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

Aaron C.Q. Thomas [a,b], Connor A. Stead [b], Jatin G. Burniston [b], Stuart M. Phillips [a,*]

[a] Protein Metabolism Research Lab, Department of Kinesiology, McMaster University, Hamilton, ON, Canada
[b] Research Institute for Sport & Exercise Sciences, Liverpool John Moores University, Liverpool, United Kingdom

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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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* Corresponding author.
E-mail address: phillis@mcmaster.ca (S.M. Phillips).

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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-1α – 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
STRADα – 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 VO_2 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 VO_2 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., VO_2 peak) are underpinned by muscle adaptions, 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 quadricep 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 adaption 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 (S^{2448}, S^{2481}, and S^{2446}) 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 S^{2448} phosphorylation also occurs following acute AE [37], indicating mTOR S^{2448} 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 T^{389} of ribosomal protein S6 kinase beta-1(70S6K), T^{37}/T^{46} of eukaryotic translation initiation factor 4E (eIF4E)-binding protein 1 (4E-BP1), and T^{56} of eukaryotic elongation factor-2 kinase (eEF2K; a.k.a. CaM-KIII). Further, mTORC1 is known to phosphorylate master regulators of the autophagy-lysosomal pathway, such as S^{757} of Unc-51-like autophagy-activating kinase 1 (ULK1) and S^{211} 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 S^{2448} 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 S^{2448} 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 T^{389} 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 T^{308}/S^{478} by PDK1 [45]. Phosphorylation of AKT S^{478} alone is not sufficient to increase AKT activity; instead, it induces a conformational change, stabilizing the active structure and promoting phosphorylation of AKT T^{308} for full activation [45]. In human muscle, following RE, AKT S^{478} and T^{308} 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 S^{477}/T^{479} and AKT S^{473}, hypothesized to stabilize active AKT [48]. Active AKT directly phosphorylates TSC2 T^{1462}, a GTPase activating protein for Rheb, preventing signal transduction through mTOR [49]. At the same time, Rheb bound to GTP increases mTOR S^{2448} phosphorylation [50]. In non-muscle, HEK293 cells, phosphorylation of mTOR S^{2159}/T^{2164} has also been demonstrated to alter interaction with raptor and PRAS40, found to be required for mTORC1 associated mTOR autophosphorylation at S^{2481}, promoting downstream signaling and growth [51]. However, phosphorylation of mTOR S^{2159}/T^{2164} 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

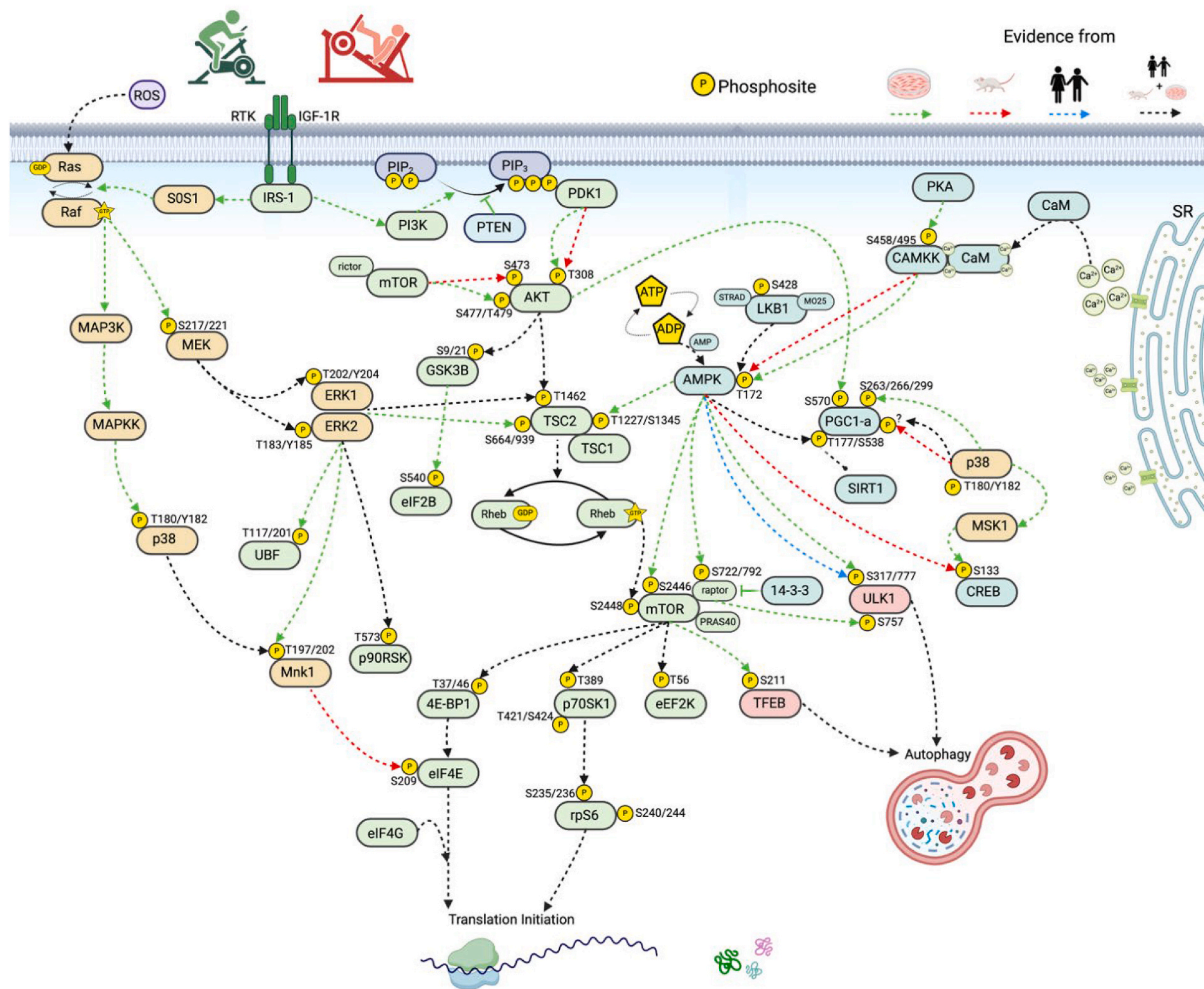

Fig. 1. Simplified signaling network of the key proteins, kinases, and transcription factors involved in the regulation of translation initiation and autophagy following acute exercise. Targeted PTM studies following exercise have identified AKT-mTOR, MAPK, and AMPK cascades as primary regulators of 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 T^{421}/S^{424} and eEF2 T^{56} [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 S^{2448} phosphorylation to regulate phosphorylation of p70 T^{389} 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 T^{389}, 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 T^{389} 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 S^{473} and mTOR S^{2448} are still significantly phosphorylated following RE after 10 weeks of RT [25]. Additionally, in 8 chronically resistance-trained men, the phosphorylation of p70S6K T^{389} 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 S^{473} 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 S^{473} or AKT T^{308} following resistance exercise (8 sets × 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 % $VO2_{max}$), 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 S^{209}, 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}/Y^{204} phosphorylation is well correlated to the intensity of the exercise stimulus [71], which has been demonstrated for both ERK1/2 T^{202}/Y^{204} and ERK2 T^{183}/Y^{185} 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 S^{217} and S^{221}, a known activator of ERK [73]. MAPK singling 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}/Y^{182}, 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-1a 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 Y^{182}, thought to be regulated via the Src family of tyrosine kinases and Ras [83]. In human muscle, p38a T^{180}/Y^{182} 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 S^{133} via MSK1, initiating the transcription of genes involved in energy metabolism and mitochondrial biogenesis [85,86].

In human muscle, ERK1/2 T^{202}/Y^{204} and p38MAPK T^{180}/Y^{182} 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}/Y^{204} and p38MAPK T^{180}/Y^{182} 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}/Y^{204} 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}/Y^{204}, p38MAPK T^{180}/Y^{182}, and JNK T^{183}/Y^{185}) phosphorylation immediately post-exercise with significant Mnk1 $T^{197/202}$ phosphorylation but no significant increase in eIF4E S^{209} phosphorylation [90]. The lack of increase of eIF4E S^{209} 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 S^{664} 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 T^{1462}) and shown to regulate mTOR activity as assessed by downstream S6K1 phosphorylation at T^{389} and T^{421}/S^{424} [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}/Y^{204} is significantly phosphorylated above rest in human skeletal muscle 1 h after RE, coinciding with increased phosphorylation of p70S6K T^{389} [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 T^{117} and T^{201} 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) T^{573}, 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 knockdown has been shown to reduce ERK1/2 T^{202}/Y^{204} 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}/Y^{204} and ERK T^{183}/Y^{185} 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}/Y^{182} phosphorylation still occurs in response to AE (1.6-fold increase); nonetheless, the magnitude of the response may be attenuated in comparison to naïve 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}/Y^{182} 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 AMPKa1 and AMPKa2, and activity is primarily exercise-regulated via phosphorylation at sites T^{183} and T^{172}, respectively [103,104]. AMPKa1 and a2 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 a1 and a2 activity [106]. AMPKa2 T^{172} 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 T^{172} phosphorylation 16-fold immediately post-exercise [37]. Additionally, phosphorylation of AMPK(a1 and a2) T^{172} 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-1a) [84]. A potent upstream regulator of AMPK T^{172} phosphorylation in skeletal muscle is LKB1 [109–111]. Pseudokinase STRADα promotes the active conformation of LKB1, which is stabilized by MO25α interacting with the LKB1 activation loop [112]. LKB1 S^{428} phosphorylation alters cellular location and affinity for AMPK activation [113]. When LKB1 expression in muscle is knocked down by ~90 %, AMPK T^{172} demonstrates significantly reduced phosphorylation following muscle contraction [114]. Downstream, the acute increase in AMPK T^{172} activity regulates SIRT1 affinity for PGC-1a by phosphorylating PGC-1a T^{177}/S^{538} in addition to elevating the intracellular NAD + concentration, which in turn modulates mitochondrial protein synthesis [115–118]. Furthermore, chronic (repeated) activation of AMPK T^{172}, 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, AMPKa2 activity is significantly increased during and immediately following (1–2 h) exercise [108]. AMPK phosphorylates TSC2 T^{1227}/S^{1345} and heightens its ability to suppress translation regulation via the mTORC1 pathway [125]. Additionally, work performed in TSC2-depleted cells identified raptor S^{722}/S^{792} 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 T^{2446}, which has been demonstrated to reduce activity via attenuation of phosphorylation at mTOR S^{2448} [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 p70SK1 T^{389} and p70SK1 T^{421}/S^{424} [127]. However, although mixed MPS is repressed during RE while AMPKa2 is known to be active, there is a robust increase (75 %) in MPS following RE (1–2 h) despite AMPKa2 remaining significantly elevated from basal levels [108]. Further, an elevation in protein synthesis has been shown to occur concomitantly with an increase in mTOR S^{2448} 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 T^{172} 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 S^{317} and S^{777} [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 S^{466}, 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 T^{172} 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 AMPKa 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 T^{180}/Y^{182} and ERK1/2 T^{202}/Y^{204} phosphorylation, which is associated with a reduction of gene expression of mitochondrial transcription factor A (mTFA) but does not alter PGC-1a 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 AMPKa T^{172} through a CAMKKb-dependent mechanism [144,145].

In addition to ROS, nitric oxide production is increased during muscle contraction [146]. Nitric oxide signaling can regulate AMPKa T^{172} and CAMK T^{286} phosphorylation [147,148] and, therefore, may influence downstream GLUT4, PGC-1a, 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 perturbance, 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 bisphosphate conversion by phospholipase Cγ1 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 T^{308} [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 T^{308} phosphorylation [159]. Watt et al. (2015) [162] demonstrated that inhibition of YAP by mutation of S^{79} 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 S^{2448} 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 Y^{397} 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 Y^{397} may also regulate mTORC1 activity through direct phosphorylation inhibition of TSC2 T^{1462} [171,172]. However, another mechanism has been identified for FAK Y^{397} 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 S^{411} and p70S6K T^{421}/S^{424} 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 S^{2234}/S^{2237}, 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 S^{143} 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 S^{26}, S^{34}, S^{170}, S^{496}, T^{70}, T^{80}, T^{117} [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 Ibata 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]. Therefor, 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 myofilaments 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 altogether, 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 subfractions, 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 ± 38 %) and 180–360 min (184 ± 28 %). Whereas, after a single session of AE, bulk (mixed muscle protein) MPS is elevated by ~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 (~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 p70SK6K1) 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 S^{2448} 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 attemtped to sumarize 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 S^{2448} phosphorylation and its downstream target 4EBP1 $T^{37/46}$ [212]. However, the magnitude of mTORC1 S^{2448} signaling downstream targets S6K1 T^{389} and 4EBP1 $T^{37/46}$ at 1–4 h post-exercise is less (~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 S^{2448} 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

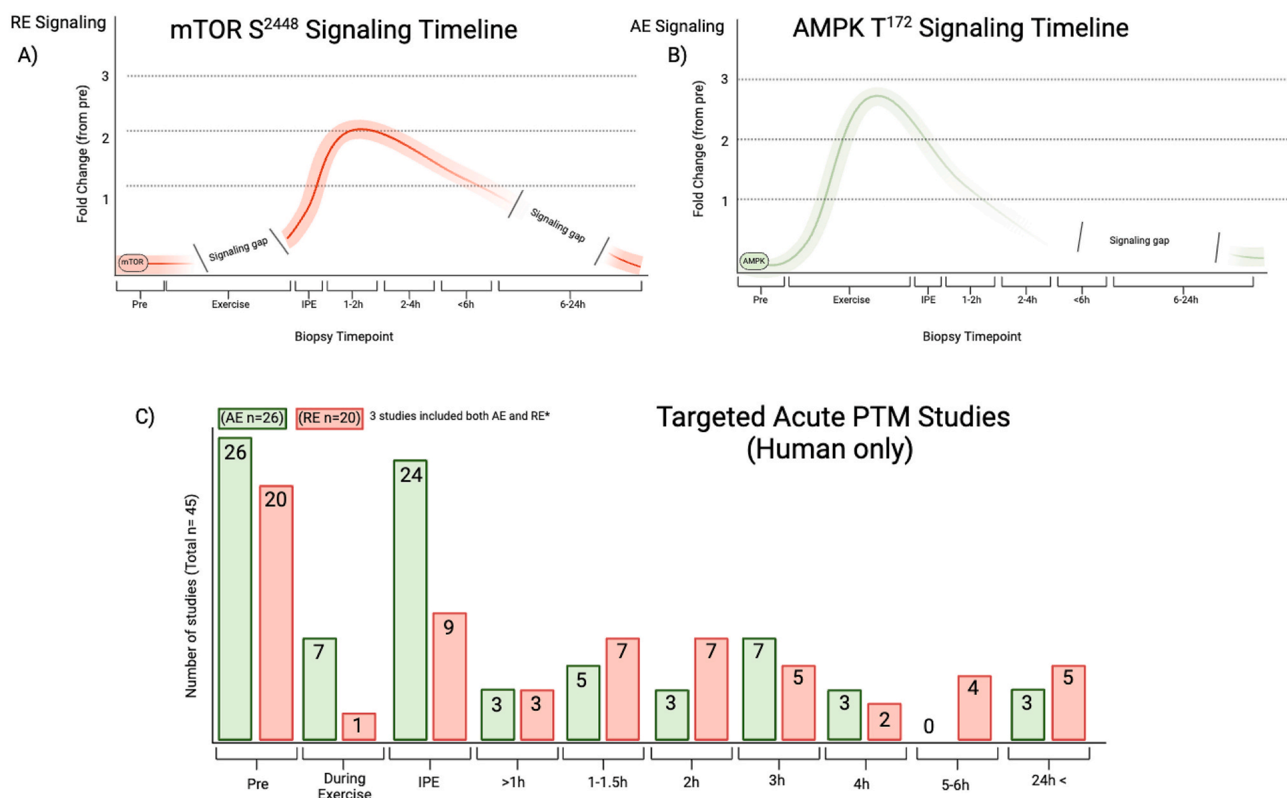

Fig. 2. Acute exercise PTM signaling timelines for representative proteins of resistance (mTOR) and aerobic (AMPK). Sixteen studies each for resistance and aerobic exercise were identified from a systemic search of the literature to have assessed (A) mTOR S2448 and (B) AMPK T172 phosphorylation following 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, AMPKa1, has 50 known sites of post-translational modification, including 36 phospho-sites, 12 ubiquitylation sites, 1 acetylation, and 1 SUMOylation site (Phosphosite, AMPKA1 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 (T^{183}). Similarly, 34 modification sites are known on the human isoform of AMPKa2, and only 12 sites have been studied by LTP targeted analyses, including the exercise responsive T^{172} mapped with 222 targeted studies from a total of 249 mapped to the entirety of AMPKa2. 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 S^{2448} and S^{2481} 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 underexploited. 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 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.

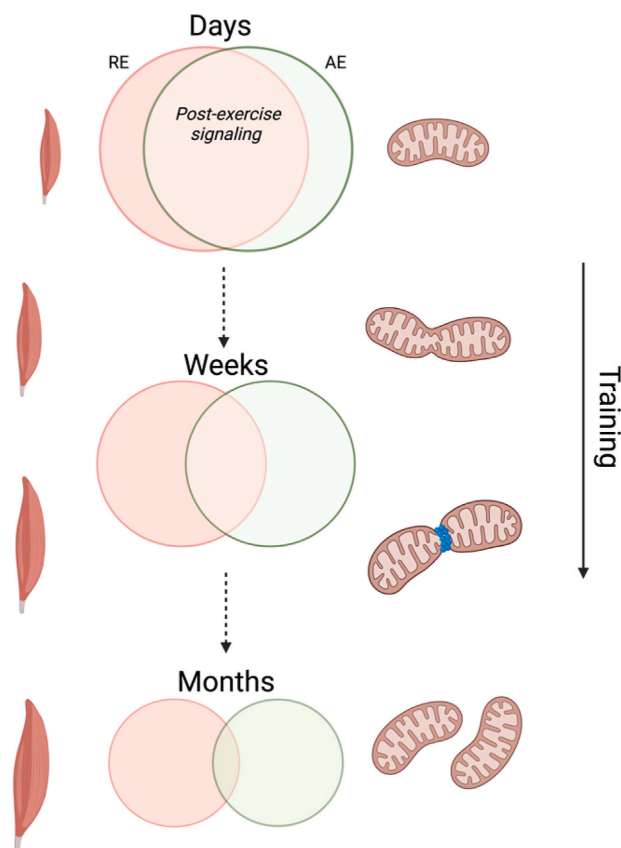

Fig. 3. Exercise perturbation in unaccustomed muscle elicits a less specific signaling (PTM, transcription, and translation) response that becomes more refined with chronic training. With repeated exercise of a specific modality, the molecular response to exercise has a lesser but more efficient PTM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

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 T^{172} and FAK $Y^{576/577}$ and eIF4E S^{209} 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.

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