletal muscle superoxide generation that occurs during contractile activity, *Antioxidants Redox Signal.* 18 (6) (2013) 603–621.
- [140] E.C. Hinchey, et al., Mitochondria-derived ROS activate AMP-activated protein kinase (AMPK) indirectly, *J. Biol. Chem.* 293 (44) (2018) 17208–17217.
- [141] J.W. Zmijewski, et al., Exposure to hydrogen peroxide induces oxidation and activation of AMP-activated protein kinase, *J. Biol. Chem.* 285 (43) (2010) 33154–33164.
- [142] M. Torres, H.J. Forman, Redox signaling and the MAP kinase pathways, *Biofactors* 17 (1–4) (2003) 287–296.
- [143] G.D. Wadley, et al., Xanthine oxidase inhibition attenuates skeletal muscle signaling following acute exercise but does not impair mitochondrial adaptations to endurance training, *Am. J. Physiol. Endocrinol. Metab.* 304 (8) (2013) E853–E862.
- [144] P.T. Mungai, et al., Hypoxia triggers AMPK activation through reactive oxygen species-mediated activation of calcium release-activated calcium channels, *Mol. Cell Biol.* 31 (17) (2011) 3531–3545.
- [145] C. Hidalgo, et al., A transverse tubule NADPH oxidase activity stimulates calcium release from isolated triads via ryanodine receptor type 1 S-glutathionylation, *J. Biol. Chem.* 281 (36) (2006) 26473–26482.
- [146] D.M. Pattwell, et al., Release of reactive oxygen and nitrogen species from contracting skeletal muscle cells, *Free Radic. Biol. Med.* 37 (7) (2004) 1064–1072.
- [147] V.A. Lira, et al., Nitric oxide increases GLUT4 expression and regulates AMPK signaling in skeletal muscle, *Am. J. Physiol. Endocrinol. Metab.* 293 (4) (2007) E1062–E1068.
- [148] G.K. McConnell, et al., Central role of nitric oxide synthase in AICAR and caffeine-induced mitochondrial biogenesis in L6 myocytes, *J. Appl. Physiol.* 108 (3) (1985) 589–595, 2010.
- [149] R.J. Bloomer, A.H. Goldfarb, Anaerobic exercise and oxidative stress: a review, *Can. J. Appl. Physiol.* 29 (3) (2004) 245–263.
- [150] L.L. Spriet, Anaerobic metabolism in human skeletal muscle during short-term, intense activity, *Can. J. Physiol. Pharmacol.* 70 (1) (1992) 157–165.
- [151] F. He, et al., Redox mechanism of reactive oxygen species in exercise, *Front. Physiol.* 7 (2016) 486.
- [152] H. Wackerhage, et al., Stimuli and sensors that initiate skeletal muscle hypertrophy following resistance exercise, *J. Appl. Physiol.* 126 (1) (1985) 30–43, 2019.
- [153] M. Vanmunster, et al., Mechanosensors control skeletal muscle mass, molecular clocks, and metabolism, *Cell. Mol. Life Sci.* 79 (6) (2022) 321.
- [154] S. Mathes, et al., Evidence for skeletal muscle fiber type-specific expressions of mechanosensors, *Cell. Mol. Life Sci.* 76 (15) (2019) 2987–3004.
- [155] N.D. Steinert, et al., Mapping of the contraction-induced phosphoproteome identifies TRIM28 as a significant regulator of skeletal muscle size and function, *Cell Rep.* 34 (9) (2021) 108796.
- [156] S. Dupont, et al., Role of YAP/TAZ in mechanotransduction, *Nature* 474 (7350) (2011) 179–183.
- [157] A. Csibi, J. Blenis, Hippo-YAP and mTOR pathways collaborate to regulate organ size, *Nat. Cell Biol.* 14 (12) (2012) 1244–1245.
- [158] K. Tumaneng, et al., YAP mediates crosstalk between the Hippo and PI(3)K-TOR pathways by suppressing PTEN via miR-29, *Nat. Cell Biol.* 14 (12) (2012) 1322–1329.
- [159] C.A. Goodman, et al., Yes-Associated Protein is up-regulated by mechanical overload and is sufficient to induce skeletal muscle hypertrophy, *FEBS Lett.* 589 (13) (2015) 1491–1497.
- [160] Z. Meng, et al., RAP2 mediates mechanoresponses of the Hippo pathway, *Nature* 560 (7720) (2018) 655–660.
- [161] K.A. Gunay, et al., Myoblast mechanotransduction and myotube morphology is dependent on BAG3 regulation of YAP and TAZ, *Biomaterials* 277 (2021) 121097.
- [162] K.I. Watt, et al., The Hippo pathway effector YAP is a critical regulator of skeletal muscle fibre size, *Nat. Commun.* 6 (2015) 6048.
- [163] C.G. Hansen, et al., The Hippo pathway effectors YAP and TAZ promote cell growth by modulating amino acid signaling to mTORC1, *Cell Res.* 25 (12) (2015) 1299–1313.
- [164] Y. Nagata, et al., Entry of muscle satellite cells into the cell cycle requires sphingolipid signaling, *J. Cell Biol.* 174 (2) (2006) 245–253.
- [165] K.I. Watt, et al., Yap is a novel regulator of C2C12 myogenesis, *Biochem. Biophys. Res. Commun.* 393 (4) (2010) 619–624.
- [166] S. Bell, E.M. Terentjev, Focal adhesion kinase: the reversible molecular mechanosensor, *Biophys. J.* 112 (11) (2017) 2439–2450.
- [167] Z.A. Graham, P.M. Gallagher, C.P. Cardozo, Focal adhesion kinase and its role in skeletal muscle, *J. Muscle Res. Cell Motil.* 36 (4–5) (2015) 305–315.
- [168] M.D. Boppart, Z.S. Mahmassani, Integrin signaling: linking mechanical stimulation to skeletal muscle hypertrophy, *Am. J. Physiol. Cell Physiol.* 317 (4) (2019) C629–C641.
- [169] J.T. Parsons, Focal adhesion kinase: the first ten years, *J. Cell Sci.* 116 (Pt 8) (2003) 1409–1416.

- [170] H.C. Chen, et al., Phosphorylation of tyrosine 397 in focal adhesion kinase is required for binding phosphatidylinositol 3-kinase, *J. Biol. Chem.* 271 (42) (1996) 26329–26334.
- [171] B. Gan, Y. Yoo, J.L. Guan, Association of focal adhesion kinase with tuberous sclerosis complex 2 in the regulation of *src* kinase activation and cell growth, *J. Biol. Chem.* 281 (49) (2006) 37321–37329.
- [172] H. Crossland, et al., Focal adhesion kinase is required for IGF-I-mediated growth of skeletal muscle cells via a TSC2/mTOR/S6K1-associated pathway, *Am. J. Physiol. Endocrinol. Metab.* 305 (2) (2013) E183–E193.
- [173] S. Klossner, et al., Mechano-transduction to muscle protein synthesis is modulated by FAK, *Eur. J. Appl. Physiol.* 106 (3) (2009) 389–398.
- [174] A. Ulbricht, et al., Cellular mechanotransduction relies on tension-induced and chaperone-assisted autophagy, *Curr. Biol.* 23 (5) (2013) 430–435.
- [175] Z. Mao, F. Nakamura, Structure and function of filamin C in the muscle Z-disc, *Int. J. Mol. Sci.* 21 (8) (2020).
- [176] N.J. Hoffman, et al., Global phosphoproteomic analysis of human skeletal muscle reveals a network of exercise-regulated kinases and AMPK substrates, *Cell Metabol.* 22 (5) (2015) 922–935.
- [177] B. Kathage, et al., The cochaperone BAG3 coordinates protein synthesis and autophagy under mechanical strain through spatial regulation of mTORC1, *Biochim. Biophys. Acta Mol. Cell Res.* 1864 (1) (2017) 62–75.
- [178] L. Reimann, et al., Phosphoproteomics identifies dual-site phosphorylation in an extended basic motif regulating FILIP1-mediated degradation of filamin-C, *Commun. Biol.* 3 (1) (2020) 253.
- [179] A. Kontogianni-Konstantopoulos, et al., Muscle giants: molecular scaffolds in sarcomerogenesis, *Physiol. Rev.* 89 (4) (2009) 1217–1267.
- [180] N. Ibatá, E.M. Terentjev, Why exercise builds muscles: titin mechanosensing controls skeletal muscle growth under load, *Biophys. J.* 120 (17) (2021) 3649–3663.
- [181] M. Gautel, et al., A calmodulin-binding sequence in the C-terminus of human cardiac titin kinase, *Eur. J. Biochem.* 230 (2) (1995) 752–759.
- [182] C.G. Hidalgo, et al., The multifunctional Ca(2+)-calmodulin-dependent protein kinase II delta (CaMKII δ) phosphorylates cardiac titin's spring elements, *J. Mol. Cell. Cardiol.* 54 (2013) 90–97.
- [183] S. Lange, et al., The kinase domain of titin controls muscle gene expression and protein turnover, *Science* 308 (5728) (2005) 1599–1603.
- [184] R. van der Pijl, et al., Titin-based mechanosensing modulates muscle hypertrophy, *J Cachexia Sarcopenia Muscle* 9 (5) (2018) 947–961.
- [185] W.A. Linke, M. Kruger, The giant protein titin as an integrator of myocyte signaling pathways, *Physiology* 25 (3) (2010) 186–198.
- [186] M.J. Gibala, et al., Myofibrillar disruption following acute concentric and eccentric resistance exercise in strength-trained men, *Can. J. Physiol. Pharmacol.* 78 (8) (2000) 656–661.
- [187] P.M. Clarkson, M.J. Hubal, Exercise-induced muscle damage in humans, *Am. J. Phys. Med. Rehabil.* 81 (11 Suppl) (2002) S52–S69.
- [188] K.W. Jorgenson, S.M. Phillips, T.A. Hornberger, Identifying the structural adaptations that drive the mechanical load-induced growth of skeletal muscle: a scoping review, *Cells* 9 (7) (2020).
- [189] I. Markus, et al., Exercise-induced muscle damage: mechanism, assessment and nutritional factors to accelerate recovery, *Eur. J. Appl. Physiol.* 121 (4) (2021) 969–992.
- [190] R.L. Lieber, Biomechanical response of skeletal muscle to eccentric contractions, *J. Sport Health Sci.* 7 (3) (2018) 294–309.
- [191] J. Friden, M. Sjøstrom, B. Ekblom, Myofibrillar damage following intense eccentric exercise in man, *Int. J. Sports Med.* 4 (3) (1983) 170–176.
- [192] S.M. Roth, et al., Ultrastructural muscle damage in young vs. older men after high-volume, heavy-resistance strength training, *J. Appl. Physiol.* 86 (6) (1985) 1833–1840, 1999.
- [193] J.G. Yu, L. Carlsson, L.E. Thornell, Evidence for myofibril remodeling as opposed to myofibril damage in human muscles with DOMS: an ultrastructural and immunoelectron microscopic study, *Histochem. Cell Biol.* 121 (3) (2004) 219–227.
- [194] S.M. Phillips, et al., Mixed muscle protein synthesis and breakdown after resistance exercise in humans, *Am. J. Physiol.* 273 (1 Pt 1) (1997) E99–E107.
- [195] K.D. Tipton, D.L. Hamilton, I.J. Gallagher, Assessing the role of muscle protein breakdown in response to nutrition and exercise in humans, *Sports Med.* 48 (Suppl 1) (2018) 53–64.
- [196] D.R. Moore, et al., Myofibrillar and collagen protein synthesis in human skeletal muscle in young men after maximal shortening and lengthening contractions, *Am. J. Physiol. Endocrinol. Metab.* 288 (6) (2005) E1153–E1159.
- [197] D.L. Morgan, U. Proske, Popping sarcomere hypothesis explains stretch-induced muscle damage, *Clin. Exp. Pharmacol. Physiol.* 31 (8) (2004) 541–545.
- [198] F. Damas, et al., Resistance training-induced changes in integrated myofibrillar protein synthesis are related to hypertrophy only after attenuation of muscle damage, *J. Physiol.* 594 (18) (2016) 5209–5222.
- [199] C. Lim, et al., An evidence-based narrative review of mechanisms of resistance exercise-induced human skeletal muscle hypertrophy, *Med. Sci. Sports Exerc.* 54 (9) (2022) 1546–1559.
- [200] F. Damas, et al., Early resistance training-induced increases in muscle cross-sectional area are concomitant with edema-induced muscle swelling, *Eur. J. Appl. Physiol.* 116 (1) (2016) 49–56.
- [201] K.L. Flann, et al., Muscle damage and muscle remodeling: no pain, no gain? *J. Exp. Biol.* 214 (Pt 4) (2011) 674–679.
- [202] T.N. Lueders, et al., The α 7 β 1-integrin accelerates fiber hypertrophy and myogenesis following a single bout of eccentric exercise, *Am. J. Physiol. Cell Physiol.* 301 (4) (2011) C938–C946.
- [203] S. Joannis, et al., Evidence for the contribution of muscle stem cells to nonhypertrophic skeletal muscle remodeling in humans, *Faseb. J.* 27 (11) (2013) 4596–4605.
- [204] R.R. Wolfe, Skeletal muscle protein metabolism and resistance exercise, *J. Nutr.* 136 (2) (2006) 525S–528S.
- [205] C.J. Mitchell, et al., Acute post-exercise myofibrillar protein synthesis is not correlated with resistance training-induced muscle hypertrophy in young men, *PLoS One* 9 (2) (2014) e89431.
- [206] M.P. Harber, et al., Muscle protein synthesis and gene expression during recovery from aerobic exercise in the fasted and fed states, *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 299 (5) (2010) R1254–R1262.
- [207] D.J. Cuthbertson, et al., Anabolic signaling and protein synthesis in human skeletal muscle after dynamic shortening or lengthening exercise, *Am. J. Physiol. Endocrinol. Metab.* 290 (4) (2006) E731–E738.
- [208] N.A. Burd, et al., Low-load high volume resistance exercise stimulates muscle protein synthesis more than high-load low volume resistance exercise in young men, *PLoS One* 5 (8) (2010) e12033.
- [209] N.A. Burd, et al., Enhanced amino acid sensitivity of myofibrillar protein synthesis persists for up to 24 h after resistance exercise in young men, *J. Nutr.* 141 (4) (2011) 568–573.
- [210] R. Ogasawara, et al., Resistance exercise-induced hypertrophy: a potential role for rapamycin-insensitive mTOR, *Exerc. Sport Sci. Rev.* 47 (3) (2019) 188–194.
- [211] Y.C. Long, U. Widegren, J.R. Zierath, Exercise-induced mitogen-activated protein kinase signalling in skeletal muscle, *Proc. Nutr. Soc.* 63 (2) (2004) 227–232.
- [212] C.E. Mazo, et al., The effects of acute aerobic and resistance exercise on mTOR signaling and autophagy markers in untrained human skeletal muscle, *Eur. J. Appl. Physiol.* 121 (10) (2021) 2913–2924.
- [213] J.G. Burniston, Investigating muscle protein turnover on a protein-by-protein basis using dynamic proteome profiling, in: J. Burniston, Y.W. Chen (Eds.), *Omics Approaches to Understanding Muscle Biology. Methods in Physiology*, Springer, New York, NY, 2019. https://doi.org/10.1007/978-1-4939-9802-9_9Omics.
- [214] N. Stansfield, et al., Turnover rates of human muscle proteins in vivo reported in fractional, mole and absolute units, *Ben, BurnistonbioRxiv* (2024) 576451. <https://doi.org/10.1101/2024.01.21.576451>.
- [215] D.M. Camera, et al., Dynamic proteome profiling of individual proteins in human skeletal muscle after a high-fat diet and resistance exercise, *Faseb. J.* 31 (12) (2017) 5478–5494.
- [216] K. Srisawat, et al., People with obesity exhibit losses in muscle proteostasis that are partly improved by exercise training, *Proteomics* (2023) e2300395.
- [217] K. Højlund, et al., In vivo phosphoproteome of human skeletal muscle revealed by phosphopeptide enrichment and HPLC-ESI-MS/MS, *J. Proteome Res.* 8 (11) (2009) 4954–4965.
- [218] G.K. Potts, et al., A map of the phosphoproteomic alterations that occur after a bout of maximal-intensity contractions, *J. Physiol.* 595 (15) (2017) 5209–5226.
- [219] R. Blazev, et al., Phosphoproteomics of three exercise modalities identifies canonical signaling and C18ORF25 as an AMPK substrate regulating skeletal muscle function, *Cell Metabol.* 34 (10) (2022) 1561–1577 e9.
- [220] B.L. Parker, et al., Quantification of exercise-regulated ubiquitin signaling in human skeletal muscle identifies protein modification cross talk via NEDDylation, *Faseb. J.* 34 (4) (2020) 5906–5916.
- [221] N.J. Pillon, et al., Transcriptomic profiling of skeletal muscle adaptations to exercise and inactivity, *Nat. Commun.* 11 (1) (2020) 470.
- [222] P.A. Makhnovskii, et al., Transcriptomic signatures and upstream regulation in human skeletal muscle adapted to disuse and aerobic exercise, *Int. J. Mol. Sci.* 22 (3) (2021) 1208.
- [223] O.C. Witard, L. Bannock, K.D. Tipton, Making sense of muscle protein synthesis: a focus on muscle growth during resistance training, *Int. J. Sport Nutr. Exerc. Metabol.* 32 (1) (2022) 49–61.
- [224] V.G. Coffey, J.A. Hawley, Concurrent exercise training: do opposites distract? *J. Physiol.* 595 (9) (2017) 2883–2896.
- [225] B. Egan, A.P. Sharples, Molecular responses to acute exercise and their relevance for adaptations in skeletal muscle to exercise training, *Physiol. Rev.* 103 (3) (2023) 2057–2170.
- [226] V. Kumar, et al., Human muscle protein synthesis and breakdown during and after exercise, *J. Appl. Physiol.* 106 (6) (1985) 2026–2039, 2009.
- [227] C.G. Perry, et al., Repeated transient mRNA bursts precede increases in transcriptional and mitochondrial proteins during training in human skeletal muscle, *J. Physiol.* 588 (Pt 23) (2010) 4795–4810.
- [228] C. Broholm, et al., Exercise induces expression of leukaemia inhibitory factor in human skeletal muscle, *J. Physiol.* 586 (8) (2008) 2195–2201.
- [229] M.J. Arkinstall, et al., Regulation of metabolic genes in human skeletal muscle by short-term exercise and diet manipulation, *Am. J. Physiol. Endocrinol. Metab.* 287 (1) (2004) E25–E31.
- [230] P.A. Makhnovskii, et al., Regulation of proteins in human skeletal muscle: the role of transcription, *Sci. Rep.* 10 (1) (2020) 3514.
- [231] E.B. Emanuelsson, et al., Remodeling of the human skeletal muscle proteome found after long-term endurance training but not after strength training, *iScience* 27 (1) (2024) 108638.

- [232] J. Kuang, et al., Interpretation of exercise-induced changes in human skeletal muscle mRNA expression depends on the timing of the post-exercise biopsies, *PeerJ* 10 (2022) e12856.
- [233] C.G.R. Perry, J.A. Hawley, Molecular basis of exercise-induced skeletal muscle mitochondrial biogenesis: historical advances, current knowledge, and future challenges, *Cold Spring Harb Perspect Med* 8 (9) (2018).
- [234] D.J. Bishop, et al., Discordant skeletal muscle gene and protein responses to exercise, *Trends Biochem. Sci.* 48 (11) (2023) 927–936.