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**TITLE**

# Molecular Responses to Acute Exercise and Their Relevance for Adaptations in Skeletal Muscle to Exercise Training

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Molecular responses to acute exercise

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## ABSTRACT

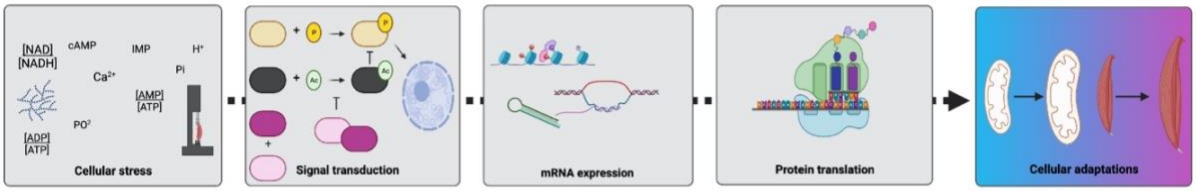
Repeated, episodic bouts of skeletal muscle contraction undertaken frequently as structured exercise training is a potent stimulus for physiological adaptation in many organs. Specifically in skeletal muscle, remarkable plasticity is demonstrated by the remodeling of muscle structure and function in terms of muscular size, force, endurance, and contractile velocity as a result of the functional demands induced by various types of exercise training. This plasticity, and the mechanistic basis for adaptations to skeletal muscle in response to exercise training, is underpinned by activation and/or repression of molecular pathways and processes induced in response to each individual acute exercise session. These pathways include the transduction of signals arising from neuronal, mechanical, metabolic, and hormonal stimuli through complex signal transduction networks, which are linked to a myriad of effector proteins involved in the regulation of pre- and post-transcriptional processes, and protein translation and degradation processes. This review therefore describes acute exercise-induced signal transduction and the molecular responses to acute exercise in skeletal muscle including emerging concepts such as epigenetic pre- and post-transcriptional regulation, and the regulation of protein translation and degradation. A critical appraisal of methodological approaches and the current state of knowledge informs a series of recommendations offered as future directions in the field.



## KEYWORDS

aerobic; gene expression; metabolism; resistance; transcription; translation;


## GRAPHICAL ABSTRACT




## GRAPHICAL ABSTRACT LEGEND

Overview of the working model for how exercise-induced signal transduction and the associated molecular responses to a single session of acute exercise are proposed to underpin adaptations in skeletal muscle to exercise training. Biochemical and biophysical responses to acute exercise activate and/or repress signal transduction pathways to alter gene expression, rates of protein synthesis and other downstream processes. With repeated bouts of exercise (i.e. exercise training), changes in protein content yield cellular adaptations that alter skeletal muscle phenotype and improve exercise performance.

## CLINICAL HIGHLIGHTS

The development and maintenance of functional capacity through aerobic fitness and skeletal muscle strength can delay the onset of lifestyle-related chronic diseases, and are therefore central to performance and healthy ageing across the life course. These benefits are mediated in part by extensive remodeling of skeletal muscle by exercise, the mechanistic bases of which are informed by almost half a century of investigation of molecular responses in skeletal muscle to acute exercise and exercise training. Understanding of the molecular responses to acute exercise as a means to explain the adaptation to different types of exercise, e.g. aerobic, resistance, high intensity interval, can assist in fine-tuning exercise training prescription and additional co-intervention strategies. As such, a better understanding of these physiological processes and molecular networks may facilitate the further development of personalized exercise medicine. By identifying important molecular networks that are potentially “druggable” targets, this may also provide foundation for developing pharmacotherapies that could be applied in isolation, or as compounds serving as adjunct treatments to exercise that potentiate or augment the benefits of acute exercise and/or adaptations to exercise training.

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# 1. INTRODUCTION

## *A. Historical context*

Although the application of various types of exercise training to produce improvements in performance has long been known (1-5), *how* exercise produces these improvements has been the subject of investigation for a little over half a century (6). The birth of the modern study of the molecular basis of adaptations in skeletal muscle to exercise training can be traced to a seminal work by Dr. John Holloszy that demonstrated remarkable adaptive changes in mitochondrial protein content and enzyme activity in the skeletal muscle of rats subjected to intense exercise training (7). Prior to this study, a number of exercise training studies on rats subjected to 30 minutes of swimming daily for 5 days per week for 5 to 8 weeks had failed to observe changes in activity of various enzymes involved in cellular energy pathways (8-10). Holloszy (7) hypothesized that the differences in mitochondrial proteins and enzyme activity observed in different muscle types and with different activity levels was a consequence of an “adaptive process”, but that the exercise training stimulus to induce adaptive changes in skeletal muscle was required to be “more vigorous and prolonged” than the previous training studies. Therefore, rats were subjected to 5 days of treadmill running of progressively increasing work until after 12 weeks they were running for 120 minutes continuously (31 m/min) interspersed with 30 sec “sprints” (42 m/min) once every 10 min. Compared to sedentary control rats, mitochondrial protein abundance, the capacity to oxidize pyruvate, and the enzymatic activities of cytochrome oxidase and succinate oxidase were all increased ~1.5 to 2.0-fold in trained skeletal muscle (7).

Aside from that focus on the mitochondrion, adaptations in skeletal muscle (and other organs and systems) to exercise training had been described in detail by the 1930s here in *Physiological Reviews* (1). However, in 1964 a model of resistance exercise using a pulley device that allowed for progressive overload on the biceps brachii in mice resulted in an ~30% increase in fiber cross-sectional area of these muscles (11). Around the same time, the plasticity of skeletal muscle in response to different frequencies of electrical stimulation had been demonstrated in the soleus muscle of rabbits (12). Thus, by the late 1960s the hallmark phenotypical features of adaptation to aerobic and resistance exercise training, namely mitochondrial and hypertrophic adaptations respectively, had been established. Understanding the mechanistic and molecular basis of these adaptations remains a major focus of efforts in the field to the present day, and hence the focus of this review.

With the introduction of the skeletal muscle biopsy technique for the study of intramuscular physiology in humans from the early 1960s (cf. (13, 14)), alongside several independent groups applying various rodent models of exercise training, the next decade

provided enormous insight into the adaptive changes taking place in skeletal muscle in response to exercise training (albeit largely focused on aerobic exercise). Indeed, Holloszy's 1976 review (15) concluded "*Now that the adaptive responses to endurance exercise have, to a large extent, been characterized, it seems likely that the main emphasis of future research in this area will be to determine the mechanisms by which these adaptations are brought about.*" A decade later, in his perspective on the nascent field of molecular and cellular exercise physiology in 1988, Dr. Frank Booth echoed the sentiment on the interest in "mechanisms" and thereby beckoned the next 35 years of research with the observation that "*if exercise physiologists intend to delineate the mechanisms that permit an acute response to a single bout of exercise and the mechanisms that produce adaptations to chronic physical training, then the application of new and future techniques in molecular and cell biology will greatly assist this quest.*"

These "mechanisms" underpinning adaptation, rather than the adaptive changes themselves that occur in skeletal muscle in response to exercise training, will be the major focus of this review. The remodeling of muscle structure and function, with respect to alterations in muscular size, force, endurance, and contractile velocity as a result of changes in functional demand induced by various types of exercise training have been reviewed extensively elsewhere (4, 16-21). Instead, this review will focus on the changes to molecular pathways and processes induced in response to acute exercise, which in turn are proposed as the mechanistic basis for adaptations in skeletal muscle in response to exercise training. As will be described throughout this review, this model (Figure 1) for how exercise-induced signal transduction and the associated molecular responses to a single session of acute exercise are proposed to underpin adaptations in skeletal muscle to exercise training remains a working model and is not fully validated, because many important gaps in knowledge and caveats exist around how well data garnered from rodents and untrained human participants in response to acute exercise sessions can explain the adaptations in skeletal muscle to exercise training.

The understanding of these molecular pathways and processes has developed in parallel with the emergence of modern molecular biology techniques, such that from the late 1970s, the field of molecular and cellular exercise physiology emerged to compliment traditional exercise biochemistry in which earlier observations on adaptations in skeletal muscle to exercise training had been made (6, 22-24). Some of the first observations on the molecular basis of adaptations in skeletal muscle to exercise training were changes in mRNA abundance in response to various models of contractile activity (25-30), and aerobic (31) and resistance (32) exercise training. However, these studies could not definitively distinguish whether changes in mRNA abundance in response to chronic contractile activity or exercise

training were the result of a new steady-state rate of transcription, or a transient increase in transcription during recovery. However, several studies performed in rodents subjected to a single session of exercise demonstrated acute, transient increases of several-fold in mRNA abundance in the first few hours of recovery after exercise, before returning to baseline values within 24 hours (33-36). The same acute, transient patterns were soon confirmed in human skeletal muscle in response to various experimental designs involving aerobic exercise (37-42), and latterly, resistance exercise (43, 44). Together, these data suggested that transient increases in transcription during recovery from consecutive sessions of exercise resulted in a gradual accumulation of mRNA and, therefore, represented the underlying kinetic basis for the cellular adaptations associated with exercise training i.e. enhanced abundance of gene transcripts are the information for the synthesis of protein components that provoke structural remodeling and functional adjustments in the long term (cf. (45)).

Within a few years, the deployment of microarrays for gene expression analysis on a broader scale (than the previous approaches of investigating candidate genes), revealed the large numbers of genes whose mRNA abundance was altered by acute exercise in human skeletal muscle (46, 47). Investigating changes in mRNA abundance by transcriptomics (i.e. gene expression profiling using expression microarrays or RNA-sequencing) has provided enormous insight into the effect of acute exercise on the transcriptional profile of skeletal muscle, as well as the effects on skeletal muscle induced by inactivity or exercise training. Recently, data collected from human studies of aerobic and resistance exercise, including acute exercise sessions and chronic exercise training, have been integrated using meta-analysis methods to create an online open access database known as MetaMEx (www.metamex.eu), alongside an interface to readily interrogate the database (48). This database at the time of writing contained 66 transcriptomic datasets from human skeletal muscle, including 13 studies of acute aerobic exercise, and 8 studies of acute resistance exercise. Therefore, the transcription of exercise-responsive genes remains central to the model of exercise training-induced adaptations in skeletal muscle with hundreds of genes exhibiting altered expression patterns both at various time points in the first 24 hours after a single exercise session, and at rest after a period of exercise training (48-64). In the last decade the discovery of novel roles for pre- and post-transcriptional processes particularly as they relate to epigenetics, namely histone modifications (65), DNA methylation (66, 67), and miRNA (68, 69), in addition to the regulation of protein translation, the biogenesis and activity of organelles including ribosomes (translational capacity and efficiency) and lysosomes (autophagy), and the role of skeletal muscle satellite cells, have all emerged as important regulators of the adaptive response to exercise (70, 71).

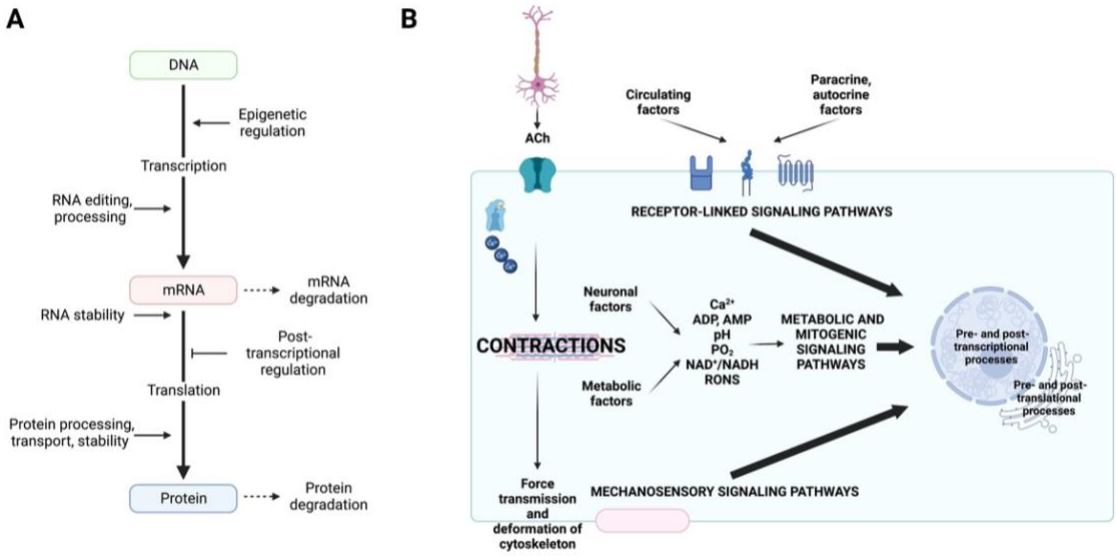


**FIGURE 1. The flow of genetic information in a cell and regulation by muscle contraction.**

(A) Flow of information from DNA via mRNA to protein. A gene (DNA) is transcribed to the various forms of RNA, first to pre-mRNA that may be edited and then processed to one or by alternative splicing to several forms of mRNAs. The mRNAs are then transported out of the nucleus to the cytosol. In the cytosol, the mRNA may be degraded or translated into protein. The activities of the proteins are controlled. They may be synthesized as inactive proteins that later are reversibly or irreversibly activated, or alternatively synthesized as active proteins that later are inactivated. Proteins are ultimately the effecting molecules producing physiologic effects in virtually every mechanism in the cell.

(B) The model proposes that factors intrinsic and extrinsic to muscle cells in exercising skeletal muscle are under the influence of neuronal, mechanical, metabolic, and hormonal stimuli that constitute perturbations to homeostasis, which in turn produce molecular signals that activate and/or repress signal transduction pathways and downstream effector proteins involved in the regulation of pre- and post-transcriptional processes, and protein translation and degradation processes. This model attempts to provide continuity and integration between biochemical and biophysical responses to acute exercise and the expression of genes and activity of processes that ultimately dictate changes in skeletal muscle phenotype in response to exercise training. Adapted from (45).

Following the initial observations of transient changes in mRNA abundance in response to acute exercise, the question remained as to the mechanisms that linked the onset of muscle contraction to changes in the rate of transcription, while also acknowledging that regulation beyond mRNA abundance must also be considered as part of this model (45). In their seminal review in 1996, Williams & Neufer (45) developed the earlier perspective by Booth (22) to describe a working model identifying several steps at which gene expression could be controlled (Figure 1A), and most specifically suggesting that rates of transcription, mRNA degradation, and protein turnover, were regulated by upstream signal transduction pathways activated or repressed in response to muscle contraction (Figure 1B). At that time, signal transduction pathways in skeletal muscle were not well-described, only a handful of protein kinases had been identified as responsive to muscle contraction, and the full spectrum of posttranslational modifications remained to be identified. Nevertheless, Williams & Neufer (45) proposed a number of “primary messengers” arising within skeletal muscle during

contraction, namely changes in intracellular calcium concentration ($[Ca^{2+}]_i$), declines in intracellular pH, declines in energy charge or phosphorylation potential ([ATP]/[ADP][$P_i$]), changes in redox state, declines in oxygen tension, and changes in mechanical stretch or tension. Indeed, in the aforementioned work by Holloszy (7), the following prescient observation was made on the nature of intracellular signals in the regulation of adaptation, "*The intracellular concentrations of numerous substances, including pyruvate, lactate, $P_i$, ADP, and AMP increase in muscle during exercise. Whether or not one of these acts as an inducer of the biosynthesis of the enzymes involved in mitochondrial electron transport is not known.*" Additionally and importantly, Williams & Neufer (45) recognized the potential for factors external to contracting myofibers, including peptide hormones and other factors in circulation (including those acting in an autocrine or paracrine manner), as other primary messengers acting through receptor-linked signaling pathways (Figure 1B).

### *B. Major themes and structure of this review*

With an increasing adoption of cellular and molecular analytical techniques by exercise physiologists including protein kinase activity assays, and the widespread application of the Western blot method with antibodies specific to posttranslational modifications, early discoveries of a variety of signaling kinases induced by acute exercise or contractile activity included, among others, protein kinase C (72), AMP-activated protein kinase (AMPK) (73), mitogen-activated protein kinases (MAPKs) (74), $Ca^{2+}$/calmodulin-dependent protein kinases (CaMKs) (75), and ribosomal protein S6 kinase (S6K1) (76), which were subsequently confirmed in human skeletal muscle (77-81). As a result, the model described by Williams & Neufer (45) was soon updated with description of a myriad of putative signal transduction pathways, their upstream regulators and downstream targets (82-84). With much new information being discovered in the interim, this working model for molecular regulation of adaptations in skeletal muscle to exercise has been the subject of several more recent reviews (65, 67, 85-92).

The purpose of this review is therefore to provide an update and contemporary perspective on acute exercise-induced signal transduction and the molecular response to acute exercise in skeletal muscle, including the emerging concepts around epigenetic pre- and post-transcriptional regulation, and the regulation of protein translation, as well as novel discoveries arising from the application of 'omics' technologies. We will focus predominantly on data garnered from human experiments where it is available and appropriate. However, where key mechanistic insights have come from transgenic mouse models and *in vitro* cell culture experiments that help inform human experimentation, these data will also be described. A challenge of a review of this nature with this intended breadth is to then cover in adequate depth the main players and pathways involved in regulating skeletal muscle

phenotype in what remains a rapidly expanding field of research. For instance, most if not all of the main acute exercise-induced signal transduction pathways and associated downstream effector proteins and processes describe here have been the subject of their own detailed reviews (68-71, 93-118). Therefore, our intention is to parse the salient features of these pathways and processes in relation to exercise and skeletal muscle with reference to original research, while referring to detailed reading elsewhere for further depth. Although there is some overlap between the acute response to a single session of exercise, responses to subsequent exercise sessions early in an exercise training intervention, and ultimately adaptations to prolonged exercise training, our focus will predominantly be on the molecular events occurring in the first 24 hours after a single, acute exercise session.

We initially describe the current model for how acute exercise-induced signal transduction and the associated molecular responses to a single session of acute exercise are proposed to underpin the adaptations in skeletal muscle to exercise training. We then describe the nature of the exercise stimulus as a stressor to homeostasis, as well as the similarities and differences between types of exercise and the how these exercise stimuli can create divergent molecular responses to exercise in skeletal muscle. Prior to delving more deeply into the molecular pathways and processes as they are currently understood, we provide a perspective on the experimental models in cells and rodents that are often employed to explore these concepts. Next, we propose an updated framework for the 'processing' of molecular information after exercise wherein exercise 'stimulus' generates molecular 'signals' that are sensed by 'sensor' proteins, computed and conveyed by 'signal transduction' proteins, and how 'effector' proteins in turn regulate transcription, translation or protein synthesis, protein degradation and other cellular processes. All these processes provide the molecular basis for how skeletal muscle (and perhaps other organs) adapt to exercise over time. Examples of some of the key players in skeletal muscle that are currently thought to be regulators of adaptation to exercise are provided, as well as consideration of emerging techniques to identify novel players in this model of regulation. Given that there are many detailed reviews on specific targets and pathways, we will not exhaustively review each, but instead focus on the salient aspects of regulation and function relevant to the discussion of acute exercise and exercise training. Importantly, as we will describe, while molecular responses do exhibit some specificity in response to different types of acute exercise, at present these responses do not fully explain the divergent adaptive responses to different types of exercise training. We will then consider the translational potential for the investigation of this molecular regulation in exercise physiology, and critically-appraise the excellence of knowledge described throughout the review, before lastly, providing some thoughts on future directions for this field of research.

### *C. Categorization of types of exercise*

Exercise can be categorized under three broad types: 1) aerobic (or endurance) training; 2) resistance (or strength) training; and 3) various intermediate combinations including, but not limited to, circuit training, concurrent training, high intensity interval training, sprint interval training, and sprint training (Figure 2). While all types of exercise share the same fundamental characteristics such as the initiation of contractile activity, elevated energy expenditure and ensuing perturbations to homeostasis, the specific characteristics differ in terms of the force (load), velocity, duration, frequency, and number of contractions. These parameters are important determinants of the neuronal, mechanical, metabolic, and hormonal stimuli acting on skeletal muscle (Section 3), and therefore in turn the perturbations to homeostasis induced by any single session of exercise.

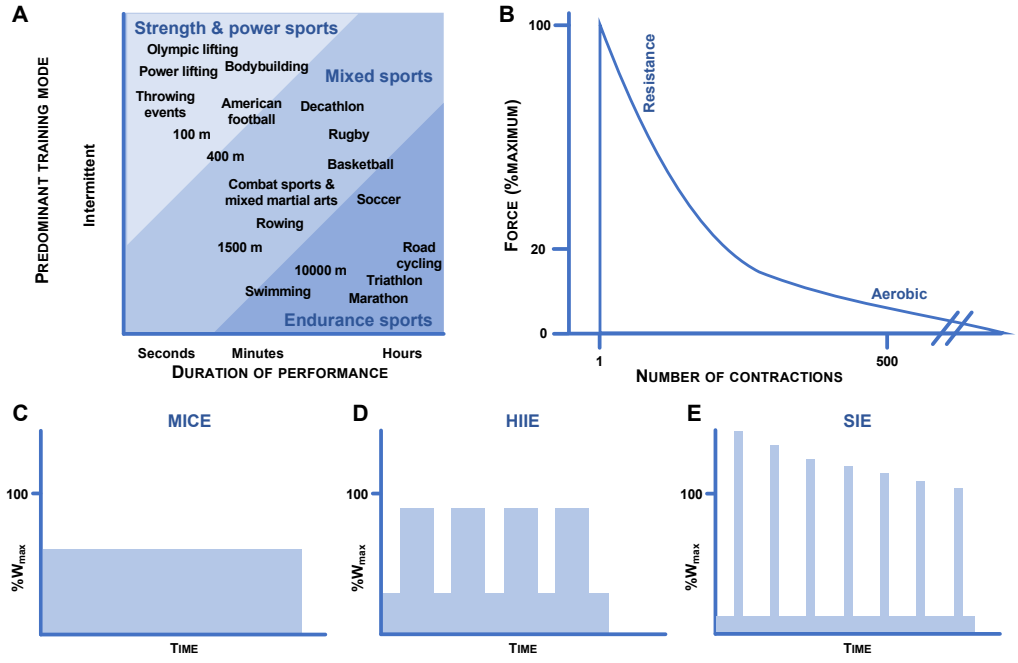


**FIGURE 2. Overview of exercise types/categories and nature of the exercise stimulus**

A) Conceptual representation of various sporting activities along continuums of the predominant training undertaken and the duration of performance in competition.

B) A major distinction between aerobic and resistance exercise is the force of each contraction and the number of contractions that are performed during typical training sessions or that can be performed at a given intensity before fatigue.

Representations of (C) moderate intensity continuous exercise (MICE), (D) high intensity interval exercise (HIIE), and (E) low volume sprint interval exercise (SIE) (adapted from (21)). The intensity is depicted as a percentage of the maximal power output (%$W_{max}$) achieved during a typical incremental exercise test to assess $\dot{V}O_{2max}$ on a cycle ergometer. A similar representation could be made using the percentage of velocity at $\dot{V}O_{2max}$ (v $\dot{V}O_{2max}$) instead of %Wmax for testing and training using running as the exercise mode. Adapted from (119).

Aerobic exercise training generally encompasses exercise durations of several minutes up to several hours at various exercise intensities incorporating repetitive, low resistance exercise such as running, cycling, and swimming, and in the classical sense can be considered as moderate intensity continuous exercise (MICE), or training (MICT). Exercise intensity and training prescription is typically expressed relative to $\dot{V}O_{2max}$ or the power output ($W_{max}$) or velocity ($v\dot{V}O_{2max}$) that corresponds to this parameter, and anchored to measurements of lactate threshold and critical power, as well as being based on the three recognized intensity domains: moderate (below lactate threshold), heavy (between lactate threshold and critical power) and severe (above critical power) (120).

Resistance exercise training generally encompasses short-duration activity at heavy, severe, or maximal exercise intensities comprising of high force contractions of single or relatively few repetitions such as with Olympic weightlifting or powerlifting, and/or as somewhat lower force contractions but performed in greater number as practiced for greater emphasis on maximizing muscle hypertrophy such as with bodybuilding-type exercise training. Exercise intensity and training prescription is typically expressed as a percentage relative to one repetition maximum (1RM), which is the highest load that can be lifted or moved through a range of motion in one lifting effort, or relative to maximum voluntary isometric contraction (MVIC), which is the maximum force that can be generated in an isometric contraction at a specific joint angle. For example, resistance training to develop strength of a particular muscle or muscle group may typically encompass 1 to 5 repetitions at 80-95%1RM repeated for 3 to 5 'sets', whereas to promote hypertrophy of the muscle, this would more likely comprise a protocol including 6 to 12 repetitions at 60-85%1RM repeated for 4 to 6 sets. However, even so called 'low-load' resistance training (typically less than 60%1RM and even as low as 30%1RM) that involves lower force contractions repeated more frequently (typically greater than 15 repetitions to volitional failure) can still evoke similar increases in gross muscle size when compared to higher load resistance exercise (greater than 60%1RM) of fewer repetitions to failure over a period of 6 weeks or greater (121, 122). Maximal strength increases are likely to be greater after higher load resistance training, and therefore the similar gross hypertrophy demonstrated between low and high load resistance training maybe influenced by changes in fiber type (123, 124). For a more exhaustive review of this topic, readers are referred elsewhere (125).

For aerobic exercise, the duration of time spent in each intensity domain in a given session is the key determinant of the physiological demands of that training session, whereas for resistance exercise, the number of repetitions per set (usually as a function of the %1RM lifted), number of sets per exercise, and number of exercises per session are the key determinants of the physiological demands of that training session. In turn, whether for aerobic

or resistance exercise, the accumulation of these individual exercise training sessions, and the characteristics thereof, determine the *volume* of exercise training being undertaken, with this training volume being the key determinant of the type and magnitude of the various adaptations to exercise training (126, 127).

Therefore, muscular contraction during aerobic exercise is of higher frequency (repetition), lower power output (force) demand, whereas during resistance exercise is of lower frequency, higher force demand. These divergent demands are reflected to a certain extent in the nature of the adaptations observed following prolonged aerobic exercise training compared to resistance exercise training when either are performed in isolation (Section 1.D) (Figure 3). Therefore, one postulation has been that the type of exercise stimulus (88), and the contemporaneous environmental conditions (128-130), are reflected in both the specificity and divergence of the molecular signatures that are induced, and these signatures underpin the specificity of adaptation in skeletal muscle to exercise. In fact, we and others have previously illustrated aerobic and resistance exercise at opposite ends of the spectrum for exercise type in order to conceptualize the breadth of differences in the acute metabolic and molecular responses, and chronic adaptations (85-88, 131). However, as discussed later in Section 5.J, while this conceptualization is useful in theory and as a didactic tool, it must also be said that for several reasons it is too simplistic as a framework to fully explain the specificity and continuity between acute molecular responses, and the physiological and functional adaptations manifested as the endurance and hypertrophy phenotypes.

Intermediate types of exercise are best exemplified by both the concurrent aerobic and resistance training, and the high intensity intermittent exercise (HIIE) models of exercise that have increased in popularity in the past two decades. Concurrent training was initially of considerable interest in the early 1980s when a phenomenon known as the “concurrent training” or ‘‘interference” effect was first reported (132). Simply stated, training for both endurance and strength outcomes through concurrent aerobic and resistance exercise training resulted in a compromised adaptation to resistance exercise training in terms of strength, power, and hypertrophy when compared with training using resistance exercise alone. However, with public health guidelines now advocating the participation in three to five sessions of aerobic exercise, and at least two sessions of resistance exercise training per week (133, 134), concurrent training is effectively the default form of exercise training that is required in order to fulfil these recommendations. Indeed, investigation of the molecular and phenotypic responses to concurrent exercise training within the same session, day or intervention has been the subject of considerable recent interest in both health and performance contexts (135-139).

Intermittent or “interval training” has also been well-established for many decades as

another type of aerobic exercise training. The more recent emergence of, and interest in, HIIE models of exercise training have centered largely on high intensity interval training (HIIT) and sprint interval training (SIT) (Figure 2). In their comprehensive review on the topic, MacInnis & Gibala (2017) (21) define HIIE as "near maximal" efforts generally performed at an intensity that elicits ≥80% of maximal heart rate, whereas SIE is characterized by efforts performed at power outputs that are equal to or greater than those that correspond to $\dot{V}O_{2max}$. SIE is, therefore, often characterized by "all-out" or "supramaximal" efforts. Again, this general classification scheme is imperfect given that HIIE could involve a range of exercise modes including those used for aerobic (e.g., cycling, running) and resistance exercise (e.g., weightlifting, plyometrics) as well as novel forms such as bodyweight exercise or resisted sled sprinting, or may target sport-specific repeated sprint ability characterized by short sprints (≤10 seconds), often including a change of direction, and interspersed with brief recovery periods (usually ≤60 seconds) (140, 141).

### *D. Overview of adaptations in skeletal muscle to exercise training*

While a detailed description of the adaptations in skeletal muscle to exercise training is beyond the scope of this review, a brief summary is required given the major theme concerning the mechanistic regulation of those adaptations. An overview of these adaptations at the level of cellular/regulatory, tissues/systems, and the whole body is depicted in Figure 3 and summarized as follows.

The hallmarks of adaptation to aerobic exercise training are, at a whole body level, improvements in aerobic *fitness* measured by maximal oxygen uptake ($\dot{V}O_{2max}$) and/or improvements in exercise *performance* e.g. time-to-exhaustion or time-trial performance, and a shift towards increased contribution from lipid oxidation (and decreased carbohydrate oxidation) at the same absolute exercise intensity as before training (142, 143). These improvements largely reflect enhancements in the intrinsic oxidative capacity of skeletal muscle (mitochondrial respiration), and the delivery and utilization of substrates in exercising muscle during subsequent exercise sessions. These enhancements are underpinned at a cellular level, by increased abundance of proteins involved in oxygen delivery to and extraction from skeletal muscle (144), calcium handling and excitation contraction coupling (145, 146), antioxidant capacity (147), glucose transport and glycogen synthesis (148), glycolytic metabolism (149), lactate transport and pH regulation (150), mobilization, transport and oxidation of fatty acids (151, 152), the TCA cycle (153), and mitochondrial ATP production (7). A largely similar range of adaptations are elicited by HIIT and SIT (154-164). Mitochondrial biogenesis is another well-established response to aerobic exercise training in the form of MICT (165) and HIIT (163, 166, 167), as defined by an increase in muscle mitochondrial

number and volume, as well as concomitant changes in organelle composition (101), and whose regulation is briefly described in Section 6.D.

The hallmarks of adaptation to resistance exercise training are a range of morphological and neurological adaptations that contribute to changes in muscle function with respect to size (muscle hypertrophy), strength and power (17, 168, 169), whereas a greater capacity for non-oxidative energy provision is also observed after resistance exercise training (18, 143). The major morphological adaptations include (i) an increase in cross-sectional area (CSA) of muscle fibers (or the muscle tissue as a whole), often preferentially occurring in type IIa fibers, (ii) a change in the angle of pennation of individual muscle fibers, and (iii) increases in the proportion of non-contractile tissue such as collagen (170-174). Neurological adaptations favor increased muscle strength via improvements in motor unit activation, firing frequency, and synchrony of high threshold motor units (175). Neural adaptations occur rapidly and tend to precede hypertrophic adaptations (176), which occur at a slower rate since the rate of muscle protein synthesis (MPS) must exceed the degradation of muscle proteins (muscle protein breakdown; MPB) for a period of time before a measurable accretion of contractile protein occurs (177). Myofibrillar protein accretion is proposed as the primary mechanism by which the CSA of individual muscle fibers increase in size (169, 178). Consequently, the regulation of MPS (114), and MPB (degradation) (117), have become a central focus for understanding the mechanistic basis of adaptation to resistance exercise training (92, 129). However, these processes are also likely to important for the adaptations to aerobic exercise given the robust response in MPS to MICE, HIIE and SIE (179, 180), and the proposed role of mitochondrial protein synthesis in exercise training-induced mitochondrial biogenesis (181, 182). More details on the regulation of MPS and MPB are described in Sections 2.E and 6.F, respectively.

Despite the common approach to the conceptualize aerobic and resistance exercise at opposite ends of the exercise continuum and various associated adaptations as specific to each type of exercise, there are caveats to this approach. Rather than describing adaptations as specific to a given type of exercise, it is more accurate to state that the contribution of various adaptations is greater in one type of exercise compared to another. For example, aerobic exercise training can produce modest hypertrophy of muscle fibers (183) and neural adaptations in terms of muscle recruitment are evident in endurance athletes (184, 185), whereas resistance exercise training can produce improvements in mitochondrial function (186, 187) and whole-body aerobic fitness measured by $\dot{V}O_{2max}$ (188). Some longitudinal studies of heavy resistance exercise training have generally reported either no change or a decrease in mitochondrial volume density and maximal activities of marker enzymes such as citrate synthase (189-191). However, several studies have observed enhanced mitochondrial

protein content and function (186, 187, 192, 193), and like aerobic exercise training, an altered metabolic response to submaximal exercise (194), and improvements in fatigue resistance have been observed (18, 195).

On a related theme, short-sprint training, which is generally recognized as brief (<10 second efforts) but intense exercise, with the goal to develop speed and power, can produce a broad range of adaptive responses, including some that resemble the traditional endurance or strength/power phenotypes (20, 143). Again, the specifics of the adaptive responses in skeletal muscle are highly dependent on the duration of sprint efforts, recovery between sprints, and total volume within sessions (20). Additionally, despite consisting of a small number of repeated, intermittent sets of high (force) power output, all-out sprint activity (e.g. repeated all-out 30-second Wingate test efforts interspersed with a few minutes of recovery), repeated sprint or SIT demonstrates a remarkable capacity to produce an endurance phenotype in skeletal muscle (157, 158, 196-199), and improve endurance performance (21). The observation that various forms of HIIE should have such effects is should not be surprising since these adaptations are consistent with SIE inducing molecular responses largely similar to those associated with moderate-to-heavy intensity aerobic exercise (200-202).

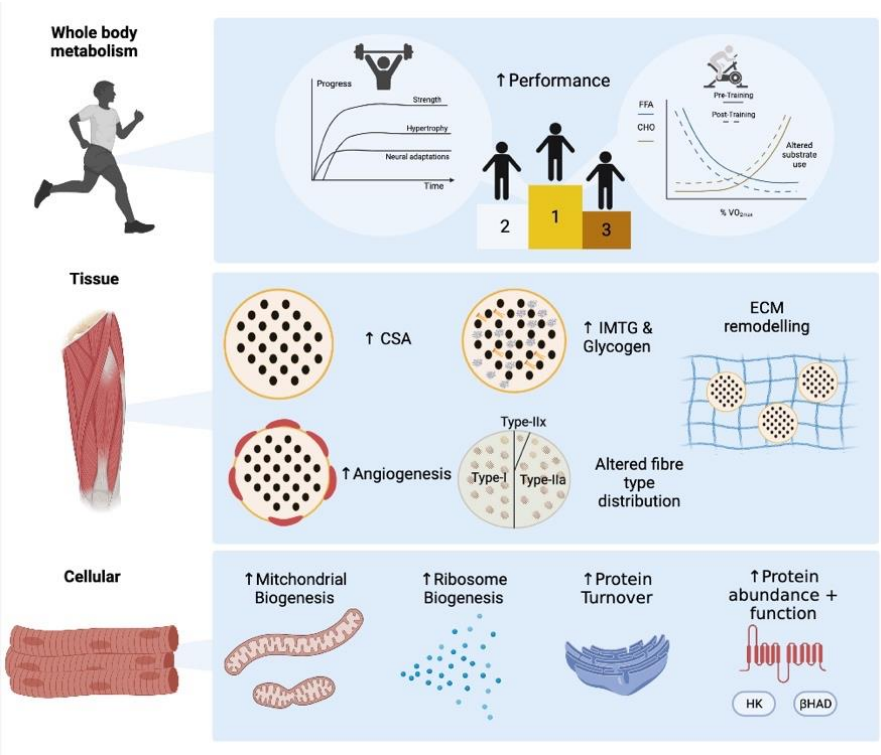


**FIGURE 3. Overview of adaptations in skeletal muscle to exercise training.**

At a cellular and regulatory level in skeletal muscle, adaptations to exercise training take many forms, including changes in abundance, regulation and/or maximal activity of key proteins involved in energy provision, the remodeling of cellular components such as contractile proteins and the extracellular matrix, and the biogenesis of organelles such as ribosomes and mitochondria (70, 86,

88, 92, 101). Consequent to these cellular changes are alterations at the level of tissues and systems such as angiogenesis, muscle hypertrophy and altered substrate metabolism (4, 16-21). The teleological understanding of these coordinated changes in skeletal muscle form and function is that they occur in order to minimize perturbations to cellular homeostasis, with this better maintenance of cellular homeostasis likely contributing to improved fatigue resistance during future sessions of exercise (17).

In simplified terms, exercise training-induced adaptations are a consequence of the training stimulus, which is largely determined by the volume of training (as a function of frequency, intensity, and duration of sessions) and the type(s) (e.g. aerobic, resistance, interval) and mode(s) (e.g. running, cycling, rowing) of training performed. However, the question remains as to how variations in these parameters of exercise prescription have the potential to produce somewhat divergent adaptive responses and phenotypes. The contemporary view is that a network of molecular responses activated by exercise occur in a manner that, broadly speaking, induce adaptive responses following repeated exercise sessions, and that these networks are sensitive enough to the specifics of the training stimulus to ultimately explain divergence in training adaptations. However, as described in later sections, unequivocal evidence for this level of regulation remains somewhat elusive.

## 2. FOUNDATIONAL CONCEPT: THE MECHANISTIC BASIS OF EXERCISE TRAINING-INDUCED ADAPTATIONS IN SKELETAL MUSCLE

The concept of homeostasis is central to the why and how cells, tissues and organs adapt to exercise. As classically-defined, homeostasis is the ability to maintain relative constancy or uniformity in the internal environment in the face of significant changes in the external environment. In the context of this review, this definition can be reframed as the ability of the whole body (broadly) and/or the skeletal muscle (locally) to maintain constancy under the challenge of exercise. Of notable relevance here is that the definition of exercise speaks to this concept, i.e. exercise is “any and all activity involving generation of force by the activated muscle(s) that results in disruption of a homeostatic state” (203). Many perturbations to homeostasis occur with the onset of, and recovery from, exercise, and are manifested in virtually every organ system, but particularly so in the skeletal muscle being exercised (204, 205). These perturbations consequently induce acute responses and long-term adaptations that act as compensatory mechanisms to re-establish and/or preserve homeostasis as required.

The principle of progressive overload suggests that regular and progressive exercise training induces repeated perturbations to homeostasis, which are a necessary stimulus for the adaptations that later defend against perturbations to homeostasis in future sessions of

exercise i.e., this explains *why* tissues and organs adapt to exercise. However, this principle does not explain the mechanisms by which skeletal muscle or other organs respond and adapt i.e., *how* do the adaptations to exercise training occur? Moreover, how does skeletal muscle adapt in a divergent manner to specific *types or modes* of exercise training i.e., how is specificity conferred? Thus, while there is little doubt about the effects of exercise training to produce wide-ranging adaptations in skeletal muscle, the mechanistic bases for how these changes occur remains a topic of much investigation. The model initially proposed by Williams & Neufer (45) and updated by us and others (65, 67, 82-92) posits a series of steps or phases from the onset of the exercise stimulus to a change in protein abundance and/or activity (Figure 4), but as described later, there are temporal and molecular overlaps, as well as feedback and feedforward actions between the various steps rather than a clear sequential pattern.

The contemporary view of this model centers on signal transduction i.e. stimuli, stressors and signals originating from the either inside or outside of a cell resulting in the activation of the intracellular transfer of signals, usually characterized by changes in kinase or phosphatase cascades, or other posttranslational mechanisms and signaling processes, to protein targets in various cellular locations (e.g. cytosolic, nuclear, mitochondrial) that regulate downstream processes. These downstream processes include the regulation of gene transcription and protein translation, such that pre- and post-transcriptional processes, and the regulation of protein translation and degradation, are evidently all regulated by acute exercise through the modulation of the activity of transcription factors and coregulators, translation initiation and elongation factors, and proteolytic regulation.

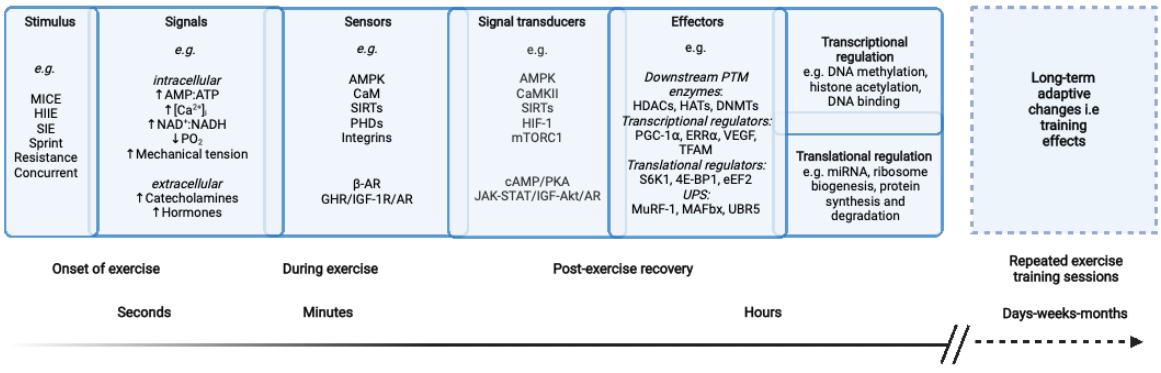


**FIGURE 4. Molecular events and their approximate time course in skeletal muscle in response to exercise**

Schematic representation of the molecular response to exercise and associated exercise-induced signal transduction pathways and downstream effector proteins that underpin the adaptation in skeletal muscle to exercise. Examples, but a far from exclusive list, of the respective signals, sensors, signal transduction proteins and effectors are provided. Adapted from (119).

### *A. Exercise signals*

The onset and continuation of exercise, and ensuing post-exercise recovery period, result in responses both intrinsic and extrinsic to the contracting skeletal muscle that act as important *signals* initiating the molecular response to exercise (Figure 4). Returning to the concept of homeostasis, in a resting myofiber, homeostasis is a function of competing processes including, but not limited to, substrate flux though the various metabolic pathways, ion distribution across the plasma membrane, $Ca^{2+}$ sequestration in the sarcoplasmic reticulum, and cycling of contractile proteins. However, the stimulus of acute exercise induces a myriad of challenges to the maintenance of homeostasis including electrolyte imbalances across cell membranes; changes in muscle cell and tissue volume; changes in the regulators of the various energy-producing pathways and ATP turnover, such as increases in $[Ca^{2+}]_i$ in the sarcoplasmic reticulum (SR), metabolites related to the cytosolic phosphorylation potential ([ATP]/[ADP][$P_i$]), and the mitochondrial reduction/oxidation (redox) state [$NAD^+$]/[NADH]; declining concentrations of muscle glycogen; declining pH; reduced intracellular partial pressure of oxygen ($PO_2$); increased oxygen free radical production including reactive oxygen species and nitrogen species (RONS); increased muscle temperature; and intermittent mechanical load/tension and associated sarcolemmal disruption.

Those listed above are responses intrinsic (intracellular) to the contracting muscle, but extrinsic (circulating) factors that influence the molecular responses to exercise including the prevailing hormonal and substrate milieu such as concentrations of catecholamines (e.g. adrenaline, noradrenaline), of cytokines including tumor necrosis factor $\alpha$ (TNF-$\alpha$) and interleukin 6 (IL-6), of hormones including growth hormone (GH), insulin-like growth factor I (IGF-I) and testosterone, of substrates including glucose, amino acids and free fatty acids, and/or the potential contribution of exercise factors acting in an autocrine or paracrine manner (e.g. IL-6, IGF-I). The magnitude of change induced by exercise to any given factor is determined to a large extent by the intensity, duration, and type of exercise (Section 3). Moreover, many acute responses continue in the hours after the cessation of an exercise session as part of the restoration of homeostasis, and include inflammatory and anti-inflammatory responses, restoration of fluid balance, lactate oxidation, resynthesis of muscle glycogen and intramuscular triglyceride (IMTG) utilized during exercise, and elevations in MPS (129, 206-211). Together these intrinsic and extrinsic factors represent many, but unlikely all, of the responses that constitute perturbations to homeostasis induced by the stimulus of acute exercise, which in turn produce molecular signals that lead to the initiation of signal transduction pathways described in Section 5.

### *B. Sensor proteins*

Collectively, these perturbations to homeostasis during and after an exercise session are sensed by a panoply of *sensor* proteins found within cells and on cell membranes including small molecule sensors, transmembrane receptors, and other cellular sensors/receptors, which detect alterations in the molecular signals described above (Table 1). For example, the heterotrimeric serine/threonine protein kinase AMP-activated protein kinase (AMPK) and the protein deacetylases of the sirtuin family (e.g. SIRT1) are intracellular sensors of changes in [ATP]/[ADP][$P_i$] (109), and [$NAD^+$]/[NADH] (96), respectively. The calcium-binding messenger protein calmodulin (CaM) is a sensor of the changes in [$Ca^{2+}$]$_i$ flux in the SR that are required for muscle contraction (94). The heterodimeric transcription factor hypoxia-inducible factor (HIF) is sensitive to intracellular $PO_2$ (100). Mechanosensors are a diverse (and somewhat elusive) set of proteins that can sense changes in mechanical load (tension) through muscle, and downstream pathways often, although not exclusively, converge on mechanistic target of rapamycin complex 1 (mTORC1) activation (92).

Sensor proteins also include receptors present on the cell membrane that can sense specific peptide hormonal signals arising from changes in circulating concentrations. Examples include GH, IGF-I and adrenaline and their activation of the GH receptor (GHR) (108), IGF-I receptor (IGF-IR) (95), and β-adrenergic receptor (β-AR) (98, 212), respectively. However, receptors as sensor proteins can also include intracellular receptors for steroid-based hormone signals such as the androgen receptor (AR), which is generally located in the cytoplasm but can translocate to the nucleus upon activation (e.g. by binding with testosterone) and thereby regulate gene expression (103).

**Table 1.** Selected sensor proteins and receptors, their function and role in skeletal muscle, and their associated downstream targets, pathways, and processes relevant to exercise-induced signal transduction

| SENSOR PROTEIN OR RECEPTOR | REGULATION | DOWNSTREAM TARGETS, PATHWAYS & PROCESSES |
|---|---|---|
| **Sensors of intrinsic/intracellular signals** | | |
| **$Ca^{2+}$/Calmodulin-dependent protein kinases (CaMKs)** | - Ser/Thr kinases activated in a CaM-dependent<br>- Elevations in [$Ca^{2+}$]$_i$ are decoded by CaM, a multifunctional signal transducer that activates downstream kinases and phosphatases | - Modulates adaptive changes through regulation of the activity of transcriptional regulators |
| **Mitogen-activated protein kinases (MAPKs)** | - Three main MAPK subfamilies in human skeletal muscle: (i) ERK1/2, (ii) JNK, and (iii) p38 MAPK<br>- Sense changes in growth factors, cytokines, tension, and cellular stress including ROS | - Regulate transcriptional events by phosphorylation of diverse substrates localized in the cytoplasm or nucleus, including transcriptional regulators<br>- Proposed to regulate protein translation through mTORC1-independent pathways |
| **Mechanosensors** | - diverse class of proteins involved in cytoskeletal structure and force transmission, and can sense stretch/length, tension/force, and electrical potentials in muscle cells | - Modulate the phosphorylation of downstream targets in mechanotransduction pathways leading to enhanced protein synthesis primarily through mTORC, a critical regulator of protein synthesis |

| | | |
|---|---|---|
| | - include focal adhesion kinase (FAK), Yes-associated protein (Yap), phosphatidic acid synthesized by phospholipase D or diacylglycerol kinase zeta (DGKζ), the $\alpha_7\beta_1$-integrin isoform, titin, Bag3, filamin-C, and stretch-activated ion channels | and myofiber growth in response to anabolic stimuli |
| **Sirtuins (silent mating type information regulation 2 homolog) (SIRTs)** | - A family of NAD+-dependent protein deacetylases<br>- Sense fluctuations in $NAD^+$ as well as the ratio of $[NAD^+]/[NADH]$<br>-Increases in $[NAD^+]$ increase the enzymatic activity of SIRTs | - Catalyze lysine deacetylation of both transcriptional regulators (SIRT1) and mitochondrial enzymes (SIRT3)<br>- Couple redox state to gene expression and enzyme function |
| **Prolyl hydroxylases (PHDs)** | - Enzymes that sense intracellular $PO_2$<br>- Low intracellular $PO_2$ concentrations inhibit the hydroxylase activity of PHDs<br>- Hydroxylation of HIF-1α marks HIF-1α for proteasomal degradation | - Declining intracellular $PO_2$ concentrations and consequent inhibition of PHD activity result in declining hydroxylation of HIF-1α and upregulation of HIF-1-dependent gene expression |
| **AMP-activated protein kinase (AMPK)** | - A heterotrimeric (catalytic α, and regulatory β and γ subunits) Ser/Thr kinase<br>- Senses energy status of the cell via AMP/ATP and Cr/PCr ratios, and depletion of muscle glycogen | - Modulates metabolism acutely through phosphorylation of metabolic enzymes<br>- Modulates adaptive changes through regulation of the activity of transcriptional and translational regulators |
| **Sensors of extrinsic/extracellular signals** | | |
| **β-adrenergic receptors (β-AR)** | - Members of the large family of GPCRs, and have a seven-transmembrane helix topology<br>- Sense and transduce signals encoded in catecholamine hormones and neurotransmitters to activate intracellular signaling<br>- Signal via G protein-dependent pathways to control key physiological functions | - Acutely activates adenylate cyclase to convert ATP to cAMP, then activating several different cAMP-dependent protein kinases that phosphorylate target proteins to alter cell processes including substrate utilization and force potentiation<br>-Chronic activation associated with β-agonists associated with skeletal muscle hypertrophy |
| **Androgen receptor (AR)** | - Member of the steroid receptor superfamily and a ligand-dependent nuclear transcription factor<br>- Senses concentrations of androgens/steroid hormones including testosterone | - Regulate transcription by testosterone binding to ARs and acting as a transcription factor by binding to specific androgen response elements<br>- Androgen-mediated increase in protein synthesis proposed through mTORC1 via IGF-1/Akt and/or ERK1/2<br>- ARs are expressed in satellite cells and may also activate and increase the number of satellite cells for growth and regeneration of muscle |
| **Growth hormone receptor (GHR)** | - Transmembrane receptor that belongs to the class I cytokine receptor family<br>- Senses circulating concentrations of growth hormone | - Direct effects exerted by binding of growth hormone to skeletal muscle GHRs activating JAK-STAT signaling and increasing protein synthesis through mTORC1<br>-Indirect effects exerted through increased production of IGF-1 from the liver and consequent IGF-1R signaling in skeletal muscle |
| **Insulin-like growth factor 1 receptor (IGF-1R)** | - Transmembrane receptor that belongs to the large class of tyrosine kinase receptors<br>- Activated by IGF-1 in circulation and/or IGF-1 and MGF acting in autocrine/paracrine manner | - Binding of IGF-1 to IGF-1R results in phosphorylation of the intracellular adaptor proteins Shc or insulin receptor substrate 1 (IRS-1), which results in the activation of MAPK and PI3K/Akt signaling, respectively<br>- Downstream effects are to stimulate protein synthesis largely through mTORC1 signaling |

### *C. Signal transduction*

This array of sensor proteins acting as, and in concert with, primary and secondary messengers then transduce these signals arising from neuronal, mechanical, metabolic, and hormonal stimuli that amplify and/or dampen the initial signals through complex *signal transduction* networks. This signal transduction of molecular information mainly includes protein-protein interactions, post-translational modifications (e.g. phosphorylation, acetylation,

ubiquitination, SUMOylation) and protein translocation, which lead to the activation and/or repression of another diverse array of proteins that relay the sensed molecular signal inside the cell, and are ultimately coupled to downstream effector proteins, mostly in the form of transcriptional and translational regulators. The best-established acute exercise-induced signal transduction pathways in human skeletal muscle are described in Section 5.

### *D. Effector proteins and processes*

The downstream targets of these signal transduction pathways are *effector* proteins (Table 2), again of which there are many, such as transcription factors and coregulators on the regulation of both pre- and post-transcriptional processes, and translation initiation and elongation factors in the regulation of protein translation, in addition to regulators of protein degradation. Indeed, at each level of the flow of genetic information in a cell, acute exercise has been demonstrated to exert an effect in skeletal muscle, including the accessibility of genes through DNA modifications such as increased acetylation, methylation and phosphorylation of histones (213-215) and hypomethylation of gene promoters (216-219); nucleosome repositioning (220); the DNA binding activity of transcription factors such as HIF-1, AR, ATF2, NF-κB, Forkhead transcription factors, O-box subfamily 1 (FOXO1), cAMP response element binding protein (CREB), myocyte enhancer factor 2 (MEF2), GLUT4 enhancer factor (GEF), and nuclear respiratory factors (NRFs) (221-230); the protein stability and subcellular localization of transcriptional regulators such as HDACs, MEF2, HIF-1, p53, small ubiquitin-related modifier (SUMO) 1, transcription factor EB (TFEB), nuclear factor of activated T cells (NFAT), and the peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) (201, 202, 214, 224, 228, 231-235); post-transcriptional regulation via changes in miRNA abundance (236-242); and differential regulation of protein translation and degradation processes through canonical pathways (cf. (114, 117)).

In elucidating the regulation of skeletal muscle phenotype, reductionist approaches often employ, by design, a focus on an individual target gene and its physiological action in isolation. However, by the very nature of physiological regulation, these targets invariably act in concert with many other proteins as part of broader cellular processes. As a result, it should be recognized that, rather than individual proteins as central or “master regulators” of adaptation to exercise, processes such as the balance between MPS and MPB including autophagy and mitophagy, mitochondrial biogenesis, ribosomal biogenesis and the activity of skeletal muscle satellite cells are the most important determinants of adaptations in skeletal muscle to exercise training. These processes are therefore described in more detail in Section 6.

722 **Table 2.** Selected effector proteins and processes proposed as regulators of exercise-induced
723 adaptation in skeletal muscle in response to modulation of their activity by acute exercise

724

| PROTEIN | FUNCTION & REGULATION |
|---|---|
| **Regulators of transcriptional processes** | |
| **Estrogen-related receptors (ERRs)** | - ERRα and ERRγ are constitutively-active orphan nuclear receptors with high expression in oxidative tissues<br>- regulate OXPHOS, fatty acid oxidation and mitochondrial DNA genes, and angiogenesis<br>- ERRα is regulated by PGC-1α; necessary for PGC-1α-induced mitochondrial biogenesis and VEGF-induced angiogenesis |
| **Forkhead transcription factors, O-box subfamily (FOXOs)** | - regulated by PTMs including acetylation and phosphorylation<br>- FOXO1 regulates genes involved in energy metabolism and shifts in fuel selection, often in concert with ERRα<br>- FOXO3 drives muscle atrophy through upregulation of the muscle-specific ubiquitin ligases MAFbx and MuRF1 |
| **Histone acetyltransferases (HATs) and deacetylases (HDACs)** | - antagonistic enzymes regulating gene expression by altering histone acetylation status<br>- transcriptional activators (HATs) and repressors (HDACs) by catalyzing acetylation and de-acetylation, respectively<br>- rate of transcription determined by balance between HATs and HDACs |
| **Hypoxia-inducible factor (HIF)** | - heterodimeric transcriptional factor composed of HIF-1α and HIF-1β<br>- normoxia: HIF-1α is hydroxylated PHDs and degraded<br>- hypoxia: PHD activity inhibited allowing stabilization of HIF-1α, activation in concert with HIF-1β<br>- activated HIF-1 induces transcription of target genes involved in erythropoiesis, energy metabolism and angiogenesis |
| **Mitochondrial transcription factor A (TFAM)** | - nuclear-encoded transcription factor essential for the replication, maintenance and transcription of mitochondrial DNA and normal mitochondrial function<br>- expression regulated by PGC-1α coactivation of NRF-1 and -2 binding to its promoter |
| **Myocyte enhancer factor (MEF) 2** | - MADS-box transcription factor involved in muscle remodeling through binding sites present in promoters of a wide range of metabolic and myogenic genes<br>- association with class II HDACs represses MEF2 activity<br>- works in concert with PGC-1α to enhance transcription |
| **Nuclear respiratory factor (NRF) 1 and 2** | - nuclear-encoded transcription factors linked to regulation of many mitochondrial genes<br>- NRF-1 regulates genes of all five electron transport chain complexes<br>- regulates mitochondrial DNA transcription through TFAM activation<br>- coactivation by PGC-1α with NRF-2 and ERRα regulates their own and NRF-1 expression |
| **Peroxisome proliferator-activated receptors (PPARs)** | - family (PPARα, β/δ and γ) of ligand-dependent nuclear hormone receptors<br>- regulate transcription by dimerizing with the retinoid X receptor and binding to PPAR response elements (PPREs)<br>- regulatory roles in lipid metabolism and whole-body fuel turnover |
| **PPARγ coactivator (PGC) family** | - family of transcriptional coactivators: PGC-1α, PGC-1β and PGC-1α-related coactivator (PRC)<br>- regulated by PTMs in response to environmental cues governing pathways of thermogenesis, gluconeogenesis, muscle differentiation and cell growth<br>- orchestrators of cellular metabolism through network of transcriptional activators and repressors<br>- dominant effects observed in nucleo-mitochondrial regulation of mitochondrial biogenesis, but specific isoforms may be involved in regulation of glycolytic metabolism and hypertrophy e.g. PGC-1α4 |
| **Regulators of translational efficiency and capacity** | |
| **Mechanistic target of rapamycin complex (mTORC)** | - senses a variety of inputs including growth factors, insulin, amino acids, and factors relating to mechanotransduction, and acts through canonical pathway in the control of protein translation and cellular growth<br>- mTOR is a Ser/Thr kinase that serves as a core component in two functionally and structurally distinct multimeric protein complexes, mTORC1 and mTORC2<br>- the regulatory-associated protein of mTOR (RAPTOR) and the proline-rich Akt substrate of 40 kDa (PRAS40) specifically regulate mTORC1 activity, whereas rapamycin-insensitive companion of mTOR (RICTOR), stress-activated protein kinase-interacting protein 1 (mSIN1), and the RICTOR-binding proteins Protor-1/2 are unique to mTORC2<br>- the two complexes are responsive to different signals and exhibit specificity for different downstream effectors and thereby serving somewhat different roles in cellular metabolism, with mTORC1 a primary coordinator of protein synthesis and autophagy, and mTORC2 a primary regulator of cytoskeletal structure and cell survival<br>- most focus in skeletal muscle and exercise has been on mTORC1 activity |
| **Ribosomal protein/p70 S6 kinase (S6K1)** | - substrate of mTORC1, and a key regulator of MPS through canonical pathways of protein translation and ribosome biogenesis, whose kinase activity is increased by mTORC1 activation leading to phosphorylation of downstream targets including the 40S ribosomal protein S6 (rpS6) |

| | |
|---|---|
| | - activation of S6K1 is proposed to result in increased translation initiation through eukaryotic translation initiation factor eIF4B and eIF4E binding protein (4E-BP1), and translation elongation through elongation factor 2 (eEF2) and eEF2 kinase (eEF2K) |
| **Eukaryotic initiation factors (eIFs)** | - eIFs are group of factors regulating translation initiation through the recognition of mRNA, priming of ribosomal subunits and the creation of pre-initiation and initiation complexes<br>- eIF3 and eIF4 are bound to the signaling proteins S6K1 and 4E-BP1, respectively, which are phosphorylated after mTORC1 activation, resulting in the detachment of eIF3 and eIF4 from S6K1 and 4E-BP1 and steps towards the assembly of ribosomes and translation initiation |
| **Eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1)** | - translation initiation factor that is another major target of phosphorylation by mTORC1<br>- phosphorylation of 4E-BP1 suppresses binding and inhibition of eIF4E by 4E-BP1, with removal of 4E-BP1 from eIF4 allowing the preinitiation complex to be recruited to the mRNA strand before translation initiation<br>- this derepression allows eIF4E to directly bind the 5' end of mRNA to ultimately form an active eIF4F complex, a rate-limiting step in translation initiation.<br>- phosphorylation of 4E-BP1 (alongside S6K1 and rpS6) is often used as an indicator of mTORC1/anabolic signaling |
| **Eukaryotic elongation factors (eEFs)** | - eEFs aid the translation of mRNA to protein by enhancing the recruitment of loaded transfer RNAs to the ribosome and accelerating the shift of the ribosome to the next codon once a peptide bond has been formed<br>- the phosphorylation status of eEF2 is primarily regulated by eEF2K, which is phosphorylated and inhibited by S6K1 (downstream of mTORC1 activation), thereby activating eEF2<br>- may also be regulated by mTORC1-independent mechanisms |
| **Ribosome biogenesis** | - a complex, multistep process involving the *de novo* synthesis of ribosomes initiated by ribosomal DNA (rDNA) transcription with subsequent processing, maturation, and assembly of rRNAs and r-proteins<br>- requires the activity of all three RNA polymerases I, II, and III (Pol I, Pol II, and Pol III), with Pol I serving as a primary control point in concert with the transcription initiation factors TIF-IA and TIF-IB, and upstream binding factor UBF, which are key in forming the pre-initiation complex (PIC) at the rDNA promoter and regulating rDNA transcription<br>- regulation of TIF-IA and UBF occurs through multiple signaling proteins including ERK, AMPK, mTORC1 and S6K1<br>- *de novo* ribosome synthesis is assessed by markers including 45S pre-rRNA abundance as a readout of rDNA transcription, and phosphorylation of TIF-IA and UBF |
| **Regulators of muscle protein degradation** | |
| **Autophagy** | - the specific degradation and recycling of dysfunctional proteins or organelles through the autophagosome-lysosomal system<br>- macro-autophagy involves entire regions of the cytosol or specific organelles and protein complexes being engulfed by a vacuole known as an autophagosome, which then fuses with the lysosome<br>- micro-autophagy involves the direct uptake of cytosolic components into lysosomes<br>- more selective types of autophagy, known as chaperone-mediated autophagy and chaperone-assisted selective autophagy can degrade specific proteins.<br>- the transcription factor EB (TFEB) has been viewed as a master regulator of lysosome biogenesis and therefore central to the regulation of autophagy through its role in determining lysosomal pool size and activity |
| **Mitophagy** | - mitophagy largely occurs through activation of AMPK-Unc-51 autophagy activating kinase 1 (ULK1) signaling, in cooperation with the activation of other mitochondrial-specific kinases and ubiquitin ligases, such as Parkin under the influence of recruitment by PTEN-induced putative kinase 1 (PINK1)<br>- activation of this pathway is also crucial for the formation of the autophagosomal membrane, which occurs through the ATG7-mediated lipidation of microtubule-associated protein 1 light chain 3 (LC3)-I with phosphatidylethanolamine (PE), to form LC3-II<br>- mitophagy receptors BCL2/adenovirus E1B 19-kDa protein-interacting protein 3 (BNIP3) and NIP3-like protein X (NIX) present on mitochondrial membranes can also directly interact with LC3-II to induce mitophagy<br>- the autophagosome subsequently fuses with a lysosome, and its cargo is degraded and recycled within the cell |
| **Ubiquitin-proteasome system (UPS)** | - involves ubiquitination of target proteins followed by their degradation in the 26S proteasome, a protein complex of more than 30 proteins<br>- proteins are selected for degradation by attaching ubiquitin to the ε-amino group of a lysine residue, or in some cases to the N-terminus of the protein<br>- ubiquitination is an energy-requiring process carried out by a series of three types of enzymes, named E1, E2 and E3<br>- ubiquitin is first 'primed' by an E1-activating enzyme and then transferred to one of several E2-conjugating enzymes, which in turn interact with numerous E3 ligases. The actual ubiquitination of target proteins is performed by E2-E3 pairs, but it is the E3 ligase that confers specificity to the process<br>- two muscle-specific E3 ligases are muscle RING finger 1 (MuRF1) and muscle atrophy F-box (atrogin-1/MAFbx) and are key regulators of skeletal muscle proteolysis under catabolic conditions |
| **Endoplasmic reticulum stress and the unfolded** | - exerts proteostatic control via signal transduction pathways responsive to cellular processes induced by the abnormal accumulation of unfolded or misfolded proteins at the ER, and acts to adjust protein folding capacity or induce apoptosis |

| | |
|---|---|
| **protein response ($UPR^{ER}$)** | - sensors of misfolded proteins and ER stress are the three major $UPR^{ER}$ branches, namely (i) the protein kinase R-like ER kinase (PERK)-eukaryotic translation initiation factor 2 subunit $\alpha$ (eIF2$\alpha$)-ATF4 pathway, (ii) the inositol-requiring enzyme 1$\alpha$ (IRE1$\alpha$)-X-box binding protein 1 (XBP1) pathway, and (iii) the ATF6 pathway<br>- other important proteins in the $UPR^{ER}$ include the chaperone protein BiP/GRP78, which under basal conditions, BiP constitutively binds to the luminal domains of the three sensors, thus preventing their activation, and CCAAT/enhancer-binding protein homologous protein (CHOP), which is a proapoptotic factor downstream of ATF4 |

### *E. Role of muscle protein synthesis*

The dynamics of protein turnover and proteostasis are central to phenotypic plasticity. In human skeletal muscle, proteins display global turnover rates of ~1.0 to 1.5%/day in young healthy individuals (243, 244). A dynamic equilibrium exists in that MPB exceeds MPS in the fasted state and during exercise, whereas MPS exceeds MPB in the fed state and in the post-exercise period (129, 245). The net positive balance induced by exercise can be augmented by ingestion of protein and carbohydrate, through the combined impact of transient essential aminoacidemia (>~1500 $\mu$M) to increase MPS, and modest insulinemia (~15 to 30 mU/L) to markedly attenuate MPB. Thus, aerobic and resistance exercise, either alone or combined with appropriate nutrition strategies, are determinants of skeletal muscle protein turnover and remodeling by acutely augmenting the degree by which MPS exceeds MPB, in addition to MPS being also increased in the resting state after a period of resistance exercise training (246-251). Central to muscle hypertrophy in response to resistance exercise training is the contention that repeated transient increases in MPS through exercise and appropriate nutrition intake result in the accumulation of myofibrillar proteins predominantly, and thereby increased size of the exercise-trained muscle (129) (Section 1.D).

Because MPB is challenging to measure (117), and likely to be less consequential than MPS in the regulation of resistance exercise-induced muscle hypertrophy (129), there has been much focus on understanding the regulation of MPS in that context (245). As a result, molecular investigations to date have predominantly centered on the activation of canonical regulators of protein translation, namely mTORC, S6K1, 4E-BP1, and related parallel and downstream targets (114) (Section 5.C) (Table 2).

The term MPS refers broadly to whole or ‘mixed’ muscle protein synthesis and acutely reflects protein translation efficiency as defined as the rate of translation per ribosome (252, 253). The integrated response of hypertrophic and non-hypertrophic remodeling, including tissue repair and myonuclear and mitochondrial protein turnover, is captured by this global measure (254). However, given that myofibrillar proteins comprise ~75% to ~85% of muscle fiber volume (255), analyses of MPS at the level of bulk subfractions including myofibrillar (myoPS), sarcoplasmic (sarcPS) and mitochondrial (mitoPS) (249, 256-260), and individual

proteins via proteome profiling (259, 261-263), are likely to provide greater insight into the specifics of adaptive responses than those provided by mixed MPS.

Muscle hypertrophy requires accretion of a greater volume of muscle protein *per se*, and this is largely in the form of myofibrillar protein accretion (169, 178), yet the process of adaptation to *any* type of exercise will require an increase in protein synthesis i.e. the synthesis of new proteins from existing, or acute exercise-induced changes in, mRNA abundance. For example, SIE is a potent stimulus to stimulate MPS and skeletal muscle anabolism in a general sense (179), and robust increases in MPS in response to MICE have also been observed (249, 256, 258, 264-269) ((reviewed in (180)). The suggestion that specificity of adaptation may be conferred from the stimulation of subfractions of MPS is because of observations of an increase in myoPS after resistance, but not aerobic, exercise (249, 256), and the increase in mitoPS after a period of aerobic, but not resistance, exercise training (249). Moreover, when acute aerobic and resistance exercise are performed concurrently, an increase in myoPS is indeed observed (256). One caveat is that slower turnover rate, or latency, of skeletal muscle mitochondrial proteins compared to myofibrillar proteins, coupled with sometime short measurement periods for MPS in the post-exercise period, may contribute to observations of between-fraction differences in exercise-induced alterations in MPS (180). Nevertheless, a working model is that acute aerobic and resistance exercise produce consequent effects to preferentially augment mitoPS and myoPS, respectively, and the cumulative effects over time are central to exercise training-induced mitochondrial biogenesis (181, 182) and myofibrillar protein accretion (169, 178), respectively.

Recent advances in stable isotope methodology with protein labeling with deuterium oxide ($D_2O$) (270) and dynamic proteome profiling (271) has produced a number of studies detailing the contribution of synthesis and degradation to changes in protein abundance of individual proteins (259, 261-263, 272, 273). The maintenance of cellular homeostasis is a dynamic process, and therefore to optimize survival, a cell must be able to rapidly respond to intrinsic or extrinsic changes in its environment. Thus, proteins that control these responses must be able to increase or decrease in short time frames, by altering the rate of translation of existing mRNA rather than necessary relying on being preceded by acute exercise-induced changes in mRNA abundance. Once MPS has been stimulated above basal rates by a session of exercise, translation of specific proteins is primarily dependent on translational efficiency and capacity (Section 6.E). Unsurprisingly, at the level of individual proteins, there is considerable variation in turnover rates and the balance between synthesis and degradation in the regulation of resting and adapted abundance (271). By way of illustration, data from rat skeletal muscle describe a range in resting synthetic rates from 0.58%/day (Collagen alpha-1 chain; CO1A1) to 5.40%/day (N-myc downstream-regulated gene 2 protein; NDRG2), and

eight proteins having different synthetic rates in soleus (type I fiber dominant) compared to plantaris (type II fiber dominant) muscle (273). In humans in response to two weeks of resistance exercise training under energy restriction, the ~26% increase in bulk myoPS coincided with increased synthetic rates of 175 of the 190 measured myofibrillar, sarcoplasmic, and mitochondrial proteins in skeletal muscle (259). Synthetic rates of measured proteins ranged from as low as ~0.2%/day to as high as ~15.0%/day in that analysis (259).

An advance on this method is dynamic proteome profiling, which in addition to synthetic rates also incorporates measures of degradation rates and protein abundance (271). This method has revealed novel insights into individual protein kinetics in response to resistance exercise in humans (three sessions over nine days) (261), and voluntary wheel running in rats (daily sessions over two weeks) (263). Of particular note is that dynamic proteome profiling allows for the identification of responses that go beyond the simple metric of synthesis e.g. (i) proteins that increased in abundance as expected because their synthetic rate was elevated; (ii) increased synthesis and degradation (i.e. turnover) but no change in protein abundance; (iii) changes in abundance that are primarily driven by degradation (i.e. increases in abundance in the absence of increases in synthetic rate or decreases in abundance despite increases in synthesis) (271). The intricacies are exemplified by observations of increases in synthetic rates of bulk fractions, but both increases and decreases in abundance of individual proteins within these fractions (261, 263). Undoubtedly, wider application of these methods will provide a greater understanding of individual protein kinetics, which in turn will provide enormous insight into the role of proteostasis and protein turnover in response to acute exercise and exercise training. Ultimately, regardless of whether there is a change in mRNA abundance (increase or decrease) in response to exercise (and recognizing there are discrepancies between mRNA and protein kinetics (274-278)), phenotypic and functional consequences of exercise are entirely dependent on change at the protein level; be that in the form of altered abundance, maximal activity, and/or sensitivity to the regulatory mechanisms determining activity or function.

### *F. Time course of molecular events*

The time course of molecular events in the model described above is such that homeostatic perturbations, molecular signals, signal transduction and transcriptional regulation mostly occur during the exercise session and in the early phase of recovery (minutes to hours), whereas changes in mRNA and protein abundance occur in the hours and day(s) that follow (Figure 5). In broad terms, the activation and/or repression of intracellular signal transduction pathways associated with transcriptional regulation occurs with the onset of exercise and into recovery but return to resting levels within approximately the first 3 hours

after exercise session, whereas pathways involved in the regulation of protein translation tend to have delayed but sustained activation for several hours into recovery. In the 24 hours after the cessation of an exercise session, the modulation of transcriptional and translational processes results in hundreds of genes and proteins exhibiting changes in expression, posttranslational modifications, and function. However, the time course of change in specific targets or cellular processes depends on various regulatory factors and molecular events (Sections 5 and 6), and in the case of changes in mRNA and protein abundance are largely dictated by the kinetics of transcriptional and translational regulation on a gene-specific basis (275, 277, 279-283).

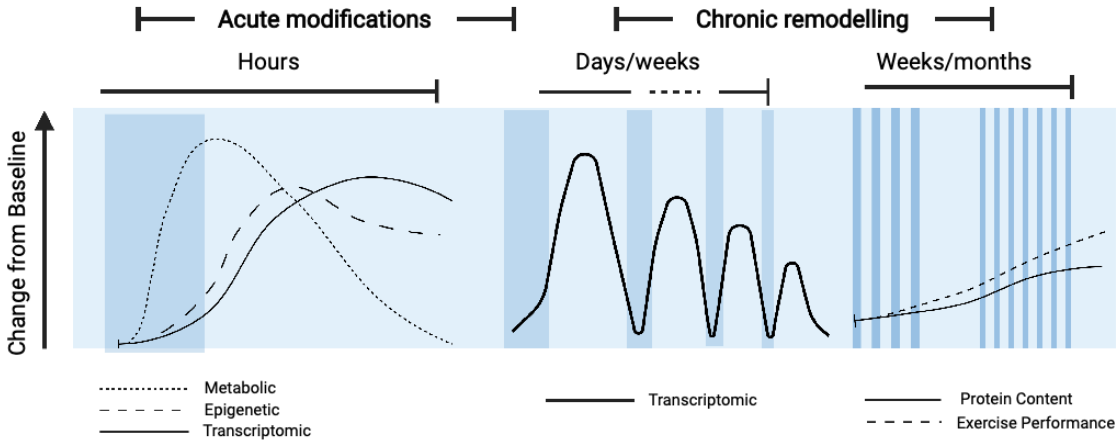


**FIGURE 5. Schematic representation of changes in mRNA and protein abundance over time as a consequence of acute exercise and exercise training.**

Acute exercise induces transient changes in the concentrations of substrates, metabolites, and cofactors, which in turn leads to activation and/or repression of signaling pathways, fluctuations in epigenetic status, and subsequent transcriptional changes. Changes in mRNA abundance several-fold from basal levels are typically greatest at 3 to 12 hours after cessation of exercise and generally return to basal levels within 24 hours. Although the visual depiction appears as an increase in a given parameter, decreases are also observed i.e. conceptually change from baseline is can occur in either direction. Moreover, the temporal pattern is specific to a given gene (see Figure 6 for further illustration) and is strongly influenced by the nature of the exercise challenge. During the following days and weeks of frequent exposure, repeated exercise leads to fluctuations in the transcriptomic response, resulting potentially in either attenuated or augmented expression of given genes in response to acute exercise. These transcriptomic changes lead to functional consequences at the level of protein content after weeks and months of exercise training, orchestrating phenotypic changes in skeletal muscle tissue including altered patterns of substrate utilization, better defense of perturbations to homeostasis, and enhanced exercise performance. As protein half-lives are usually much longer than those of mRNA, changes in protein content or activity are more readily observed in response to exercise training as opposed to the robust changes in transcript abundance observed after acute exercise. Changes in protein content are dependent on the balance between the many levels of regulation of protein translation (synthesis) and degradation (breakdown). Adapted from (67, 88).

For example, increased AMPK activity and/or phosphorylation is evident during and/or immediately after aerobic or resistance exercise, but returns to resting levels within the first 3

hours of recovery (284-293), whereas signaling regulating protein translation can be suppressed during aerobic exercise (294, 295), but exhibits a delayed and sustained increase over the next 3 to 24 hours of recovery from aerobic and resistance exercise (257, 285, 296-299). Similarly, a several-fold increase in mRNA abundance of PGC-1$\alpha$ is evident early into recovery and peaks between 2 to 4 hours before returning to resting levels after ~10 hours (reviewed in (127)), yet many genes exhibit differential time courses of expression in the first 8 hours after exercise (41, 51, 291, 299-313) (illustrated in Figure 6), and changes in gene expression are observed as long as 16, 24, 48, 72, 96 and 120 hours after a single exercise session (47, 51, 60, 300, 301, 303, 304, 307, 310, 312, 314-325).

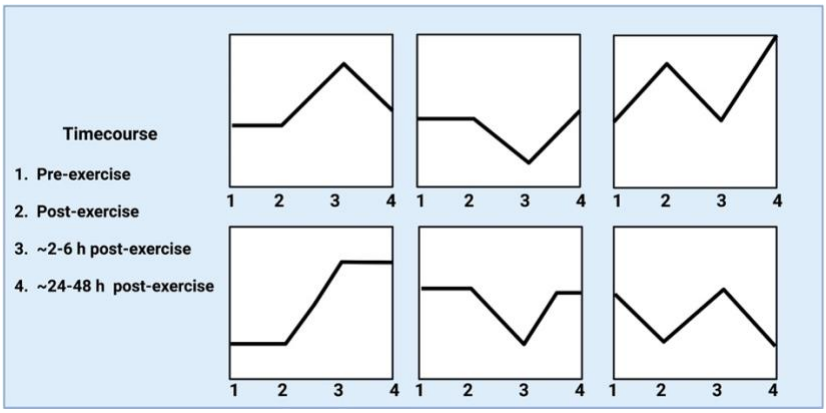


**FIGURE 6. Illustration of possible temporal and directional changes in mRNA abundance in response to a single session of acute exercise.**

Acute exercise induces transient changes in mRNA abundance in skeletal muscle, the temporal and directional changes in which are proposed to be specific to a given gene and strongly influenced by the nature of the exercise challenge. The examples illustrated are not exhaustive, but represent some the most commonly-observed responses.

While these molecular events in Sections 2.A, 2.B, 2.C and 2.D are often depicted as linear or sequential from the onset of the exercise stimulus to a change in protein abundance and/or activity (Figure 4), there are likely to temporal and molecular overlaps between the various events *in vivo* (Figure 5). Moreover, as described in Section 5, these pathways also ostensibly demonstrate a degree of dependence, crosstalk, interference, and redundancy in their regulation, making the exact contribution of each pathway to exercise training-induced adaptations difficult to isolate. Nevertheless, changes that ultimately result in functional improvements in exercise capacity and performance occur in the following days, weeks, and months consequent to cumulative effect of frequent, repeated sessions of exercise and their associated molecular events (317, 318, 326). Hence, while each individual session of exercise is necessary as a stimulus for the adaptive process in skeletal muscle and other organs, the long-term adaptation to exercise (i.e. exercise training) is the result of the progressive and

cumulative effects of each acute session of exercise leading to a new functional threshold for a given parameter.

## 3. THE NATURE OF THE EXERCISE STIMULUS AS A STRESSOR AND ITS CONSEQUENCES FOR MOLECULAR RESPONSES TO ACUTE EXERCISE

Acute exercise-induced signal transduction and associated molecular responses to acute exercise are proposed to be a consequence of a myriad of neuronal, mechanical, metabolic, and hormonal factors that act as initial signals (or “primary messengers”) for these pathways (45, 84-88). A prevailing view is also that differential contributions from these various factors with different types of acute exercise may explain, in large part, the specificity of adaptations to different types of exercise training. Therefore, it is important to first consider the nature of the exercise stimulus given its important consequences for molecular responses to acute exercise.

At present, exercise type and intensity have been the independent variables most often explored in the context of the molecular response to exercise, and the effect of duration is less well-defined. However, broadly speaking, divergent types (e.g. aerobic versus resistance) and divergent intensities (e.g. low versus heavy/severe) induce both overlapping and distinct physiological demands on skeletal muscle, the extent of which is largely dependent on variables such as force, velocity, duration, frequency, and number of contractions (Figure 2). Aside from determining the physiological demands of an exercise session and therefore the influence of neuronal, mechanical, metabolic, and hormonal factors, these variables also influence the recruitment profile of different fiber types in skeletal muscle. Fiber type is likely to have an important role in the molecular response to exercise, but for the purposes of this review, we will focus almost exclusively on the responses in mixed muscle. The caveats of only considering mixed muscle samples are explored in Section 4.D. The training status of the individual is also an important consideration for the nature of the responses to an exercise stimulus, so design issues and the modulating effects of exercise training or trained status on the molecular response to acute exercise are considered in further detail in Section 7.

### *A. Neuronal and mechanical factors influencing the molecular response to acute exercise*

All forms of exercise, by definition, involve contractile activity in skeletal muscle. In accordance with the sliding filament model of skeletal muscle contraction (327), hydrolysis of adenosine triphosphate (ATP) by myosin ATPase provides the immediate energy source for

actin-myosin crossbridge cycling, but is also consumed to a large extent by the ATP-consuming enzymes sodium-potassium ($Na^+/K^+$)-ATPase and sarcoendoplasmic reticulum $Ca^{2+}$ ATPase (SERCA), which regulate, respectively, the dynamics of $Na^+/K^+$ and $Ca^{2+}$ exchange necessary for contraction. Specifically, $Ca^{2+}$ is essential for facilitating the cross-bridge interaction between myosin and actin during myofibrillar contraction. During a voluntary muscle contraction, the neuronal input to muscle excitation via the propagation of an action potential through to muscle contraction and the generation of force is described by the process of excitation-contraction coupling (328-331). Central to this process is the release of $Ca^{2+}$ from the SR (where concentrations are in the mM range) into the cytosol (where concentrations are 1000-10000-fold lower) and reuptake of $Ca^{2+}$ to the SR (330). As a result, $[Ca^{2+}]_i$ can be elevated by up to five- and fifty-fold in type I and type II muscle fibers, respectively, during contraction (332, 333).These $[Ca^{2+}]_i$ oscillations are translated into discrete signals that modulate the enzymatic activity of the CaMK family of protein kinases and the protein phosphatase calcineurin, both of which implicated in muscle plasticity as $Ca^{2+}$ "decoders" (94, 334). Because the amplitude and duration $[Ca^{2+}]_i$ oscillations are a function of the level of force output by the muscle (328), and because these $Ca^{2+}$ decoders are sensitive to the pattern of $Ca^{2+}$ oscillations and/or muscle contraction (335-338), $Ca^{2+}$-related signaling may be an important contributor to the specificity of molecular responses to acute exercise and exercise training (Section 5.A). For example, aerobic exercise would be likely to mimic the slow-type prolonged low frequency stimulation resulting in sustained and moderately-elevated $[Ca^{2+}]_i$, whereas resistance exercise would be likely to mimic the fast-type brief, infrequent bursts of high frequency stimulation resulting in shorter duration and higher $[Ca^{2+}]_i$, but to our knowledge divergent $[Ca^{2+}]_i$ oscillations and $Ca^{2+}$-related signaling in response to different types of exercise in humans remains to be demonstrated.

Any form of contractile activity will also impart mechanical stress on the extracellular matrix, and cytoskeletal and sarcolemmal components of skeletal muscle fibers, but the relationship between $[Ca^{2+}]_i$ and force production during contraction makes it difficult to separate the contribution of neuronal and mechanical factors in influencing the molecular response to acute exercise. Indeed, mechanical factors acting as stimuli being sensed by the muscle cell encompass a number of interrelated parameters including mechanical stretch and the direction of that stretch (339, 340), concentric versus eccentric contractions (177, 341), slow versus fast contractions (342), low versus high force contractions (343), and the interrelated aspects of the frequency and load pattern (344, 345). The sensing of these signals is broadly described by the concept of mechanosensory regulation (or mechanotransduction) (Section 5.C), whereby a group of proteins involved in cytoskeletal structure and force transmission in skeletal muscle are linked to the transduction of these signals to downstream

962 targets (92, 111, 346-348). These pathways have been traditionally associated with resistance
963 exercise and muscle hypertrophy due to their convergence on mTORC-related signaling
964 (113), but intuitively their contribution to aerobic exercise adaptations cannot be discounted,
965 especially given the capacity of aerobic exercise to produce hypertrophy in skeletal muscle,
966 albeit modest in magnitude (183).

967 On a whole-body level, features of an exercise session are again likely to impact on
968 the role of mechanical factors in influencing the molecular response to acute exercise. One
969 example is the differences in muscle activity of the quadriceps muscle group during cycling
970 compared to running exercise, with greater eccentric muscle contractions performed in the
971 latter (349). Another example is to consider differences in the force, velocity, duration,
972 frequency, and number of contractions of the quadriceps between aerobic cycling exercise
973 and knee extension resistance exercise. In the case of cycling, MICE performed at a cadence
974 of 60 rpm for 30 minutes would require 30 contractions of ~0.5 seconds duration per leg per
975 minute (900 contractions in total) at an intensity <10%1RM. A SIE session of 7 repetitions of
976 30 second all-out sprints at ~120 rpm would require 30 contractions of ~0.25 seconds duration
977 per leg per sprint (210 contractions in total) at an intensity ~10-20%1RM. In contrast, as
978 recently demonstrated (342), a knee extension resistance exercise session undertaken at
979 either 30% or 80%1RM and performed as either “regular (R)” 3 second contractions (1:1:1
980 eccentric:pause:concentric) or “slow (S)” 7 second contractions (3:1:3) until failure in each of
981 the three sets performed resulted in marked differences in the characteristics of the session.
982 In addition to intended differences between the load lifted per repetition and the repetition
983 duration, the number of repetitions per set ranged from 6±1 (80S) to 20±4 (30R) (i.e. ~18 to
984 60 contractions in total), and the total time under load per session ranged from 76±20 (80R)
985 to 225±52 seconds (30S). To summarize, these respective aerobic and resistance exercise
986 sessions differ markedly in their number, intensity, and duration of contractions, in addition to
987 the absence of loaded eccentric contractions in cycling exercise. These differences are likely
988 to produce divergent neuronal and mechanical responses between different types of exercise
989 session, influence muscular efficiency and ATP turnover (350), and modulate the contributions
990 of energy systems and the metabolic response to acute exercise.

991 ***B. Metabolic factors influencing the molecular response to acute exercise***

992 *1. Energy provision and pathways of ATP resynthesis in skeletal muscle*

993 Despite the importance of ATP to cellular function, and that ATP turnover rate within the
994 exercising skeletal muscle can be more than 100-fold greater than at rest (351), relatively
995 small amounts of ATP are stored in skeletal muscle. Yet intramuscular [ATP] remains largely
996 stable during most forms of exercise, only decreasing by ~20-40% during very intense

997 exercise (352-354), even though *in vivo* rates of ATP hydrolysis could theoretically deplete
998 total muscle ATP stores within ~2 seconds at maximal intensity (353), and ~15 seconds at
999 submaximal intensity (355). Thus, to mitigate declines in [ATP], skeletal muscle relies on a set
1000 of metabolic pathways (ATP-phosphagen system, anaerobic glycolysis, and aerobic
1001 metabolism of substrates coupled to oxidative phosphorylation) for rapid resynthesis and
1002 provision of ATP in proportion to the metabolic demands placed on the contracting muscle by
1003 a given exercise challenge (Figure 7). The bioenergetics of skeletal muscle during exercise
1004 are reviewed extensively elsewhere (355, 356), but are briefly summarized here given the
1005 importance of metabolic factors influencing the molecular response to acute exercise (65, 67,
1006 88). In attempting to match the resynthesis of ATP to the rate of ATP demand, again
1007 collectively a mix of neuronal, metabolic, and hormonal signals coordinate the activities of
1008 various pathways. Among the many factors that regulate ATP resynthesis are $[Ca^{2+}]_i$; ADP,
1009 AMP, creatine and $P_i$ concentrations; adrenaline; ratios of oxidized to reduced coenzymes
1010 (e.g., $[NAD^+]$/[NADH] and [FAD]/$[FADH_2]$); and substrate/product concentrations (355, 356).
1011 At the onset of exercise, [ADP], [AMP], and $[P_i]$ increase via ATP hydrolysis and the adenylate
1012 kinase (ADK) reaction, whereas phosphocreatine (PCr) provides an immediate supply of ATP
1013 by transferring a phosphate to ADP (catalyzed by the near-equilibrium creatine kinase (CK)
1014 reaction) as [ADP] increases (354, 357, 358). Accordingly, [PCr] declines, largely in proportion
1015 to exercise intensity (357, 358), i.e. [PCr] continually decreases if the exercise intensity is
1016 severe (352, 358), but for moderate and heavy intensity exercise, [PCr] stabilizes (354, 358),
1017 as the rate of ATP resynthesis from aerobic pathways eventually increases to reach an
1018 equilibrium with the rate of PCr hydrolysis.

1019 Rates of glycogenolysis and glycolysis increase at the onset of exercise (353, 359),
1020 yielding ATP through substrate phosphorylation and resulting in lactate production (reviewed
1021 in (360)). Increasing rates of ATP turnover have a feedforward effect on rates of muscle
1022 glycogenolysis through a decrease in cellular energy charge, again in proportion to the relative
1023 exercise intensity (357, 358). The increases in glycolytic flux are consequent to the increased
1024 activity of glycogen phosphorylase (PHOS) and phosphofructokinase (PFK), with the
1025 reversible lactate dehydrogenase (LDH) enzyme controlling the interconversion of pyruvate to
1026 lactate, and pyruvate dehydrogenase (PDH) regulating the entry of pyruvate into the
1027 mitochondria for oxidation by transforming pyruvate to acetyl CoA. The regulation of these
1028 enzymes is beyond the scope of this review but is described in detail elsewhere (355).

1029 In the mitochondria, the majority of acetyl CoA enters the tricarboxylic acid (TCA) cycle,
1030 and the remainder is shunted to acetylcarnitine (361). Key enzymes of the TCA cycle are
1031 activated by $[Ca^{2+}]_i$, linking the TCA cycle to muscle contraction, and inhibited by NADH,
1032 preventing flux through the TCA cycle when demand for electrons is low (reviewed elsewhere

(355). However, direct ATP production from the TCA cycle is minimal. The main pathway of ATP resynthesis is through oxidative phosphorylation consequent to the reduction of $NAD^+$ and FAD molecules (forming NADH and $FADH_2$) and subsequent passage of electrons to oxygen via the electron transport chain. The control of oxidative phosphorylation is complex but largely dependent on [ADP] at rest. During exercise, the matching of rates of ATP resynthesis and ATP hydrolysis are influenced by the phosphorylation potential, ([ATP]/[ADP][Pi]) and the mitochondrial redox potential ([NADH]/[$NAD^+$]), which both decline in proportion to exercise intensity (357).

When the rate of glycolysis exceeds the rate of PDH flux, intracellular lactate concentrations increase. Lactate, if not converted back to pyruvate for oxidation, can be shuttled out of the skeletal muscle cell via monocarboxylate transporters in the plasma membrane to undergo oxidation in a variety of other cells including neighboring muscle cells, or circulate to the liver where it serves as a substrate for gluconeogenesis (362). Free fatty acids (FFAs) derived from triglycerides stored in adipose tissue and skeletal muscle are the other main sources of fuel for oxidative metabolism during exercise. Fatty acids liberated by lipolysis in adipose tissue are transported in plasma bound to albumin and enter skeletal muscle via fatty acid transporters, e.g., CD36 and fatty acid binding protein (FABPpm), or through diffusion. Lipolysis of IMTG is controlled by the integrated actions of adipose triglyceride lipase (ATGL) and hormone sensitive lipase (HSL), and several other proteins, including the perilipin (PLIN) family, and increases [FFA] in the sarcoplasm (363). Regardless of source, FFAs are chaperoned by FABPc, converted to fatty acyl CoA molecules via acyl-CoA synthetase (ACS), and subject to the availability of free carnitine are transported into the mitochondria via carnitine palmitoyltransferase 1 and 2 (CPT1/CPT2). The β-oxidation pathway yields acetyl CoA, NADH, and $FADH_2$, which contribute to ATP resynthesis via oxidative phosphorylation, as described above.

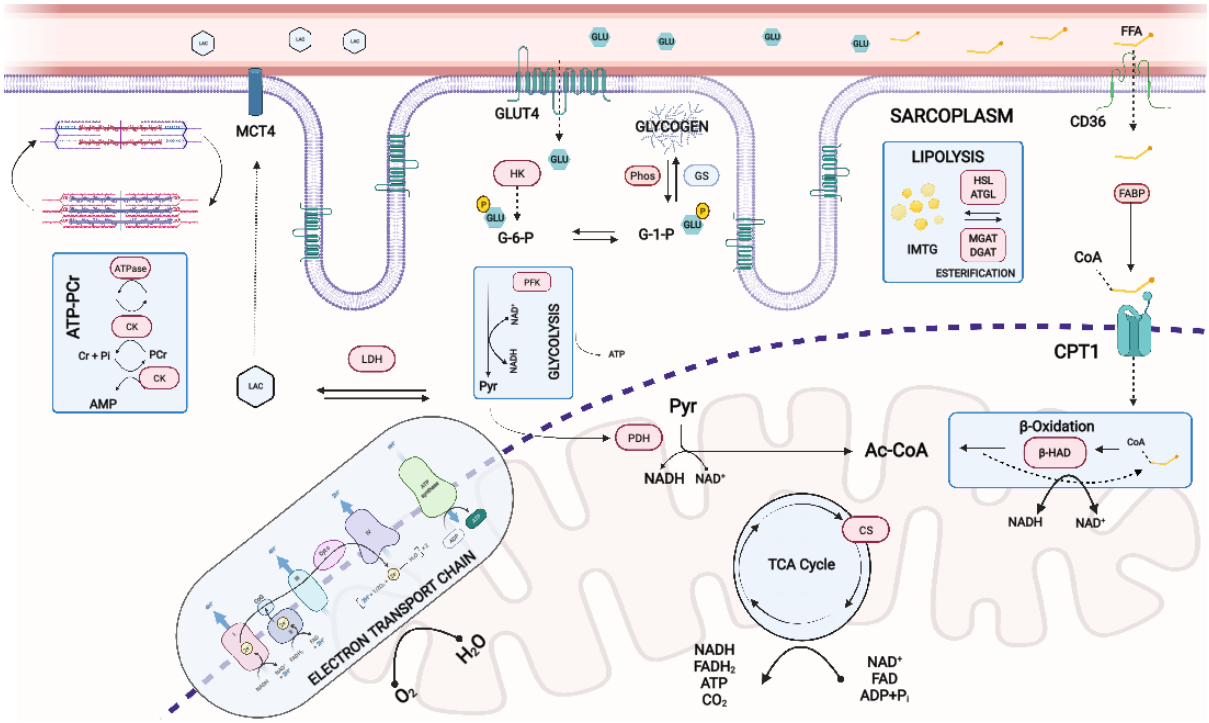


**FIGURE 7. Pathways of energy provision via ATP resynthesis in skeletal muscle during exercise.**

ATP hydrolysis, catalyzed by myosin ATPase, powers skeletal muscle contraction. Metabolic pathways of ATP generation in skeletal muscle include (1) the ATP-phosphagen system wherein the degradation of PCr by creatine kinase (CK) produces free Cr and $P_i$, which is transferred to ADP to re-form ATP; the adenylate kinase (AK) (myokinase) reaction catalyzes the formation of ATP and AMP from two ADP molecules; (2) anaerobic glycolysis, where glucose-6-phosphate derived from muscle glycogen (GLY) (catalyzed by glycogen phosphorylase, PHOS) or circulating blood glucose (GLU) (catalyzed by hexokinase, HK), is catabolized to pyruvate (PYR), which is reduced to lactate (LAC) by lactate dehydrogenase (LDH), and produces ATP by substrate level phosphorylation; (3) processes of carbohydrate (glycolysis) and lipid (b-oxidation) metabolism producing acetyl-CoA (Ac-CoA), which enters the tricarboxylic acid (TCA) cycle in the mitochondria, coupled to oxidative phosphorylation in the electron transport chain (ETC). The two main metabolic pathways, i.e., glycolysis and oxidative phosphorylation, are linked by the enzyme complex pyruvate dehydrogenase (PDH). GLUT4 facilitates glucose uptake to the sarcoplasm, which may undergo glycolysis or during rest/ inactivity, be stored as glycogen via glycogen synthase (GS). Fatty acyl translocase (FAT/CD36) facilitates long-chain fatty acid transport at the sarcolemma, and, in concert with fatty acid binding protein (FABPpm) and carnitine palmitoyltransferase 1 (CPT1), across the mitochondrial membrane. FFAs entering the cell may be oxidized via β-oxidation or be diverted for storage as IMTG via esterification by monoacylglycerol acyltransferase (MGAT) and diacylglycerol acyltransferase (DGAT). Liberation of FFAs from IMTG stores via lipolysis in skeletal muscle during exercise occurs via the activities of HSL and ATGL. In addition to its role as a feedforward signal of muscle contraction, $[Ca^{2+}]_i$ also provides gross control of metabolic regulation, whereas feedback related to ATP demand provides fine-tuning of the contribution of various pathways such that all pathways of ATP generation are active during exercise, but the relative contribution of each is determined by the intensity and duration of contraction, as a function of the relative power (rate of ATP production) and capacity (potential amount of ATP produced). CS, citrate synthase; Cyt c, cytochrome c; PFK, phosphofructokinase.

### *2. Substrate utilization during exercise*

During acute exercise, the contribution of various metabolic pathways (i.e., ATP-PCr, glycolytic, and aerobic) to energy provision are determined by the *relative intensity* and *absolute power output* of the exercise session (358, 364, 365); reviewed in (355, 356). In

general, the anaerobic processes have small capacities for ATP resynthesis but can yield ATP at higher rates, whereas the aerobic system resynthesizes ATP at a lower rate but with a much larger capacity (366). Net ATP resynthesis is almost entirely supported by aerobic metabolism during steady-state exercise, but anaerobic pathways are an important source of ATP for transitions to higher intensities of aerobic exercise (including from rest), and for severe intensities of aerobic exercise, as well as maximal efforts in resistance exercise and sprinting. The *absolute* power output determines the rate of ATP demand and energy expenditure; whereas the *relative* exercise intensity influences the relative contributions of CHO and lipid sources, and circulating (extramuscular) and intramuscular fuel stores, to energy provision (355, 356). Other factors influencing patterns of substrate utilization include biological sex, exercise duration, dietary intake before and during exercise, and percentage of type I muscle fibers (367).

The partitioning of fuel sources utilized from extra- or intramuscular substrates is coordinated quantitatively and temporally to meet the metabolic demands of exercise. At low-to-moderate intensities of exercise, the primary fuel sources supplying skeletal muscle are glucose, derived from hepatic glycogenolysis (or gluconeogenesis) or oral ingestion, and free fatty acids (FFAs) liberated by adipose tissue lipolysis. The contributions of liver and adipose tissue to substrate provision to contracting muscle during exercise are described in detail elsewhere (368, 369), and are largely under the influence of hormonal responses to the onset of exercise (e.g. adrenaline, noradrenaline, glucagon, insulin, cortisol) (370). As exercise intensity increases, muscle utilization of circulating FFAs declines modestly, whereas utilization of circulating glucose increases progressively up to near maximal intensities (365). When exercise at a *fixed* intensity is prolonged (>60 minutes), an increasing energy contribution is derived from lipid oxidation. Consequently, the proportion of energy derived from muscle glycogen declines and is replaced by a progressive increase in plasma FFA oxidation (364).

Muscle glycogen is the predominant CHO source during moderate-to-severe intensity exercise and the rate of glycogenolysis is proportional to the relative exercise intensity (355, 360). Increased glycogenolysis during exercise occurs via activation of glycogen phosphorylase, which reflects alterations in $[Ca^{2+}]_i$, $[P_i]$, cAMP-dependent β-adrenergic stimulation, and allosteric modulation by AMP and IMP (355, 356). Higher rates of glycogenolysis occur when initial muscle glycogen concentrations are high, but as exercise proceeds, degradation rates parallel declining glycogen levels and associated glycogen phosphorylase activity (371). The metabolic advantage is that these carbohydrate-dependent pathways produce ATP at a higher power (mol ATP/s) and at a lower rate of oxygen consumption (ATP/mol $O_2$).

Conversely, IMTGs constitute only a fraction (~1 to 2%) of whole-body lipid stores, but are the subject of intense research in exercise metabolism and metabolic disease (372). IMTGs are an important fuel source during prolonged (>90 minute), moderate intensity exercise and provide ~25% of total energy, but tend to contribute less at either higher or lower intensities of exercise (364, 365). Following exercise, IMTG stores are reduced by ~60%, predominantly in type I muscle fibers (373, 374). IMTG breakdown occurs primarily via hormone-sensitive lipase (HSL) and adipose triglyceride lipase (ATGL) (372), although the regulation of exercise-induced lipase activity remains to be fully elucidated (363).

Calculations of substrate utilization during exercise such as those described above generally assume that the contribution of protein is constant and minor (375, 376), or alternatively use a non-protein respiratory exchange ratio ($\dot{V}CO_2/\dot{V}O_2$) in these calculations (377), yet protein-based energy provision during moderate intensity exercise is estimated at 5-15% (378). Contributions closer to 15% are evident in energy-compromised states such as when muscle glycogen concentrations are low or depleted (379, 380). This energy is obtained from intramuscular protein degradation and oxidation of branched-chain amino acids by the branched-chain $\alpha$-keto acid dehydrogenase complex (379). Free amino acid flux during exercise reflects dynamic activity in *de novo* synthesis, transamination, deamination, oxidation and irreversible catabolism of specific amino acids, and thus, several amino acids (alanine, aspartate, glutamate, glutamine, and the branched-chain amino acids valine, leucine, and isoleucine) contribute to energy production in skeletal muscle (378). Moreover, alanine and glutamine, in addition to lactate, pyruvate and glycerol indirectly contribute to energy provision in skeletal muscle during prolonged exercise via their role in hepatic gluconeogenesis (381, 382). Other substrates that make minor contributions to energy provision include lactate produced locally in skeletal muscle during anaerobic glycolysis (362), and the ketone bodies acetoacetate and $\beta$-hydroxybutyrate that are elevated in states of fasting, low carbohydrate availability, or acute nutritional ketosis via exogenous ingestion (383).

The metabolic responses described above are summarized largely from the study of steady-state moderate intensity aerobic exercise, whereas the metabolic response to resistance exercise is not as well-studied. Indeed, where such studies have been performed, variations in protocols for the resistance exercise session undertaken (e.g. exercises performed including whether unilateral or bilateral, number of sets and repetitions, intensity as %1RM, tempo of exercises including eccentric and concentric contributions, and the duration of rest intervals) and the sample population (e.g. age, sex, body composition, training level, and diet) make it difficult to provide similar summaries for resistance exercise. For illustration, the extent of variations in protocols that have been investigated has been detailed in

comprehensive reviews on the blood lactate (384) and hormonal (385) responses to acute sessions of resistance exercise.

Because resistance exercise does not result in steady-state responses for $\dot{V}CO_2$ and $\dot{V}O_2$, calculation of rates of substrate utilization from expired air, as performed during aerobic exercise is not possible. However, several studies have examined such variables as energy expenditure and oxygen uptake from non-steady state data, and changes in metabolites (ATP, PCr) and substrates (glycogen, intramuscular triglyceride) in muscle biopsies in response to an acute session of resistance exercise (386-395). The energy cost of resistance exercise typically elicits a $\%\dot{V}O_{2max}$ ranging from ~30 to 60%$\dot{V}O_{2max}$, but considerable variation exists depending on the exercise protocol. That said, rates of energy expenditure, $\dot{V}O_2$ and glycolytic contributions are likely to be greater for large versus small muscle mass exercises, slow-to-moderate versus fast movement tempos, high versus moderate versus low intensity as %1RM, and high versus low volume (i.e. sets x repetitions), and short versus long rest intervals.

Declines in [PCr] are large i.e. >60%, but dependent on the intensity and volume of the exercises performed (390, 392, 394). There is an large reliance on glycolytic metabolism during resistance exercise, with as high as 80% of energy provision from these pathways when single sets are performed to failure (390). Blood lactate concentrations in the post-exercise period frequently range from 5 to 15 mM (384), whereas declines in muscle glycogen concentration are typically in the range of 25 to 40% of resting concentrations (386-391, 393, 395). Again, volume and intensity-dependent effects on the magnitude of the decline in muscle glycogen have been observed (387, 389, 390). However, on a fiber-type specific level, larger declines are observed in type II muscle fibers (387, 389, 393, 395), and recent data suggest that subcellular glycogen depots (i.e. intermyofibrillar, intramyofibrillar and subsarcolemmal) also show specific patterns of utilization i.e. many type II fibers demonstrated near-depleted levels of intramyofibrillar glycogen (395). Lastly, although data are limited, IMTG declines by ~25 to 30% on average during resistance exercise regardless of training status (388, 393). The source of intramuscular triglyceride was primarily from type I muscle fibers (393), but in both studies large inter-individual variation was noted in both resting concentrations of intramuscular triglyceride and the declines observed during the training session (388, 393).

*3. Substrate utilization and metabolic processes during the post-exercise recovery period*

After the cessation of exercise, the metabolic rate declines, but remains slightly elevated (~5-10%) for up to 24 hours (207). The extent of this 'excess post-exercise oxygen consumption' (EPOC) is proportional to the metabolic stress and determined by the intensity,

duration and type of exercise session (207, 211). This recovery period is characterized by two major phases: (i) recovery of myocellular homeostasis in the immediate hours after exercise, and (ii) cellular contributions to adaptation to exercise. Moreover, the global restoration of homeostasis includes the replenishment of oxygen stores, resynthesis of ATP and PCr, lactate oxidation, restoration of fluid balance and fuel stores, and inflammatory and anti-inflammatory responses (207, 211). In contrast to the reliance on CHO metabolism during exercise, an increase in lipid oxidation and 'sparing' of CHO sources for energy provision occurs during the recovery period (396, 397). This shift accommodates the high metabolic priority for muscle glycogen resynthesis, whereas the oxidation of lipid from both IMTG and circulating FFA sources is elevated to meet prevailing energy requirements. Lipid oxidation rates can reach 25% of that reported *during* exercise, and contribute greater than 60% of oxidative metabolism during recovery (380).

In contrast to the short time course of declines in muscle glycogen and IMTG during prolonged and/or heavy exercise, the replenishment of these intramuscular substrates to pre-exercise levels requires 24 to 48 hours (209, 210). Replenishment of muscle glycogen stores is primarily dependent on (i) the availability of substrates, both glucose and non-glucose sources including lactate and alanine, and (ii) non-insulin- and insulin-dependent enzymatic activity of glycogen synthase (206, 209). Similarly, IMTG replenishment via skeletal muscle lipogenesis is influenced by both nutrient status and activation of triglyceride synthesis via mGPAT, DGAT, and SCD1 (398). Lipid intermediaries such as diacylglycerol (DAG) and ceramides are reduced in skeletal muscle after exercise, suggesting their incorporation into IMTG during recovery (398), in addition to lipid re-esterification of previously liberated FFAs (399). The other major biosynthetic process that contribute to homeostatic recovery at a local level in skeletal muscle is elevations in MPS (129), including elevated rates of mitoPS and myoPS (249) (described earlier in Section 2.E). Although beyond the scope of this review, notably the activation of these processes and the time course of recovery from exercise can be augmented or attenuated by the timing and type of nutrition provision in the post-exercise period, and can influence the nature of molecular events during this time (128, 129, 209, 210). On the whole, these largely anabolic events during recovery from exercise are dichotomous to the catabolic nature of acute exercise, and are important cellular contributions in the regulation of adaptations in skeletal muscle to exercise training in the long-term.

### *C. Hormonal and circulating factors influencing the molecular response to acute exercise*

#### *1. Endocrine responses to acute exercise*

Given the importance of endocrine regulation of metabolism, it should not be surprising that acute exercise, whether aerobic or resistance in nature, elicits marked changes in many hormones, the full details of which are again beyond the scope of this review. Most relevant to this review are the roles of sympathoadrenal system (e.g. adrenaline, noradrenaline, cortisol) and other counterregulatory hormones (e.g. glucagon, GH) in the stress response to exercise. These roles are especially in relation to the regulation of extra- and intramuscular substrate mobilization and utilization (370, 400, 401), and those anabolic and catabolic hormones most relevant to skeletal muscle remodeling (e.g. GH, IGF-I, testosterone, cortisol) (385, 401, 402). The interaction of these hormones with their receptors and related signal transduction pathways is described in Section 5.H, but changes in concentrations of the various hormones themselves in response to exercise are briefly summarized here. These responses in terms of magnitude and temporal pattern, like other factors in this Section, are proportional to the extent of perturbations to homeostasis and therefore, the type of exercise, and the volume of exercise as a function of intensity and duration (aerobic exercise) or sets x repetitions x %1RM (resistance exercise) again are important considerations.

The circulating concentrations of the catecholamines adrenaline and noradrenaline are consistently observed to be robustly increased in response to various types of exercise including both aerobic (291, 403-405) and resistance (195, 406, 407) exercise. Acting through the β-adrenergic receptors ($\beta_1$- and $\beta_2$-ARs), the action of catecholamines is central to exercise performance, physiology and metabolism given effects on skeletal muscle contractility and glycogen metabolism, the mobilization of FFAs from peripheral adipose tissue and glucose from liver glycogen, and the cardiovascular response to exercise (reviewed in (408)). Increases in catecholamine concentrations are observed immediately with the onset of exercise (403, 404), and an anticipatory rise prior to the onset of exercise has also been observed, at least in trained participants (407, 409, 410). The magnitude and time course of the adrenaline response is intensity- and volume-dependent (291, 403, 411), with even short duration, severe exercise producing large changes in circulating [adrenaline] (293, 410, 412). On the whole, increases in circulating [adrenaline] occur in the range of ~1.5- to 20-fold resting concentrations depending on the exercise stimulus, and return to resting levels within the first 30 to 60 minutes after exercise cessation (408). The intensity-dependent effect also underpins the observation that increases in circulating [adrenaline] are attenuated after a period of aerobic or resistance exercise training when the post-training acute exercise session is performed at the same absolute (i.e. lower relative) intensity (195, 405).

Both acute aerobic and resistance exercise produce transient increases in circulating [GH], but only when exercise exceeds thresholds for intensity and duration (385, 413). For example, short duration (10 minutes) aerobic exercise must exceed lactate threshold before

changes in circulating [GH] are observed (414), whereas 30 minutes of exercise at ~70%$\dot{V}O_{2max}$ produced ~1.6-fold and 3.1-fold greater GH area under the curve (AUC) during the 2 hours of recovery compared to a 30 second Wingate sprint and 30 minute resistance exercise session, respectively (415). For resistance exercise, shorter rest periods (409, 416), heavier loads (417, 418), and greater session volume (419-421), are all associated with larger post-exercise increases in circulating [GH]. In temporal terms, circulating [GH] increases within 10 minutes of the onset of aerobic exercise, and peaks within or immediately after the exercise session, but the increase with resistance exercise tends to be delayed until ~30 to 45 minutes after the onset of exercise (413). With both types of exercise, concentrations remain elevated above baseline for ~1.5 to 2 hours after exercise (385, 413). However, an important point is that the historical data summarized here largely describe the assay of a 22 kDa form of GH from which there are different fragments that have divergent bioactivity, whereas it is now known that there are several isoforms of GH that require further investigation of their response and bioactivity in response to exercise (402).

As similar degree of complexity is present with IGF-I. Circulating IGF-I is primarily synthesized in the liver in response to stimulation of increasing [GH], but only 2% of circulating IGF-I is in its free form (422, 423). The remainder circulates bound to one of six IGF binding proteins (IGFBPs), or bound to IGFBP-3 and the acid labile subunit (ALS) (422, 423). Moreover, IGF-I can be produced locally in tissues such as skeletal muscle in response to contraction, whereby it acts in autocrine or paracrine manner (422, 424). In skeletal muscle, alternative splicing of IGF-I gene produces mechanogrowth factor (MGF) (425), which is also referred to as IGF-IEc in humans or IGF-IEb in rodents (424). This isoform is mechanosensitive and likely plays a role in adaptive changes in skeletal muscle (424). There are largely equivocal data on the circulating IGF-I response to exercise, given that a variety of methods have been used to assess the response e.g. free IGF-I, bound IGF-I, or the concentrations of IGFBPs themselves (426). Increases observed during and after exercise (416, 427, 428) may reflect local synthesis processes given that this has been observed in GH-deficient participants (427), but no change in circulating [IGF-I] after exercise has also been observed (429, 430). One point of contention is that the relatively minor changes in circulating [IGF-I] may simply reflect a hemoconcentration effect elicited by plasma volume shifts with the onset of exercise (426). Given the relationship of IGF-I synthesis with GH stimulation and the GH responses described above, it may be better to consider the circulating IGF-I response to exercise over several hours after exercise. However, total IGF-I, IGFBP-3 and ALS were unchanged over 24 hours of recovery after moderate or long duration aerobic (45 or 90 minutes) or resistance (1 or 2 hours) exercise (431). These findings should not discount the role of autocrine or paracrine effects of IGF-I and MGF given their established

stimulation of muscle anabolism (424), and evidence of local expression and production in response to muscle contraction (425, 432, 433). Similarly, there was no deleterious effect of either the *systemic* absence of IGF-I (434), or the absence of a *local* functional IGF-IR (435), on overload-induced muscle hypertrophy. However, these studies do not account for changes in IGF-II receptor (IGF-IIR) that IGF-I can also bind to (albeit with lower affinity), and there is also evidence that IGF-I/IGF-IR function is important for muscle regeneration and differentiation (436).

Both acute aerobic and resistance exercise produce transient increases in circulating [testosterone] (437-442). Again, volume as a function of intensity and duration is an important determinant of the magnitude of response (417, 442, 443) although the response in females is equivocal with both small increases (444),or no change (438), being observed. Moreover, while the increase generally peaks around the end of exercise and returns to resting concentrations within 2 hours (437, 441), declines in free [testosterone] have been observed in the post-exercise period (441, 445). The bioactivity of circulating testosterone is complicated by the fact that it is transported mostly (up to ~60%) bound with sex hormone-binding globulin (SHBG), whereas free (unbound) testosterone comprises only up to ~2% in circulation (446). Binding of free testosterone to androgen receptors (ARs) is the main route of activation of signal transduction pathways and the regulation of anabolic and gene regulatory processes (103), although there may be some role for bound testosterone or SHBG itself for activation of these pathways (447).

In contrast to these potentially anabolic hormones, cortisol is considered a catabolic hormone in the context of skeletal muscle and associated with increasing protein degradation and attenuating protein synthesis (448-450). Both acute aerobic and resistance exercise produce transient increases in circulating [cortisol] (421, 451) with the magnitude largely dependent on type, intensity and duration of exercise (418, 421, 440, 441, 452-454). Hemoconcentration is likely to be a contributor to the increase at the cessation of an exercise session (441, 453, 455), yet even when adjusted for shifts in plasma volume, the interpretation remains that an acute exercise-induced increase circulating [cortisol] occurs (441, 453, 455). The increase remains for ~1 to 3 hours in the post-exercise period before returning to resting concentrations (441, 452, 456).

*2. Exercise factors in response to acute exercise*

In addition to the response of traditional endocrine factors to exercise, a prevailing view is that skeletal muscle can act in manner analogous to an endocrine organ, such that factors released from active muscle groups during and after an exercise session exert wider metabolic effects on organs including fat, liver, gut and pancreas, and of relevance to this review, can

act locally on skeletal muscle in an autocrine/paracrine manner (116, 457). Acute exercise induces the enrichment in circulation of a vast array of factors including metabolites, several RNA species, and peptides/proteins (458-461), which can collectively be termed “exercise factors” (462, 463). Other terms used interchangeably include “myokine” for peptides and proteins released from skeletal muscle in response to exercise (116), and “exerkine” for protein or RNA factors enriched in response to exercise but with ambiguous tissue origin (464). Tissue origin is an important consideration given that many of these factors may in fact be derived from a variety of sources rather than exclusively from skeletal muscle, with prominent examples such as endothelial, cardiac, hepatic, and adipose tissues (116, 457, 465). Moreover, skeletal muscle as an organ does not simply consist only of myofibrils, but is mix of cell types (466, 467). An important distinction therefore is whether factors changed in the circulation at a whole-body level in response to acute exercise are indeed skeletal muscle-derived, skeletal muscle *fiber*-derived, or are best considered as being generally associated with the whole-body exercise response.

For each subcategory of exercise factors (i.e. metabolites, nucleic acids, proteins), the number of individual molecules that are reported to change in response to acute exercise is often estimated to be hundreds (458-461). A small number of molecules may decrease in concentration in response to acute exercise, but most changes in exercise factors are in the form of increased circulating concentration. The physiological relevance of the post-exercise enrichment of many of these factors is mostly unknown, but they likely contribute to regulation of homeostasis and substrate metabolism during and after exercise (116, 457). The cytokine/myokine interleukin-6 (IL-6), which is secreted by both immune cells and myofibers, remains the best-described in terms of kinetics of response to exercise and subsequent metabolic and/or molecular effects, and may be considered a ‘prototypical’ exercise factor. During and soon after a single session of aerobic (468) or resistance (469) exercise, circulating IL-6 is robustly observed to increase (often several-fold) (470); is transcribed, translated and released from myofibers during exercise and is therefore mostly derived from contracting skeletal muscle (471, 472); is sensitive to nutritional status (i.e. exogenous carbohydrate ingestion and endogenous glycogen concentration) (473, 474); and exerts relevant metabolic effects during and after exercise e.g. enhanced hepatic glucose output, adipose tissue lipolysis, pancreatic β-cell mass, and skeletal muscle insulin sensitivity (475-478). The details regarding subcategories of exercise factors (e.g. potential bioactivities, variability within and between subcategories, and variability in response to different types of exercise) are beyond the scope of this review, but are discussed elsewhere in relation to metabolites (479, 480), RNAs (481, 482), and proteins (463, 483). However, for most exercise factors, limited information is available beyond (sometimes inconsistent) reports that indicate a change in

circulating concentration(s) in response to acute exercise. Despite that background, there is strong speculation that exercise factors could serve as the initiating signals for the adaptations that occur in response to repeated sessions of exercise (464, 483), including as local signals in skeletal muscle (484) (Section 5.I).

### *D. Genetic factors influencing the molecular response to acute exercise*

Twin and paired sibling studies suggest that muscle size, muscle fiber type composition and number, strength, and $\dot{V}O_{2max}$ each have a large genetic contribution and vary in their degree of heritability (485-488). Underlying genetic differences between individuals are also proposed to influence the ability of an individual to respond (and the extent of that response) to acute exercise and/or exercise training interventions (often referred to as "trainability") (489, 490). Over a decade ago, a landmark study identified DNA sequence variants that predicted the trainability of $\dot{V}O_{2max}$ in response to an exercise training intervention (52). Eleven single nucleotide polymorphisms (SNPs) were identified as predicting 23% of the total variance in change in $\dot{V}O_{2max}$. Because the adaptive response in $\dot{V}O_{2max}$ to exercise training displays a heritability of ~47% (491), this method predicted approximately half of the inherited variation in trainability of $\dot{V}O_{2max}$ based on common DNA sequence variations (52). An individual's genetic profile is therefore an important factor influencing the response to exercise training, although the underpinning molecular mechanisms are presently unknown, especially because the influence of different genetic variants on the molecular response to acute exercise is largely unexplored. Such studies are methodologically very challenging to perform. Firstly, a SNP in a single gene can influence several interrelated factors with potential influence on acute molecular responses. For example, the naturally-occurring gain-of-function R225W mutation in the AMPK$\gamma$3 subunit results in increased resting AMPK activity, oxidative capacity and glycogen concentration, and decreased IMTG concentration, in skeletal muscle (492, 493). Secondly, such studies also require large n-sizes in order to adequately power for comparisons of different genotypes of single candidate genes for effects on acute molecular responses e.g. n=143 was used to investigate the influence of $\alpha$-actinin-3 (*ACTN3*) R577X gene polymorphisms on muscle fiber type composition, CSA, and glycogen concentration, yet only n=22 completed a study of the acute molecular responses to SIE as n=7 for XX, n=11 for RX, and n=4 for RR alleles when combining male and female participants (494). However, that study did suggest attenuated anabolic signaling as attenuated SIE-induced increases in mTOR and S6K1 phosphorylation in the XX genotype lacking $\alpha$-actinin-3 (494).

### *E. Summary*

In summary, the nature of the exercise response in terms of neuronal, mechanical, metabolic, and hormonal factors is largely influenced by intrinsic exercise-related parameters

namely the volume of exercise as a function of intensity and duration, and the type of exercise undertaken. Although not considered here for the sake of brevity, there are indeed other parameters that will influence the nature of these responses including environmental conditions, dietary intake and nutrition status, training status, age, biological sex, and body composition. Overall, these acute responses are largely transient with many peaking within or soon after the exercise session, and most returning to resting status within a few hours (e.g. <3 hours) after the cessation of exercise (Figure 5). Given the vast array of responses in the defense and restoration of homeostasis, it is intuitive that not all responses are in fact associated with regulation of the long-term adaptive response to exercise. However, the salient point is that the interplay between the neuronal, mechanical, metabolic, and hormonal responses to acute exercise and the activation/repression of signal transduction pathways and their downstream targets is fundamental to the mechanistic basis for exercise-training induced adaptations in skeletal muscle (67, 88, 92).

## 4. PERSPECTIVES ON MODELS EMPLOYED FOR THE STUDY OF THE MECHANISTIC BASIS OF EXERCISE TRAINING-INDUCED ADAPTATIONS IN SKELETAL MUSCLE

The performance of exercise and the multi-organ adaptations that occur with exercise training are exemplars of the integrative biology and function amongst the neuromuscular, respiratory, cardiovascular, and metabolic systems (205, 281, 495, 496), yet our understanding of molecular mechanisms that underpin these responses are, by necessity, elucidated from reductionist models (17, 89, 497-499). These reductionist models such as *in vitro* cell culture experiments and transgenic mouse models are essential for identifying and exploring mechanisms of regulation, and in theory are easier to study than the integrated system. However, methodological reductionism has its limitations when it violates assumptions of ontological reductionism i.e. the function of the integrated system (282), so here will we consider some of the features and limitations of these reductionist models in the context of exercise.

### *A. In vitro cell culture models using myotubes*

The culture of skeletal muscle cells most often involves the culture of proliferating satellite cells (myoblasts) derived from mouse, rat or human origin (500). The C2 cell line was first derived from the thigh muscles of mice subjected to crush injury (501), from which the now widely used subclone C2C12 was selected for their propensity to differentiate and form myotubes. The L6 cell line is of rat origin and first derived from the thigh muscles of newborn rats (502). Primary human myoblasts are derived from skeletal muscle biopsies by isolation

and culture of resident skeletal muscle satellite cells (503, 504). Each of these cultures begin as actively proliferating myoblasts and proceed towards confluence after which they either fuse with other myoblasts and form multinucleated myotubes, or can be deliberately shifted into differentiation to multinucleated myotubes primarily through a reduction in serum concentration in the media constituents. The phenotype of the myotubes, such as the expression of key proteins for glucose and lipid metabolism, mitochondrial function and fiber types increase during the process of differentiation, myotube formation and subsequent myotube maturation and therefore exhibit closer resemblance to adult skeletal muscle over time (503, 504) when a more striated appearance, and upregulation of adult myosin heavy chains (and downregulation of neonatal or embryonic myosin heavy chains) is evident. However, differentiated human primary myotubes have been shown in some cases to remain somewhat removed from the *in vivo* physiology of human skeletal muscle myofibers in that they are generally characterized by low mitochondrial oxidative capacity, have a preference for glucose over fatty acids as a substrate, stain positively almost exclusively as type II muscle fibers, and are randomly arranged due to lack of orientated mechanical tension rather than as parallel fibers in intact muscles (505, 506). However, other studies have demonstrated that some molecular and cellular characteristics are retained from the niche in which they were derived. Muscle cells isolated from physically-active or exercised individuals, diseased muscle such as from individuals with obesity, type 2 diabetes and cancer cachexia, as well as those from older aged donors, can 'remember' the environment that they resided in and exhibit cellular and molecular processes and phenotypes *in vitro* that are characteristic of their prior environment *in vivo* (507).

Interestingly, a comparison of rat L6, mouse C2C12, and human primary myotubes to each other and their respective intact tissues in terms of transcriptomic profiles and metabolic parameters revealed differences between species, and between cells and tissue (508). Important findings were that L6 myotubes are more appropriate for studies of glucose metabolism and mitochondrial function, while C2C12 and primary human myotubes may be more appropriate for studies of exercise models (508). In other words, depending on the experimental question and design, the selection of either L6, C2C12, or human primary myotubes can be informed by such considerations. Other considerations may include neuronal factors such as $[Ca^{2+}]_i$ transients being orders of magnitude slower in myotubes than adult muscles (334), or mechanical factors such as stretch applied *in vitro* being on the muscle fibers themselves and producing active force in contrast to the passive force and tendon-orientated nature of stretch *in vivo* (509). These limitations are most obvious when employing the respective cell lines as monolayer cultures, yet there remain several experimental advantages to cell culture over the *in vivo* condition. In recent years there have been significant

innovations in the development of 3-Dimensional (3D) cell culture systems and bioengineered skeletal muscle, and the application of electrical pulse stimulation (EPS) to mimic contractile activity. We have recently discussed these advantages and innovations in detail (500, 510), and will briefly describe them here.

The key advantages of *in vitro* experiments, especially with 3D systems, is the opportunity to isolate intrinsic and extrinsic factors influencing molecular responses to exercise in a way that is not possible in *in vivo* experiments. EPS, mechanical loading/stretching and dosing with specific growth factors, steroids, nutrients, and pharmacological agents can be performed alone and in combination, and when combined with gene silencing or overexpression can provide novel insights into the role of specific genes, pathways and networks in the molecular response to exercise, even using human cells. More extensive dose-response and time course studies relative to what can be ethically achieved in rodents or humans are also possible (albeit these are also an advantage of monolayer cultures too in this regard), whereas more recent development of bioreactors for these *in vitro* experiments will potentially lead to even higher throughput (511, 512). These bioreactors have begun to enable simultaneous mechanical and electrical stimulation that potentially provide the most physiologically-relevant *in vitro* exercise systems. When combined with 3D bioengineered skeletal muscle derived from cultured myotubes, this represents a stepwise improvement in the ability to study exercise *in vitro* (511-513). In bioengineered skeletal muscle, the myotubes are supported by a 3D matrix that allows time in culture, maturation of myotubes and contractile activity to be enhanced (514-518), and may also include co-culture with motor neurons to further improve cytoskeletal organization (519). Compared to monolayer models, these advances in 3D bioengineered skeletal muscle *in vitro* therefore replicate the structure and niche of *in vivo* skeletal muscle more closely (515, 516, 518). However, 3D culture systems are very expensive, compared to monolayer (which are also expensive experiments to run) and they require a large amount of equipment and technical skill to produce. For example, the use of gene knockdown/overexpression technologies are considerably more challenging to achieve in a 3D tissue culture compared with monolayer, and to date there have not been any studies *directly* assessing whether these 3D models are superior to monolayer cultures for mimicking exercise responses. Indeed, it is fair to say that many findings in monolayer cultures have driven hypothesis-led experimentation in humans, and *vice versa*, whereby observations in human physiology have been ‘mechanistically ratified’ using *in vitro* tissue culture. Therefore, while cell culture systems are reductionist in their very nature, their ability to compliment human experimentation in this way is a valuable part of the methodological arsenal used by exercise physiologists investigating the molecular mechanisms of exercise adaptation.

Relatedly, there are now established models to study acute and chronic contractile activity in skeletal muscle cells through electrical pulse stimulation (EPS) (498, 520, 521), whereas “muscle-specific exercise-like treatments” can also be applied using a range of pharmacological compounds (498) (Figure 8). Recent innovations also include microphysiological systems that model skeletal muscle for the same investigations with higher throughput (522, 523). However, whether EPS or pharmacological compounds are used to elicit exercise-like effects, the absence of aspects of *in vivo* physiology in cell culture is a key limitation of these models as reviewed in detail elsewhere (498, 513, 520, 521). One example is that myotubes lack the *in vivo* microenvironment and interaction with an extracellular matrix as well as other cell types such as neurons, whereas in the case of contracting myotubes as a model of exercise, the circulatory flow of substrates and the hormonal milieu of exercise are also absent. However, as described above, innovations in 3D cell culture systems and bioengineered skeletal muscle coupled to the development of novel bioreactors have gone a long way to address these limitations (510-512), and their more widespread application will undoubtedly prove fertile for discovery in the coming years. Another consideration with treatment of myotubes with pharmacological compounds or EPS is that the stimuli historically were often applied chronically over many hours or days. As described in Section 3, all exercise stimuli acting as signals or primary messengers are firstly, simultaneously present rather than active in isolation, and secondly, are transient in their activity. Therefore, pharmacological compounds and EPS models applied chronically may not accurately reflect the episodic nature of *in vivo* exercise. This limitation has been acknowledged such that now there are specific episodic protocols for EPS that better mimic acute exercise and repeated exercise sessions (516, 524, 525), as well as the transient and episodic treatment of cells with pharmacological compounds (498).

On a related point, transgenic mouse models tend to isolate individual target genes for changes in expression or protein function, with these changes tending to be constitutive, and thereby again failing to reproduce the transient and episodic nature of acute exercise and exercise training. Interpretation of observed function or phenotypes in the context of human exercise, therefore, becomes speculative (496, 497, 526). Yet transgenic mice are a powerful research tool to investigate gene function and cell biology in both health and disease. Transgenic methods can make genes non-functional (i.e. knock-out/down or loss-of-function) or increase the expression or function (i.e. knock-in or gain-of-function), and potentially do so in an inducible, tissue-specific manner (499). In two recent systematic reviews of genes whose gain- or loss-of-function produced skeletal muscle hypertrophy (527), or increased endurance performance (528), the numbers associated with these phenotypes were 47 genes (29 gain-of-function, 18 loss-of-function) and 32 genes (18 gain-of-function, 14 loss-of-function),

respectively. However, one caveat with these findings is that many discoveries about regulators of skeletal muscle phenotype are made utilizing models wherein the induced change in abundance or activity of a protein or pathway can range from complete ablation to several hundred-fold increase, whereas physiological changes in response to exercise are, most often, orders of magnitude lower that such changes. In other words, these phenotypes in isolation are potentially misleading because non-physiological modulation of a target gene may activate or repress signaling pathways or downstream effectors that are not altered under physiological conditions, and/or compensatory adjustments may occur when the target gene is knocked-out or overexpressed in embryonic development and early life course.

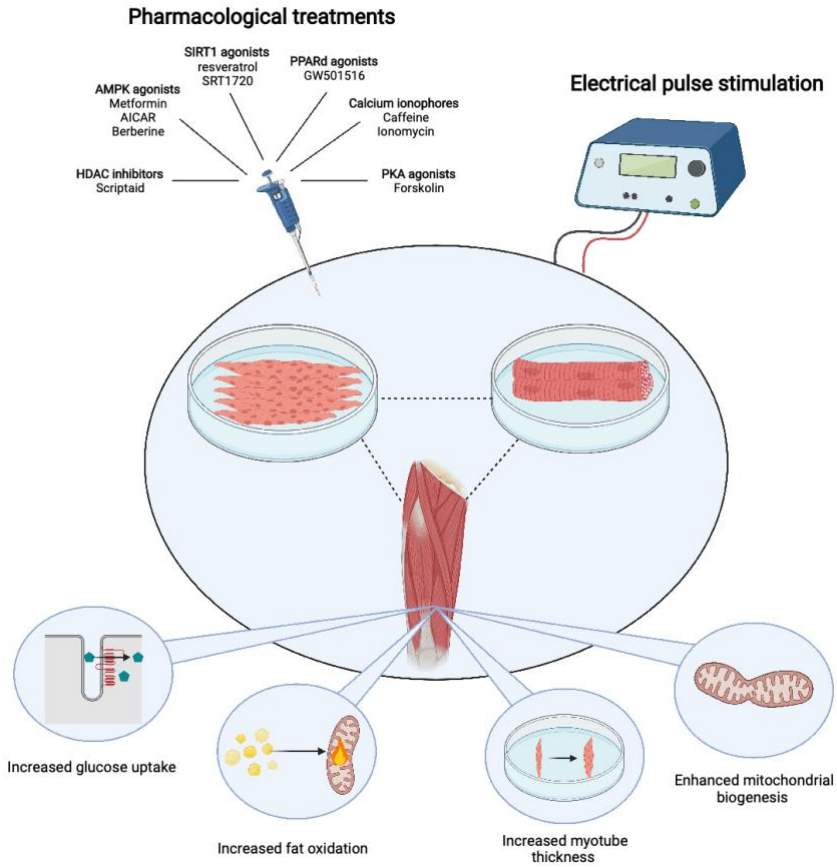


**FIGURE 8. In vitro cell culture models using myotubes to explore the effects of acute and chronic contractile activity, and pharmacological compounds to elicit exercise-like effects on skeletal muscle cells.**

Acute and chronic contractile activity can be studied through electrical pulse stimulation (EPS) (525, 529-538). “Muscle-specific exercise-like treatments” (498) include compounds such as caffeine and ionomycin acting as $Ca^{2+}$ ionophores (216, 539-544), AICAR, berberine and metformin acting as activators of AMPK (543, 545-549), forskolin acting as an activator of cAMP-dependent protein kinase A (548, 550, 551), SRT1720 and resveratrol acting as activators of SIRT1 (546, 552), Scriptaid acting as an inhibitor of HDACs (553), and GW501516 acting as activator of PPARδ (545, 554, 555). Adapted from (504).

Therefore, it seems intuitive that the following features would need to be observed before a target gene, signaling pathway or downstream process is considered as a *bona fide* regulator of exercise training-induced adaptations in skeletal muscle. Firstly, gain-of-function should result in changes in target genes, signaling pathways or downstream effectors in a manner similar to those induced by exercise. Secondly, loss-of-function should attenuate the response induced by exercise in target genes, signaling pathways or downstream effectors. Lastly, the expression or functional activity (e.g. kinase activation, DNA binding, etc) of the target protein measured in skeletal muscle *in vivo* in response to acute exercise or exercise training should be compatible with its proposed role in adaptive processes. On this theme, a notable feature of the aforementioned reviews of genes regulating skeletal muscle phenotype (527, 528) is that, through bioinformatics approaches and analysis of publicly-available datasets, these authors investigated whether in response to acute aerobic or resistance exercise the identified genes exhibited changes in mRNA abundance, or whether their protein products exhibited changes in phosphorylation status, and whether the expression of hypertrophy genes change during overload-induced hypertrophy in a way that is consistent with their function. While these data cannot conclusively prove cause-and-effect with exercise adaptations, employing triangulation approaches such as these on large scale omics datasets alongside ever-improving bioinformatics and machine learning capabilities should provide greater insight into these mechanisms of exercise training adaptation in the near future (556).

### *B. Regulator of skeletal muscle phenotype, or regulator of exercise training-induced adaptations in skeletal muscle phenotype?*

This Section does not aim to dismiss the value of these reductionist models outright, but rather to highlight that obvious limitations do exist, and should be acknowledged, in the context of appraising the quality of existing knowledge and translating findings to human physiology. One important distinction that must be made is whether a given target gene or pathway is 'simply' a regulator of skeletal muscle phenotype, or is a *bona fide* regulator of *exercise training-induced adaptations* in skeletal muscle phenotype. An illustrative example is the contrast between the aforementioned $Ca^{2+}$-sensitive enzymes calcineurin and CaMKs, both of which are implicated in muscle plasticity. Calcineurin (a protein phosphatase) and CaMKII (a protein kinase) regulate transcriptional programs downstream of signaling pathways culminating in nucleo-cytoplasmic shuttling of HDACs, and the transcription factors including serum response factor (SRF), NFAT, CREB and MEF2 (Section 5.A). Calcineurin has long been associated with the mechanisms of fiber type-switching in skeletal muscle towards a slow phenotype (557, 558), and growth factor- and overload-induced muscle hypertrophy (559, 560). In fact, its constitutive activation produces both an endurance phenotype in terms of mitochondrial function and exercise performance (561), and hypertrophy of type I muscle

fibers (562). While phosphatase activity of calcineurin is increased by acute exercise, paradoxically it is markedly decreased with exercise training (563). Ultimately, in the context of adaptive changes investigated through inhibition or knockout, calcineurin in unlikely to mediate aerobic exercise-induced changes in GLUT-4 translocation (acute) or mitochondrial biogenesis (training) (564, 565), or overload-induced hypertrophy (566, 567). On the other hand, constitutive activation of CaMKs also produces mitochondrial biogenesis, upregulation of fatty acid oxidation, and resistance to fatigue during aerobic exercise (568). Acute aerobic exercise increases CaMKII phosphorylation (79, 287, 569-573), an effect is greater at higher intensities of exercise (287, 569, 574). Three weeks of aerobic exercise training resulted in increases in *resting* CaMKII abundance and activity (575), with these changes in resting enzyme activity exhibiting a strong positive correlation with changes in the $\beta$ subunit of the mitochondrial $F_0F_1$-ATP synthase indicative of a relationship with mitochondrial adaptation (575). Similarly 8 weeks of aerobic (576), or resistance (577) exercise training also resulted in increases in resting CaMKII abundance, and in the latter study these changes in abundance were positively correlated with muscle hypertrophy (577). Conversely, disruption or inhibition of CaMKII signaling attenuates mitochondrial biogenesis *in vitro* (542) and adaptive responses to exercise *in vivo* (578, 579). Thus, it seems reasonable to conclude that calcineurin is regulator of skeletal muscle phenotype, but unlikely to have a mechanistic role in exercise training-induced adaptations, whereas CaMKII signaling is indeed likely to play a role in that context. The apparent discrepancies between calcineurin and CaMK has been proposed from a physiological perspective to be explained by the *in vivo* physiology of $[Ca^{2+}]_i$ transients and their activation of the respective enzymes (334, 580). Briefly, the stimulation of calcineurin is likely to be small in magnitude and of short duration in response to acute exercise, whereas the evident increase in resting CaMKII activity with exercise training (575) may provide the necessary stimulus to adaptation via CaMK-dependent regulation.

This distinction between the regulation of skeletal muscle phenotype and the regulation of exercise training-induced adaptations should not imply that these are mutually-exclusive types of regulation. However, we contend that conflation of these concepts has, in part, led to erroneous narratives around “master regulators” and “master switches” of exercise training-induced adaptations, such as PGC-1$\alpha$ in the context of mitochondrial adaptation to aerobic exercise, or mTORC1 in the context of skeletal muscle hypertrophy in response to resistance exercise. While the concept of master regulation can be seductive, based on current knowledge and indeed much of what is described in this review, it is highly improbable that any one protein or pathway is capable of such regulation (489). For example, PGC-1$\alpha$ has often been considered a master regulator of mitochondrial biogenesis, and therefore central to exercise training-induced adaptations in skeletal muscle (99, 581). There is little doubt

about the dramatic effects of the overexpression (582, 583) or KO (584-587) of PGC-1α on bioenergetics, mitochondrial biogenesis and skeletal muscle phenotype. Similar phenotypic effects in skeletal muscle are observed with overexpression (588, 589) or KO (590, 591) of the PGC-1α homolog PGC-1β, whereas the PGC-1α4 splice variant may be involved in the regulation of glycolytic metabolism and muscle hypertrophy (592, 593).

Yet a role of PGC-1 as this master regulator of exercise training-induced mitochondrial adaptations remains unlikely. The observations in mice that neither whole-body (586) nor muscle-specific (587) PGC-1α KO impairs aerobic exercise training-induced changes in skeletal muscle indicates that networks that are independent of PGC-1α contribute to adaptive responses in skeletal muscle. Moreover, in mice with a muscle-specific deletion of PGC-1α and PGC-1β that is inducible in adult skeletal muscle (and thereby overcoming any developmental compensatory responses to PGC-1 KO), the exercise training-induced increase in exercise performance after 4 weeks of training was similar to controls, and was associated with an increase in mitochondrial gene expression and function, even in the absence of PGC-1α and PGC-1β expression (594). Additional detailed arguments against PGC-1α being a master regulator of adaptation are described elsewhere (101, 595). Similarly, the emergence of evidence of mTORC-independent pathways regulating muscle hypertrophy (Section 5.C) also speaks against mTORC as a master regulator of that process (113). Of course, this discussion is not to suggest that PGC-1α and mTORC are not important regulators of skeletal muscle phenotype, but rather to caution against the concept of master regulation given the complexity, redundancy, and compensatory regulation in this field (86, 489, 595) (discussed further in Section 5.J). As elegantly described by Booth & Laye (497), ultimately any assertions about the function of a target gene on normal physiology and phenotype must be evaluated in the transgenic animal in the context of the sedentary state, the response during acute exercise, and at rest in the trained state. The contention is that genes function differently in the presence or absence of physical activity, such that the risk is that incorrect functions are attributed to genes by the using transgenic/KO mice with restricted physical activity (497).

### *C. Models of increased contractile activity that do not mimic human exercise*

The reference to “normal” physiology highlights another set of experimental models employed in this field; those that have been termed *in vivo* “models of increased contractile activity that do not mimic human exercise” (17). The detailed description of these models is beyond the scope of this review given our focus on acute responses, but the matter is salient given that the mechanistic role of many of the pathways and processes described throughout this review are established or investigated in such models. The general features of these

models and appraisal of their applications are reviewed in detail elsewhere (17, 89, 596-598). The two most widely-used models are synergistic ablation/functional overload for inducing skeletal muscle hypertrophy (597), and chronic low frequency electrical stimulation for inducing endurance-like adaptations including mitochondrial biogenesis (598, 599). These respective models provide enormous insight into plasticity of skeletal muscle as a general concept, but the ability to translate outcomes to equivalence in exercise training-induced adaptations is limited by the fact that the hallmark adaptations do not occur in an ecologically-valid manner (17). Specifically compared to human exercise training, in both models the application of the stimulus is chronic rather than episodic, produces adaptive changes that are much larger in magnitude in a much shorter time course, and produce some characteristics that are antithetical to human skeletal muscle. For example, synergistic ablation typically produces a type II to type I fiber transition in the predominantly type II plantaris muscle and a *decrease* in strength per unit of muscle cross-sectional area (600, 601), whereas chronic low frequency electrical stimulation can produce a marked (~25%) *loss* of muscle size with as little as three weeks of stimulation (602). Thus, as noted for all models described in this Section, some caution is warranted when interpreting aspects of mechanistic regulation from these models and extrapolating to human exercise contexts. One notable development has been the development of models of electrical stimulation that are more episodic in nature, and can be applied to deliver low frequency continuous or higher frequency intermittent stimulation to mimic various types of exercise (76, 344, 345, 603).

### *D. Limitations to the study of skeletal muscle biopsies in human participants*

There are also important limitations to the study of the molecular response to exercise in human skeletal muscle biopsies that it would be remiss not to acknowledge. These limitations are both inherent to biopsy sampling and analyses themselves, but also germane to the features of study designs that employ them. The central question throughout this review concerns mechanistic regulation of the molecular response to exercise, which intuitively would be best understood with experiments providing a high degree of temporal resolution for exploring changes mRNA and protein abundance as well as regulatory events such as protein translocation, DNA binding, and target-specific rates of transcription and translation. Yet the temporal resolution provided by muscle biopsies is a fundamental limitation because muscle biopsies only provide a static picture of these dynamic processes, in addition to the half-lives and kinetics of change in mRNA and protein being quite variable on a target-specific basis (274-278). By way of example, in addition to the aforementioned abundance of PGC-1$\alpha$ mRNA being robustly increased for ~2 to 4 hours after a session of aerobic exercise, the half-life of PGC-1$\alpha$ protein is relatively short (~2.3 h), but can be stabilized by phosphorylation by upstream kinases such as AMPK and MAPK, leading to a tripling of its half-life and an increase

in observed PGC-1α protein abundance (604). Similarly, time course studies of changes in mRNA abundance or signal transduction induced by acute exercise clearly demonstrate that biopsy timing is an essential component in the interpretation of acute exercise-induced molecular responses given the transient nature of these events and processes. For example, not taking a biopsy sample in the hour after exercise cessation would likely to erroneous conclusions about whether activation of AMPK occurs (284), or not taking a biopsy sample beyond 3 hours after exercise would likely lead to erroneous conclusions about whether changes in myogenic gene expression occur (304, 307). Similarly, no change in HIF-1α mRNA was observed immediately after (40), but was increased 6 hours after aerobic exercise (605), whereas some oxidative genes (CPT1, CD36, HAD, and ALAS1) (606) and p53 (312) only appear to be acutely-responsive to exercise when mRNA abundance is measured in samples taken 10 to 24 or 48 hours after aerobic exercise (312, 606). Slow rates of protein turnover are also observed for those mitochondrial targets (607). Therefore, the observational nature of human exercise studies means that no cause-and-effect relationship can be established for changes in skeletal muscle phenotype being explained by acute exercise-induced changes in mRNA or protein abundance. This is a point that is true of all gene targets measured in human exercise studies.

Yet there remains considerable value in time-course designs in isolation or combined with crossover designs with variables such as exercise intensity, type, or environmental condition under manipulation. If the molecular response measured is then proportional (linear or otherwise) to the nature of the perturbation to homeostasis under different conditions, then it is logical that there is more likely to be a cause-and-effect relationship between that molecular response and adaptive changes. Such designs then necessarily rely upon serial/sequential sampling with muscle biopsies, but this requires cognizance of both technical challenges and physiological responses. These considerations include the validity and repeatability of parameters measured in biopsy samples (608-622) and differences between biopsy location (620, 623, 624), the choice of biopsy needle (625, 626), and whether repeated biopsies provoke a localized physiological response independent of exercise in the skeletal muscle being sampled (302, 320, 618, 620, 627-633).

A combination of analysis costs and the reluctance of volunteers for such invasive measures has generally led to small sizes ($n \leq 15$ participants) in most human studies in the field, and the same issues tend to result in the absence of a non-exercise control condition in most study designs. Small sample sizes result in low statistical power, which is even more problematic with repeated measures and/or crossover designs where multiple comparisons are necessary. Often the relevant data are inherently noisy because of the issues with biopsy sampling highlighted above in addition to the reliance on the semi-quantitative Western blot

technique for signal transduction pathways (634). The inclusion of non-exercise control condition, although uncommon, has been included in handful of acute exercise studies either as a crossover condition (606, 635), non-exercised leg (593, 636-642), or parallel group (44, 227, 302, 310, 320, 324, 643-646). The overall pattern from this small sample of studies is that there is little effect of repeated biopsies on protein markers of signal transduction (635, 636, 638, 644, 645), at least when multiple biopsies are taken from new incisions rather than the same incision (628). However, for gene expression there can be few-to-many genes that change, albeit modestly, in the control condition (61, 302, 310, 606, 637, 640-642). In this regard, gene expression in the non-exercised leg in unilateral exercise designs is subject to the influence of changes in circulating substrates, hormones, and exercise factors associated with acute exercise (61, 640-642), whereas circadian variation, dietary factors and localized trauma may be contributing factors in other designs (61, 302, 310, 606, 637). Therefore, depending on the study aims and molecular targets, inclusion of a non-exercise control condition or group may be important in exercise studies with serial muscle biopsy sampling in order to account for the impact of non-exercise stimuli in the analysis and interpretation of the data.

Lastly, there is the consideration of fiber type specificity against the analysis of mixed muscle responses. By way of illustrative example, we have previously demonstrated an exercise intensity-dependence for the molecular response to a single session of isocaloric (~400 kcal) aerobic exercise performed at either 40% or 80%$\dot{V}O_{2max}$ (287). Specifically, the phosphorylation of several signal transduction proteins including AMPK, CaMKII, HDAC and activating transcription factor 2 (ATF2), and the mRNA abundance of PGC-1$\alpha$ were all increased to a greater extent in response to the higher intensity exercise session. A legitimate criticism of those findings was that by analyzing mixed muscle from the biopsy samples, fiber type-specific responses were not measured, and because fiber type recruitment is dependent on exercise intensity (343, 647), the differential responses observed could be influenced by the specifics of fiber type recruitment associated with each exercise intensity (648). The intensity-dependent effect on PGC-1$\alpha$ mRNA abundance has been observed in other cohorts (649-651), but one study has proposed that the lack of further increase in PGC-1$\alpha$ mRNA abundance at supramaximal exercise intensity as evidence of this fiber type-specific effect (651). Fiber type-specific responses are observed with signaling pathways such greater phosphorylation of S6K1 and lesser phosphorylation of eEF2 in type II fibers following resistance exercise (652), and greater phosphorylation of AMPK in type II fibers in HIIE compared to MICE coincident with greater muscle glycogen depletion in type II fibers in HIIE (653). That said, some studies do not observe fiber type-specific differences when comparing MICE and HIIE when the presumption is that muscle fiber recruitment patterns are different

(162, 654). One suggestion is that the absence of fiber type-specific differences may be due to a lack of difference in muscle glycogen depletion between conditions (162).

The issue of muscle glycogen is salient to the point that the analysis of mixed muscle homogenates may result in the 'masking' or 'dilution' of results that are seen specifically in type I compared to type II fibers. For example, there are fiber type-specific differences substrate utilization and contribution of energy systems in response to acute exercise that may be masked when responses are assessed based on mixed skeletal muscle biopsy samples (359, 655, 656). This phenomenon was again recently illustrated in terms of the utilization of muscle glycogen during both aerobic and resistance exercise (395, 657). In response to a high volume resistance exercise session in elite male strength athletes, mixed muscle glycogen in the vastus lateralis was depleted by ~38%, but on a fiber type-specific basis, many type II fibers demonstrated near-depleted levels of intramyofibrillar glycogen, a key depot for excitation-contraction coupling and contractile force production (395). Similarly a proteomic analysis comparing fiber types at rest identified a remarkable 471 proteins being differentially expressed between type I and type II fibers (146). In response to 12 weeks of aerobic exercise training (indoor cycling 4x per week for 1 hours at 75–90% of maximal heart rate), changes in protein abundance were observed to occur in patterns both common and divergent when between-fiber type comparisons were performed. In some cases, selected proteins were increased in one fiber type but were decreased in the other fiber type, meaning that such proteins may be indicated as unchanged in response to exercise training in analyses of mixed muscle samples as is commonly performed. Therefore, although we will focus mostly on the responses in mixed muscle in this review, fiber type-specific responses do represent an important factor influencing the molecular response to exercise, especially in relation to exercise type and intensity. Relatedly, analysis of mixed muscle will primarily consist of myofibrils, but there are many other resident cells (e.g. satellite cells, endothelial cells, immune cells, fibroblasts, and fibroadipogenic progenitors) that will contribute to measured molecular responses (466, 467). A final comment on muscle biopsies is that ~99% of studies examining fiber type-specific responses have been performed on biopsies taken from the vastus lateralis muscle of anteriolateral thigh muscle (658). This number is probably similar when considering the field of molecular exercise physiology more broadly, but whether muscle type, location, recruitment pattern, and mixed fiber type distribution have any important influence on the molecular response to exercise remains to be investigated.

## 5. ACUTE EXERCISE-INDUCED SIGNAL TRANSDUCTION PATHWAYS IN SKELETAL MUSCLE

In Sections 2 and 3, we described the model wherein the physiological stress imparted during exercise, and therefore the character of the perturbations to homeostasis, are the key determinants of neuronal, mechanical, metabolic, and hormonal factors that act as primary messengers and therefore the molecular signals for initiation of acute exercise-induced signal transduction and associated molecular responses to acute exercise. This model attempts to provide the starting point for the continuity and integration between signaling events regulated by cellular biochemical and biophysical responses and the expression of genes and processes that ultimately dictate adaptations in skeletal muscle to exercise. The obvious overlap and interrelatedness of neuronal, mechanical, metabolic, and hormonal factors makes it difficult experimentally to isolate independent and specific effects of these factors and their downstream pathways, but for the purposes of this Section, we will briefly describe each of the acute exercise-induced signal transduction pathways currently associated with the acute response to exercise and exercise-training induced adaptations in skeletal muscle.

### *A. Calcium-related signaling pathways*

As described above, changes in $[Ca^{2+}]_i$ flux that are required for skeletal muscle contraction are initially sensed and decoded by the intermediate $Ca^{2+}$-binding protein CaM. CaM is a multifunctional signal transducer that undergoes conformational changes before activating other CaM-binding proteins, primarily the downstream kinases CaMKs, and phosphatase calcineurin (94). The mechanism by which the pattern of the calcium oscillations is decoded by CaM includes changes in conformation and subcellular localization of CaM upon binding of $Ca^{2+}$ ions (659).

CaMKII is the dominant isoform in human skeletal muscle (569), and is a multi-functional serine/threonine protein kinase, comprised of 6 to 12 units with each subunit having a regulatory, catalytic, and association domain (660). Initial activation of CaMKII is facilitated by $Ca^{2+}$-dependent CaM binding, resulting in autophosphorylation on $Thr^{287}$ and activation of a $Ca^{2+}$/CaM-independent form of CaMKII (94). Phosphorylation of CaMKII makes the kinase partially independent of $Ca^{2+}$/CaM and thus, when a $Ca^{2+}$ transient ceases, the kinase retains heightened activity above basal (660). This maintenance of CaMKII enzymatic activity between $Ca^{2+}$ transients allows persistent phosphorylation of downstream substrates during repeated stimulation (334, 660). An important feature conserved in skeletal muscle CaMKII is that the activation of CaMKII is sensitive to the frequency of $Ca^{2+}$ oscillations (336), with the suggestion that intensity-dependent CaMKII activation functions as a stimulation-frequency decoder (94). Aerobic exercise increases CaMKII phosphorylation in an intensity-dependent

manner (287, 569, 574), possibly due to additional muscle fiber recruitment, or to higher $[Ca^{2+}]_i$ expected at greater force outputs (358, 647, 648).

Another suggestion is that $Ca^{2+}$ signaling may be sensitive to different *types* of exercise (i.e. aerobic vs. resistance), based on the relative amplitude and frequency of $Ca^{2+}$ transients from the respective contraction patterns, which theoretically could result in dramatically different signal transduction patterns (335). However, CaMKII phosphorylation is often unchanged in human skeletal muscle after resistance exercise (290, 297, 661), despite being increased in response to short duration, maximal intensity bouts of electrically-stimulated contractile activity in rodent skeletal muscle (662-666). Whether this divergence between the response to aerobic compared to resistance exercise is an important difference between exercise types remains to be clarified. Unfortunately, studies to date that have compared signaling pathways in response to acute aerobic and resistance exercise in either a parallel group (667), or a within-subject crossover design (249, 256, 298, 668), have not measured CaMKII phosphorylation. Given that increased resting CaMKII activity was observed in hypertrophied anterior latissimus dorsi muscles of roosters (669), in addition to increases in resting CaMKII abundance being positively correlated with hypertrophy in human vastus lateralis muscle (577), it is tempting to speculate a role for CaMKII in hypertrophic adaptations in skeletal muscle to exercise training.

That said, roles for CaMKs in skeletal muscle glucose transport, lipid uptake and oxidation, and plasticity e.g. mitochondrial biogenesis, have typically been the focus of mechanistic studies using a combination of $Ca^{2+}$-releasing agents and CaMK inhibitors (539, 540, 542, 568, 662, 670-675). The exact mechanism by which CaMKs regulate skeletal muscle gene expression and plasticity is not fully defined. Direct modulation of transcription factor function by protein phosphorylation is one possible mechanism. Another potential mechanism of CaMKII function is by modulating HDAC activity. However, transcript abundance of certain genes is not increased by elevated calcium, whereas others follow a distinct time-course of induction (540, 542, 671). Transcription factors such as SRF, CREB, and MEF2, and the HDAC family of transcriptional regulators are CaMK targets implicated in the regulation of skeletal muscle gene expression. For instance, the activation of CaMKII leads to phosphorylation and nuclear exclusion of HDAC4, which relieves repression of MEF2 (337). This mechanism would couple contraction-induced $Ca^{2+}$ signaling to an increased rate of transcription of MEF2 target genes such as PGC-1$\alpha$ and GLUT4 (94), whose mRNA abundance is robustly increased after acute aerobic exercise (225, 287).

$Ca^{2+}$-related signaling also serves as an illustrative example of the complexity of crosstalk between signal transduction pathways. Examples include CaMK kinases (CaMKKs) being identified as upstream AMPK kinases capable of inducing phosphorylation at $Thr^{172}$ and

thus activating AMPK (676, 677), and AMPK activation occurring directly as a result of activation of $Ca^{2+}$ signaling in myotubes even in the absence of contraction (671). Moreover, there is evidence that p38 MAPK lies downstream of CaMKII regulating PGC-1$\alpha$ in a pathway activated by $Ca^{2+}$ flux (542). Other examples include the inextricable links between $Ca^{2+}$-related signaling and signaling associated with PKC, RONS, and mechanosensory signal transduction as recently reviewed in the context of skeletal muscle hypertrophy (348).

### *B. MAPK signaling*

MAPKs are a family of conserved serine/threonine kinases comprising of (i) the extracellular-regulated kinase 1/2 (ERK1/2), (ii) c-jun N-terminal kinase (JNK), and (iii) p38 MAPK, whose signal transduction cascades are established in physiological processes such as cell proliferation, differentiation, hypertrophy, inflammation, gene expression and apoptosis (93, 678, 679). Activation of MAPKs is associated with the sensing of extracellular changes such as growth factors and cytokines, but via the sensing of cellular stress from diverse stimuli such as osmotic, oxidative, and mechanical stress (679). In the context of acute exercise, ERK1/2 has been demonstrated to have a mechanistic role in the regulation of fatty acid oxidation in skeletal muscle at low-to-moderate intensities of exercise via the regulation of fatty acid uptake through CD36 translocation to the plasma membrane, in addition an effect on fatty oxidation independent of effects on fatty acid uptake (680-682). In fact, this series of experiments from a perfused rat hindlimb model implicate convergence between CaMKII and CaMKK signaling via both AMPK-dependent and independent regulation and ERK1/2 signaling in both a time- and intensity-dependent manner (683).

Specific to adaptive responses, MAPKs regulate transcriptional events by through downstream signal transduction via phosphorylation of diverse substrates localized in the cytoplasm or nucleus, including transcriptional regulators (93, 678, 679). For example, during contraction p38 MAPK can activate transcription factors upstream of the PGC-1$\alpha$ gene such as ATF2 and MEF2 (684), which coincides with an increase in PGC-1$\alpha$ mRNA abundance (226, 287, 684), but can also increase PGC-1$\alpha$ protein stability and half-life through phosphorylation (685). MAPKs are also proposed to regulate protein translation and muscle hypertrophy through mTORC1-dependent and independent pathways (686-688).

Much of the initial work in skeletal muscle focused on MAPK activation in response to aerobic exercise (77, 213, 221, 689, 690), and because of the wide variety of biochemical and biophysical processes activated by muscle contraction, all three of the main MAPK subfamilies are robustly activated in human skeletal muscle (93, 678). More recently there has been an apparent divergence of focus towards p38 MAPK activation after aerobic exercise due to its role in the regulation of PGC-1$\alpha$ abundance and activity (684, 685), and the endurance

phenotype (684, 691), and towards ERK1/2 and JNK activation after resistance exercise due the purported roles of the these kinases in mechanosensory signal transduction and muscle hypertrophy (347, 348) (Section 5.C).

Constitutive activation of p38 MAPK increases markers of mitochondrial adaptation in skeletal muscle (684), whereas deletion of the p38$\gamma$ isoform, but not p38$\alpha$ or p38$\beta$, prevents exercise training-induced increases in mitochondrial biogenesis and angiogenesis (691). Given the established role of skeletal muscle satellite cells in adaptive processes (Section 6.E), it is notable that p38 MAPK signaling is also suggested as a central to satellite cell-dependent myogenesis (692) and the activation and renewal of satellite cells in the presence of muscle damage (693). Phosphorylation and activation of p38 MAPK is consistently observed in response to acute aerobic exercise including MICE (213, 287, 288, 291, 668, 694-696), and HIIE/SIE (200, 201, 288, 291, 571, 574, 695-697). These increases are sometimes observed to be greater in response to higher intensity or greater metabolic stress of exercise (287, 574), and of lower magnitude in trained individuals (213, 668). However, the effect of acute resistance exercise on p38 MAPK phosphorylation is equivocal, with several studies observing an increase (290, 292, 644, 668, 698) or no change (699-701), whereas others suggest that activation may be dependent on the intensity and/or volume of the resistance exercise session (635, 702). The predominance of eccentric or concentric contractions can also influence activation patterns of other MAPKs (703, 704), with eccentric contractions producing greater activation of the MAPKs (703-707). Notably, in a series of in situ experiments using rat plantaris muscle, ERK1/2 and JNK, but not p38 MAPK, were found to be phosphorylated in the tension-specific manner, with JNK being established as the most mechanosensitive of the three MAPKs in this context (704).

Increased ERK1/2 phosphorylation has been consistently observed in response to acute resistance exercise in human skeletal muscle (81, 293, 325, 635, 644, 699, 702, 707-717), with the implication that this pathway may increase protein translation in an mTORC1-independent manner (114). Greater ERK1/2 phosphorylation is observed with a higher number of muscle contractions (712), and higher volume (644), and higher intensity (702), during resistance exercise, but the increase in ERK1/2 phosphorylation did not differ between acute aerobic and resistance exercise (293, 668), nor did the training history (endurance- vs. strength-trained) alter the response (668). JNK phosphorylation in response to resistance exercise has been less well-studied, but several studies have observed robust increases in the post-exercise period (292, 635, 688, 707, 711, 718-721), especially after eccentric exercise (705, 707). When acute aerobic exercise (60 minutes cycling at 70%$\dot{V}O_{2max}$) and resistance exercise (8 sets of 5 repetitions of leg extensions at 80%1RM) were compared, JNK phosphorylation increased several-fold higher immediately after resistance exercise

(688). This is noteworthy because JNK has been proposed as a positive regulator of muscle hypertrophy through the inhibition of SMAD2-dependent myostatin activity, whereas muscle-specific JNK KO mice have a blunted hypertrophic response to two weeks of functional overload/synergistic ablation (688). These observations have led to strong implication of a role for JNK in mechanosensory regulation of MPS, protein translation and muscle hypertrophy described in the next section.

### *C. Mechanosensory signal transduction through mTORC-dependent and independent pathways*

As briefly mentioned in Section 2.B, a diverse class of proteins known as mechanoreceptors are involved in cytoskeletal structure and force transmission, and can sense stretch/length, tension/force, and electrical potentials in muscle cells. Therefore, the cellular perception of mechanical cues is achieved through the activation of these mechanosensing proteins. The activity of these proteins is subject of much investigation for their role in mechanosensory signal transduction (or “mechanotransduction”), especially in the context of resistance exercise and the regulation of MPS, protein translation and muscle hypertrophy (92). Broadly speaking, mechanotransduction refers to the ability of a cell to sense and respond to a mechanical stimulus and convert this stimulus into an intracellular biochemical response. However, the detailed specifics of these processes is beyond the scope of this review, have been excellently reviewed elsewhere (346) (92, 111, 347, 348), and therefore are briefly described here in the context of human exercise.

All forms of muscular contraction result in the application of tension (force) through an active muscle. However, the *adaptive* muscle hypertrophy consequent to high mechanical loads present during stimuli such as functional overload and resistance exercise is largely determined by the activation of canonical regulators of protein translation, namely mTORC, S6K1, 4E-BP1, and related parallel and downstream targets (114). S6K1 is a key regulator of MPS through canonical pathways of protein translation and ribosome biogenesis involving eukaryotic translation initiation factor 4E (eIF4E) binding protein (4E-BP1) and elongation factor 2 (eEF2). Phosphorylation of 4E-BP1 by mTOR suppresses binding and inhibition of eIF4E by 4E-BP1. This derepression allows eIF4E to directly bind the 5’ end of mRNA to ultimately form an active eIF4F complex, a rate-limiting step in translation initiation. Phosphorylation of S6K leads to the phosphorylation of the 40S ribosomal protein S6 (rpS6) and eIF4B. Collectively these events lead to the formation of the translation initiation complex and activate protein synthesis for cellular hypertrophy (114).

The list of potential regulators of hypertrophy through mechanotransduction continues to expand, and to date includes: costamere associated proteins such as focal adhesion kinase

(FAK); Yes-associated protein (Yap) of the Hippo signaling pathway; phosphatidic acid synthesized by phospholipase D or diacylglycerol kinase zeta (DGKζ); integrins and specifically the $\alpha_7\beta_1$-integrin isoform; titin; Bag3 and filamin-C; and stretch-activated ion channels encoded by the genes *PIEZO1* and *PIEZO2* (92). Evidence for each of these candidates is derived from a variety of experimental paradigms, but common features are that these are proteins that are involved in force transmission and/or responsive to mechanical deformation of cellular structures e.g. sarcolemma, nucleus. Their respective roles in muscle hypertrophy is then established via knockout, overexpression or constitutive activation, and whether their mechanism of action is mTORC1-dependent or independent is often further delineated using rapamycin as a specific inhibitor of mTORC1 (92, 111, 347, 348).

High force contractions such as those performed during resistance exercise, as opposed to low force contractions during aerobic exercise (Section 3), are likely to be essential for activation of mechanotransduction (92, 111). How “high” the force needs to be is an intriguing question because even relatively low forces (e.g. ~30%1RM), with an appropriate volume of resistance exercise training can increase rates of MPS acutely and lead to muscle hypertrophy over time (129). Whether acute aerobic exercise activates mechanotransduction to a meaningful extent requires further investigation. Neither $\alpha_7\beta_1$-integrin nor FAK signaling was increased by ~45 minutes of unilateral arm exercise at 70%$W_{max}$ (722), whereas the modulation of exercise intensity (moderate vs. heavy) and oxygen availability (normoxia vs. hypoxia) had inconsistent effects on mRNA and protein abundance of the costamere proteins integrin-linked kinase (ILK), vinculin and talin (723). Overall, while a variety of mTORC1-dependent and -independent pathways that regulate protein translation and ribosomal biogenesis are established as being activated in human skeletal muscle and associated with increases rates of MPS (114), their upstream regulation by mechanosensory proteins remains to be robustly observed in the same contexts. Conceptually, these pathways are attractive as an explanation for the divergent adaptations to aerobic versus resistance exercise in terms of muscle hypertrophy, but much work remains to be done to better establish this fact.

### *D. Redox state and $NAD^+$-related regulation*

$NAD^+$ plays a critical role in fuel metabolism as an electron carrier that couples ATP synthesis with the electron transport chain via its reduction during glycolysis and re-oxidation via lactate generation in the cytosol or mitochondrial shuttle activity, as well as reduction reactions within the TCA cycle. The deacetylase activity of the sirtuin (SIRT) family of protein deacetylases is $NAD^+$-dependent (724), with the subsequent deacetylation of lysine residues on mitochondrial enzymes (725), and transcriptional regulators (726) including PGC-1α (546), which thereby links alterations in the cellular reduction-oxidation (redox) state to the adaptive

changes in gene expression and cellular metabolism (546, 727, 728). Enhanced SIRT activity is associated with favorable adaptations in skeletal muscle metabolism, including enhanced mitochondrial function, exercise performance, and protection against obesogenic feeding and muscle atrophy (727, 729-734). Conversely, muscle-specific disruption to $NAD^+$ homeostasis causes muscle fiber degeneration and progressive loss of muscle strength and endurance capacity (735). Of particular interest to skeletal muscle metabolism and adaptation, the deacetylase activities of SIRT1 (cytoplasmic, nuclear) and SIRT3 (mitochondrial) are sensitive to changes in redox state (96, 736). In fact, SIRT1 is known to deacetylate at least 40 protein targets (737) including several with established roles in skeletal muscle phenotype such as PGC-1$\alpha$, p53 and the FOXOs.

Because the $NAD^+$/NADH dinucleotide pair is central to many redox reactions in cellular bioenergetics, it is unsurprising that dynamic fluctuations in [$NAD^+$] and the [$NAD^+$]/[NADH] ratio occur in response to stimuli such as acute exercise (357, 738-743). With increasing exercise intensity, the cytosolic $NAD^+$/NADH ratio *declines* as the lactate/pyruvate ratio increases (357, 739, 741). However, there is considerable discordance in observed changes in [$NAD^+$] and [$NAD^+$]/[NADH] between rodent and human studies, in addition to there being important differences in the measurement of [$NAD^+$] and [NADH] in terms of estimation or direct measurements, the method used for the latter, and the subcellular location i.e. cytosol vs. mitochondria (reviewed in (744)). Moreover, it remains unclear as to the significance of these fluctuations during exercise given that the largest changes in [$NAD^+$]/[NADH] would be expected to be an increase within the mitochondria driven by the increase in [$NAD^+$] (739), and recent data suggesting that SIRTs are most regulated by [$NAD^+$] and less so by [NADH] (736). One of the initial studies suggesting a role of SIRT1 activity in skeletal muscle after exercise observed elevations in SIRT1 activity (and deacetylation of PGC-1$\alpha$) peak ~3 hours after the cessation of exercise, which coincides with a dramatic *elevation* in the [$NAD^+$]/[NADH] ratio, predominantly due to an increase in [$NAD^+$] (546). Substrate shifts in the post-exercise recovery period (Section 3.B) coincide with an increase in $\beta$-oxidation and reoxidation of NADH to $NAD^+$, which could in theory explain this effect. However, even with prolonged fasting (~48 to 72 h) during which the rates of $\beta$-oxidation would be accelerated, [$NAD^+$] and the [$NAD^+$]/[NADH] ratio are largely unchanged in human skeletal muscle (745), and SIRT1 abundance and activity are also unchanged (746). Differences between rodents and humans in terms of fasting physiology are well-established, including that various aspects of the metabolic response are greater in magnitude and occur more rapidly in rodents (747), and this may be confounding factor in these discordant observations (748).

An increase in SIRT1 protein abundance (~85%) in human skeletal muscle was observed 2 hours after acute Wingate sprint effort (an effect ablated by glucose ingestion)

(749), whereas an increase in nuclear SIRT1 *activity* measured by enzymatic assay was observed in red (slow twitch/type I) gastrocnemius muscle of rats 3 hours after completing ~2 hours of treadmill running (750). Exercise-training induced PGC-1$\alpha$ has also been observed in mouse gastrocnemius muscle (751). However, to our knowledge, a increase in SIRT1 activity measured by deacetylation of target proteins has not been observed in human skeletal muscle. In fact, acetylation of p53 on $Lys^{382}$, a SIRT1 deacetylation target, was unchanged immediately and 3 hours after 60 minutes of cycle exercise at 60%$W_{max}$ (752), and was *increased* immediately after 30 minutes of moderate intensity cycle exercise (753). Additionally, the deacetylation of PGC-1$\alpha$ observed after acute aerobic exercise in rodent skeletal muscle occurred independently of SIRT1 (754), and the adaptive responses to aerobic exercise training were unaffected by muscle-specific deletion of SIRT1 (754). With these muscle-specific SIRT1 KO mice being largely similar to wild-type mice, the authors suggested that SIRT1 may be dispensable in skeletal muscle, and that acetylation of PGC-1$\alpha$ may in fact be determined by general control of amino-acid synthesis (GCN5), which had previously been identified as the dominant acetyltransferase regulating PGC-1$\alpha$ activity (729, 755). However, subsequent studies with GCN5 deletion in skeletal muscle (756), and inducible skeletal muscle-specific overexpression of SIRT1 combined with GCN5 KO (757) have not observed any effect on contractile function, mitochondrial gene expression or function, or exercise training-induced adaptations in skeletal muscle.

Contrasting findings have been observed with exercise training, with two weeks of SIT increasing SIRT1 protein abundance by ~56% (758), whereas six weeks of HIIT produced a decrease of ~20% in SIRT1 protein abundance but an ~31% increase in SIRT1 activity (759). Moreover, nicotinamide phosphoribosyltransferase (NAMPT), the rate-limiting step in the $NAD^+$ salvage pathway from which $NAD^+$ is mainly generated (760), is increased in skeletal muscle by aerobic (550, 761) and resistance exercise training (761), which would potentially serve to increase resting concentrations of $NAD^+$ and the [$NAD^+$]/[NADH] ratio as observed with exercise training (740-742). However, mice with NAMPT overexpression in skeletal muscle, and having an ~50% increase in skeletal muscle [$NAD^+$], do not exhibit changes in mitochondrial biogenesis or function suggesting that chronic elevation of [$NAD^+$] does not perturb the [$NAD^+$]/[NADH] ratio in skeletal muscle. Therefore, it remains possible that increases in the regulation of, and capacity for, SIRT1 activity occurs with aerobic exercise training, but at present changes in $NAD^+$ metabolism and/or SIRT1 activity appear to occur in parallel with remodeling of skeletal muscle by exercise training rather than being critical regulatory factors.

### *E. Hypoxia-related signaling*

HIF is a heterodimeric transcription factor composed of two subunits; a constitutively-expressed β subunit HIF-1β, and an oxygen-sensitive α subunit in the form of HIF-1α and HIF-2α (762). HIF-1α and HIF-2α when combined with HIF-1β form HIF-1 and HIF-2, respectively (762), and it is HIF-1 that has predominantly been the subject of investigation in relation to exercise (100). Genetic manipulation of HIF-1 activity has revealed roles for HIF-1α in the regulation of transcription of target genes involved in erythropoiesis, angiogenesis, and glycolytic energy metabolism (763-767), with several of these pathways broadly analogous to the response to aerobic exercise (47, 51, 768).

The intracellular $PO_2$ is the dominant factor regulating HIF activity, with changes in $PO_2$ sensed by the prolyl hydroxylase (PHD) enzymes, PHD1, 2 and 3 (762). Declines in $PO_2$ can occur with travel to higher altitude or exposure to simulated altitude, but the present discussion will be limited to the local tissue hypoxia induced during normoxic exercise. For example, $PO_2$ in resting skeletal muscle is ~1/5$^{th}$ of the oxygen pressure of inhaled air (~30 vs. ~160 mmHg) (769, 770), whereas acute aerobic exercise of moderate intensity (~50%$\dot{V}O_{2max}$) reduces $PO_2$ in the exercising muscle to ~1/50$^{th}$ of that of inhaled air (~3 mmHg) (771, 772). Notably, a threshold of ~8 mmHg has been proposed as the intramuscular $PO_2$ below which HIF-1α is stabilized (773), which suggests that most forms of exercise will impact HIF-1 protein abundance.

Under normoxic conditions, hydroxylation of HIF-1α occurs by PHD enzymes, which trigger degradation of HIF-1α via the binding of the E3 ubiquitin ligase von Hippel Lindau tumor-suppressor protein (pVHL) to HIF-1α and marking it for proteasomal degradation (774). The hydroxylase activity of PHD enzymes is inhibited by declines in $PO_2$ allowing stabilization of HIF-1α, which otherwise has a very short half-life (<1 minute). Consequently, HIF-1α translocates to the nucleus to form an active dimeric complex with HIF-1β, which activates the transcription of target genes by binding to hypoxia response elements (HREs) within target genes (e.g. the pro-angiogenic vascular endothelial growth factor-A; VEGF-A) and recruiting transacting coactivators such as p300 and CBP (CREB-binding protein) (775). Broadly speaking, HIF-dependent transcriptional regulation therefore augments survival during low $O_2$ tension either by increasing $O_2$ delivery and extraction (angiogenesis), or by enhancing the ability to obtain ATP from $O_2$-independent pathways (glycolytic metabolism) (762).

Given the declines in intramuscular $PO_2$ during exercise, unsurprisingly HIF-1α protein abundance is robustly increased in skeletal muscle during sciatic nerve stimulation in rats (776), downhill treadmill running in rats (777), and acute HIIE in mice (778). In humans, acute aerobic exercise increases nuclear HIF-1α protein abundance, which coincides with a

decrease in pVHL abundance (224). In electrically-simulated mouse soleus (779), and exercised human (224) and rat (777) vastus lateralis, enhanced DNA binding of HIF-1α is observed as part of the role for HIF-1α in transcriptional regulation. However, the effect of ambient hypoxia has little effect on the decline in intramuscular $PO_2$ during exercise (771, 776, 780), which suggests that the major effect of exercise on HIF-1α activation would be determined by the exercise stimulus itself, an effect that may be proportional to the intensity of contraction (776). In contrast, during a study of repeated sprint exercise (4 sets of 5 x10 seconds all-out sprint cycling), increases in HIF-1α mRNA and protein abundance were only observed in hypoxia, not normoxia, despite similar changes in tissue saturation index (as a surrogate measure of intramuscular $PO_2$) during exercise in both conditions (781). To our knowledge, HIF-1α protein abundance or activity has not been studied in response to acute resistance exercise. Tissue saturation index is reduced even during low intensity (~30%1RM) resistance exercise (782), but when combined with blood flow restriction, a stimulus known to induce HIF-1α abundance (783, 784), the further reduction in tissue saturation index was minor during the working sets (782). Moreover, when 8 weeks of resistance exercise training was performed in hypoxia (14.4% $O_2$), increases in muscle size and strength were similar to normoxia, but increased muscular endurance and angiogenesis were observed in the hypoxia group (785).

Despite HIF-1α being increased by acute exercise, an apparent paradox is that the phenotype of mice lacking skeletal muscle HIF-1α is to have increased reliance on lipid metabolism at rest, increased oxidative enzyme capacities, and increased capillary-to-fiber ratio, which indicates that HIF-1α *suppresses* characteristics of the endurance phenotype (764, 765). These mice also demonstrate exercise intolerance, which is consistent with what is observed in humans with Chuvash polycythemia, an autosomal recessive disorder in which degradation of HIF is impaired resulting in elevated levels of HIF at normoxic $PO_2$ (786). An explanation for this paradox is that HIF-1α-related signaling, and especially the stimulation of angiogenesis, is important in response to the early phase of an exercise training intervention, after which negative regulation of HIF-1α begins to increase (100). This hypothesis is based on the observations that there is an increase in expression of negative regulators of HIF-1α abundance and activity (i.e. PHD1-3, Factor Inhibiting HIF-1 (FIH) and SIRT6) in well-trained endurance athletes (787), as well as the increase in HIF-1α and HIF-2α mRNA abundance in response to acute exercise being ablated after only 4 weeks of aerobic exercise training (605).

### *F. Redox- and RONS-related signaling*

RONS production is constitutively-active in all cell types (788), and is amplified in skeletal muscle during exercise (789-792). RONS comprise of a plethora of diverse molecules

but during exercise, the major sources and types of RONS are (i) superoxide generated in mitochondria by complexes I and III of electron transport chain, and to a lesser extent NADPH oxidase enzymes (NOX/DUOX) and xanthine oxidase (XO) in other subcellular locations, and (ii) nitric oxide (NO) through various isoforms of nitric oxide synthase (115, 788). Additionally, superoxide may also react with NO to form peroxynitrite ($ONOO^-$), which is another potentially important RONS (788). Directly investigating RONS production in human skeletal muscle has been challenging due to the volatile nature of RONS and low bioavailability of redox probes in humans (115, 793), and so there is some debate about the extent of these sources and types during exercise in humans. Because RONS are ubiquitous and pleiotropic molecules, where they exert signaling effects in skeletal muscle is a function of their reactivity, subcellular location, and their localized removal by enzymatic and non-enzymatic antioxidant defense systems (788, 793, 794). Consequently, much of the knowledge on RONS-related signaling pathways and the regulation of skeletal muscle phenotype is from animal and cell models, whereas in human skeletal muscle, measures are often indirect (i.e. downstream signals and transcriptional responses), or are inferred from studies where antioxidant supplementations has been used to attenuate RONS-related signaling (115, 793, 794). Such designs have suggested roles for RONS in mediating adaptations in skeletal muscle because of the observation of attenuated endurance (795-799), and hypertrophic (800, 801), adaptations to exercise training in antioxidant-supplemented compared to control groups. However, the attenuation of adaptation by antioxidant supplementation or RONS inhibition is not always observed (802-806), and attenuation of acute molecular responses do not always translate into attenuated adaptations in skeletal muscle to exercise training (804, 805).

For responses to acute exercise, the mechanistic basis for the above observations is proposed as attenuated intracellular signaling resulting in consequent effects of gene expression (529, 743, 807-809), and recent evidence also suggests a role for RONS regulating epigenetic mechanisms (810). The targets of RONS-related signaling linking signal transduction to transcriptional processes that have been most prominently examined in skeletal muscle are AMPK and MAPK signaling, and transcriptional regulators including nuclear factor erythroid 2-related factor 2 (Nrf2), NF-κB and PGC-1α (115, 811). Therefore it is notable that acute MICE (812), HIIE/SIE (813), incremental exercise to exhaustion (573), and resistance exercise (227), all increase NF-κB phosphorylation, with the effect potentially being intensity-dependent (813). Acute exercise-induced activation of MAPK signaling occurs partially in a RONS-dependent manner as evidenced by attenuated JNK, but not ERK1/2 or p38, signaling during aerobic exercise with infusion of the antioxidant *N*-acetylcysteine (NAC) (808). In that experiment, increases in MnSOD mRNA abundance were attenuated in NAC, but increases in PGC-1α, IL-6 and HSP70 mRNA abundance were unaffected by NAC (808).

AMPK and CaMKII signaling is also partially under the influence of RONS given that acute ingestion of the antioxidants α-lipoic acid, vitamins C and E 2 hours prior to exercise attenuated the increase in AMPK and CaMKII phosphorylation in response to a 30 second all-out sprint on a cycle ergometer (743). The absence of effects of the antioxidant cocktail on exercise performance, indicators of aerobic and anaerobic metabolism, AMP/ATP and $NAD^+$/NADH ratios, or the degree of protein carbonylation suggested effects on AMPK and CaMKII dependent on RONS-mediated signaling (743). In rodent skeletal muscle, the effect of antioxidants is sometimes more dramatic, as evidenced by abrogation of a variety of acute exercise-induced changes in signaling and gene expression (529, 807, 809), but conversely acute molecular responses are sometimes also unaffected by the inhibition of RONS (814, 815). Therefore, it is likely that RONS-related signaling has an important role mediating the molecular response to acute exercise, but further investigation will be required using methods that specifically capture redox signaling (e.g. protein cysteine oxidation or S-nitrosylation), use targeted rather than generic antioxidants, and/or better identify the specific RONS involved.

### *G. Cellular energy status, ATP turnover and AMPK signaling*

AMPK is a heterotrimeric serine/threonine kinase that modulates cellular metabolism acutely through phosphorylation of metabolic enzymes (816), and over the longer term by modulating transcription and translation through phosphorylation of transcription factors and other signaling proteins (817, 818). The heterotrimeric complexes of AMPK comprise a catalytic α-subunit (α1 and α2) and regulatory β- and γ-subunits (β1, β2, γ1, γ2, and γ3) (819). Of the 12 possible heterotrimeric combinations, only a subset of three complexes have been identified in the vastus lateralis muscle in humans (α2β2γ1, α2β2γ3, and α1β2γ1), whereas five complexes have been identified in mouse skeletal muscle (α2β2γ1, α2β2γ3, α2β1γ1, α1β2γ1, and α1β1γ1) (109). The kinase domain is located on the α subunit, the activity of which is highly dependent on the reversible phosphorylation of $Thr^{172}$ in the α-subunit (820), with kinase activity potentially increasing >100-fold upon activation (819). For the purposes of this review, we will focus on the activity of AMPK in general terms, but discussion of the heterotrimeric complexes, their activation by different types of exercise, their influence on different downstream targets, and the role of upstream kinases is detailed excellently elsewhere (109), as is exercise-induced activation of AMPK in tissues other than skeletal muscle (821).

The kinase activity of AMPK is influenced by allosteric binding of AMP and/or ADP (activating on the γ subunit), and glycogen (inhibitory on the β subunit). Thus, AMPK activation is regulated allosterically by a cellular energy deficit, which is reflected by increases in [ADP], and the AMP/ATP and Cr/PCr ratios (109). [AMP] is considered the dominant regulator of

AMPK activity broadly (819) and during exercise (822), although *in silico* models conversely predict that [ADP] is dominant controller of AMPK activity in skeletal muscle during exercise (823). Cellular stresses that deplete ATP and raise [AMP] and [ADP] (such as metabolic poisons), or increase the cellular AMP/ATP ratio (such as glucose deprivation or oxidative stress), therefore activate AMPK (109). A robust increase in AMPK phosphorylation and enzymatic activity by exercise has been shown many times in response to various forms of exercise (78, 200, 201, 285, 287, 289, 290, 292, 293, 393, 574, 593, 695, 743, 824, 825) (reviewed in (826)), which is expected given the cellular energy deficit caused by aerobic (358, 741), resistance (386, 390, 394), HIIE/SIE (353, 827, 828) and sprint/maximal effort (656, 743, 829) exercise, and the increase in [ADP] and [AMP] as a consequence of ATP hydrolysis and resynthesis in the process of muscle contraction (355, 356) (Section 3.B). Additionally, AMPK phosphorylation in response to resistance exercise may be volume-dependent (290), whereas AMPK activity and/or phosphorylation often occurs in an intensity-dependent manner above a threshold of ~60%$\dot{V}O_{2max}$ for aerobic exercise (78, 287, 295, 649, 824, 830). This pattern probably reflects intensity-dependent effects of exercise on ATP turnover and adenine nucleotide concentrations (358, 824), as well as the greater reliance on, and therefore depletion of, muscle glycogen with increasing intensity of exercise (364, 365). Greater glycogen depletion in type II muscle fibers during HIIE compared to MICE of equal 30 minute duration is also associated with greater AMPK phosphorylation in these fibers (653). Similarly, the duration effect is such that there is a progressive increase in AMPK activity during the first 30 minutes of steady-state MICE (294, 831), and increases further from 30 to 120 minutes of steady-state MICE (822, 832), which may be related to proportional declines in muscle glycogen (831). The role of muscle glycogen concentration in the activation of AMPK by exercise is notable because of the recent findings that exercising in a state of reduced carbohydrate availability (833, 834) or low muscle glycogen (835-839) leads to greater AMPK phosphorylation, and may lead to greater adaptive changes in skeletal muscle with exercise training over time (371).

Conceptually, acute activation of AMPK acts to protect ATP concentrations by stimulating catabolic pathways to restore cellular energy stores while simultaneously inhibiting biosynthetic pathways and anabolic pathways to restore homeostasis, and also serving as a signal transducer for metabolic and mitochondrial adaptations by responding to transient perturbations to cellular energy status (819). For example, in skeletal muscle acute activation of AMPK suppresses glycogen synthesis (816) and protein synthesis (840), but stimulates glucose transport (841) and lipid metabolism (73). However, whether these metabolic effects occur during exercise specifically under the control of AMPK activation is hotly debated (842). In fact, data from inducible KO of AMPK in skeletal muscle clearly demonstrate that AMPK is

dispensable in the processes of acute exercise-induced glucose uptake, lipid oxidation and substrate utilization (843). In relation to adaptive processes, chronic AMPK activation in rodents via pharmacological intervention or genetic manipulation alters broad programs of metabolic gene expression, induces mitochondrial biogenesis, improves exercise capacity, and generally recapitulates the endurance-trained phenotype (545, 817, 818, 844-852). Of course as stated above, exercise produces only transient activation of AMPK that is largely confined to the exercise session itself and the early recovery period (<3 hours), so how well these models of chronic AMPK activation inform the mechanistic basis of exercise training-induced adaptations is questionable. As an alternative approach to the question, over the past two decades numerous AMPK KO models targeting individual subunits have been employed in which resting skeletal muscle phenotype, the response to acute exercise, and adaptations to exercise training have been explored (reviewed in (109)). One challenge has been that compensatory up-regulation, redundancy, or residual activity of other subunits has been evident with KO of AMPK $\alpha$2, $\beta$2, or $\gamma$3 subunits, which has made it difficult to determine the physiological importance of AMPK in skeletal muscle metabolism and adaptation.

Recently, double-KO models of $\alpha$1/$\alpha$2 or $\beta$1/$\beta$2 specifically in skeletal muscle have been developed to address these limitations (843, 853-856). In mice with embryonic deletion of $\alpha$1/$\alpha$2 AMPK (mdKO), the acute exercise-induced increase in COX-I, GLUT4, and VEGF mRNA abundance was ablated compared to wild-type mice, in addition to the increase in PGC-1$\alpha$ mRNA abundance being attenuated, whereas the relative changes in mRNA abundance of HKII and FATP4 were similar between genotypes (856). These results suggest that some, but not all, exercise-responsive genes are increased in an AMPK-dependent manner. Another confounding factor with AMPK KO models generally is that skeletal muscle displays a phenotype that reflects impaired oxidative capacity, and reduced maximal running speeds and exercise tolerance (109). This phenotype is also evident in $\alpha$1/$\alpha$2 AMPK mdKO (854-856), so to address this limitation, Fentz et al. (2015) performed acute exercise at the same relative intensity (%max running speed) in these mdKO mice, and in their analysis of exercise training-induced adaptations to 4 weeks of voluntary wheel running have compared subgroups matched for running distance (856). Of the 11 metabolic and mitochondrial proteins measured in quadriceps muscle as markers of exercise training adaptation, only protein abundance of UQCRC1 (mitochondrial complex III) was observed to increase exclusively in wild-type mice, whereas increases in citrate synthase activity, and protein abundance of cytochrome c, GLUT4, HKII, CD36 and FABPpm were similar in both wild-type and mdKO mice (856). This equal running sub-analysis approach still does not control for intensity and thereby overall training volume, and therefore it remains unclear whether the presence or absence of differences between genotype reflects regulation by AMPK or training volume. The

recent development of another AMPK mdKO mouse model that employs a tamoxifen-inducible deletion of α1/α2 AMPK (imdKO) and ablates AMPK activity 3 weeks after the introduction of tamoxifen (843) may be able to provide further insight into these acute and adaptive processes, but unfortunately also exhibits impairments in maximal running speeds and exercise tolerance (843). That said, overall it seems likely that there are some proteins and processes that are AMPK-dependent in response to training, whereas many others are not. One explanation is that many of the pathways described in this Section could compensate for the lack of AMPK α1/α2 activity in the models. This has been demonstrated in principle in the regulation of acute exercise-induced GLUT4 mRNA abundance, which is proposed to be regulated by a compensatory increase in protein kinase D activity and post-transcriptional reductions in HDAC5 under conditions of loss of AMPK α2 activity (857).

Regulation of transcription through inhibition of HDAC activity and consequent de-repression of MEF2 activity is one of several mechanisms by which AMPK is proposed to regulate gene expression (214, 858). These mechanisms also include increasing the transcriptional coregulator activity of PGC-1α (818, 845), potentially as a result altering [$NAD^+$] and activation of SIRT1 activity (546, 728), and increasing the DNA binding activity of transcription factors including NRF-1 and MEF2 (817, 858), as well as AMPK being an upstream kinase for phosphorylation of transcription factors including CREB (activation) (859) and FOXO1 (inhibition) (860). Therefore, the observation that acute exercise increases nuclear AMPK abundance in human skeletal muscle (835, 861, 862) is a key feature providing further evidence for a role of AMPK as a regulator of transcription through interaction with nuclear proteins. Lastly, a recent study in mouse skeletal muscle suggested a role of *mitochondrial* AMPK activity in the molecular response to acute exercise by being required for exercise-induced mitophagy (863), an important pathway for mitochondrial quality control and mitochondrial biogenesis (Section 6.D).

### *H. Peptide hormones and related signaling*

Section 3.C described transient, acute exercise-induced changes in a variety of hormones such as GH, IGF-I, testosterone, cortisol and adrenaline, and the influence of exercise type and intensity. Each of these hormones could potentially influence skeletal muscle gene expression via interaction with their respective transmembrane and intracellular receptors and consequent activation of downstream signaling pathways (95, 98, 103, 108, 118, 401). Despite anabolic or catabolic effects of these hormones being well-established, their role in exercise training-induced adaptations in skeletal muscle remains unclear (402, 864). Evidence against a role of these hormones includes marked adaptive muscle hypertrophy in synergistic ablation/functional overload experiments in the absence of change

in circulating hormones (89, 597). Notwithstanding that that model does not represent resistance exercise training in an ecologically-valid manner (Section 4), this observation is supported by hypertrophy with unilateral resistance exercise occurring without acute or training-induced increases in endogenous anabolic hormone concentrations (865). A lack of correlation between acute exercise-induced AUCs for GH, IGF-I, and free testosterone, and training-induced changes in LBM and leg strength has also been observed (866, 867), in addition to studies demonstrating that altering the systemic hormonal environment to be "pro-anabolic" via acute high volume leg resistance exercise does not have an additive effect on resistance exercise-induced rates of MPS (acute), or adaptive changes in LBM or strength of the elbow flexors (868, 869). Other studies have also demonstrated little relationship between acute hormonal changes and resistance-exercise training adaptations (870-872).

On the contrary, there remains a view that the post-exercise hormonal milieu is an important component of the anabolic response to exercise (402), not least because of the well-established anabolic effects of GH, IGF-I and testosterone when administered by exogenous means (402). The molecular pathways that link these hormones to decreased muscle protein degradation, and increased ribosome biogenesis, protein translation, extracellular matrix remodeling, and satellite cell activation, are well-described in theory (95, 98, 103, 108, 118, 401), and pharmacological targeting of several of these pathways does produce muscle hypertrophy in humans (873).

Indeed several studies do observe positive correlations between acute hormonal responses and various measures of muscle mass and strength adaptation to resistance exercise training (455, 874-880). Discrepancies between studies supporting these opposing views is evident in terms of statistical models employed, the time course of the post-exercise sampling, and the outcome measure e.g. LBM by DXA versus muscle fiber CSA. Additionally, simply measuring change in circulating concentration of a hormone may ultimately be too crude of a measure to explore these relationships *in vivo* given that likely influence of bioactivity via the dynamics of receptor concentration, interaction, and downstream signal transduction on these processes. One example is the activity and expression of the AR in skeletal muscle as the intracellular target of free testosterone (881). Acute high volume resistance exercise increases AR phosphorylation (720, 882) and AR DNA binding (230), and AR abundance is increased in response to resistance exercise training (870, 883-885). Notably, the increased abundance of AR is positively correlated with muscle hypertrophy (870, 883), and high responders to resistance exercise training have higher AR abundance before and after training compared to low responders (884). Relatedly, pharmacological *suppression* of testosterone into the hypogonadal range in young men attenuated resistance exercise

training-induced increases in LBM (886, 887), an effect that was associated with attenuation acute exercise-induced increases in anabolic signaling and MPS (887).

The influence of the post-exercise hormonal milieu on skeletal muscle gene expression is also demonstrated by marked changes in the non-exercised leg in acute unilateral exercise models (61, 640, 641). For example, one hour of single leg cycling at 50%$W_{max}$ resulted in changes in mRNA abundance in the non-exercised leg in 17 of the 20 most markedly changed mRNAs in the exercised leg (640). Because exercise increased plasma lactate, FFA, cortisol, noradrenaline, and adrenaline concentrations, it is not possible to attribute these changes in mRNA abundance to a specific factor but the authors speculated that β-AR- and PPAR-dependent signaling were likely mechanisms consequent to changes in [adrenaline] and [FFA] respectively (640). β-AR is also proposed as an important regulator of intensity-dependent exercise-induced expression of PGC-1α isoforms (888), and the regulation of the nuclear hormone receptor 4A (NR4A) family of orphan nuclear receptors (889, 890). Comprising of Nur77 (neuron-derived clone 77; NR4A1), Nurr1 (nuclear receptor related 1; NR4A2), and Nor-1 (neuron-derived orphan receptor 1; NR4A3), these are consistently among the genes most responsive to acute aerobic and repeated sprint exercise (47, 48, 219, 640, 891), and are important regulators of metabolic and mitochondrial metabolism in skeletal muscle (889, 890). An intriguing possibility is that NR4A family link the acute increase in circulating catecholamine concentrations to adaptive changes in skeletal muscle phenotype. This possibility is supported by the observation in the α1/α2 AMPK mdKO model of exaggerated increases in circulating [adrenaline] is response to acute aerobic exercise being linked to a compensatory induction of NR4A mRNA abundance (855). Similarly, in humans post-exercise cold water immersion of one leg resulted in increased PGC-1α mRNA abundance in the non-immersed leg, an effect attributed to β-adrenergic stimulation of the latter (892). β-adrenergic blockade (intravenous propranolol) does not however block the exercise-induced increased PGC-1α mRNA abundance despite lowering post-exercise mitoPS (893), whereas β-adrenergic stimulation (intravenous isoproterenol) alone does not increase mitochondrial gene expression or mitoPS in human skeletal muscle (894). Therefore, while there are some obvious indications for a hormonal influence on acute molecular responses to both aerobic and resistance exercise, there remains considerable work to be done to parse out specific regulatory roles of each.

### *I. Are exercise factors involved in the regulation of adaptations in skeletal muscle to exercise?*

In addition to the above-mentioned hormones, there is little doubt that acute exercise induces changes in circulating concentrations of hundreds of molecules considered to be

exercise factors, the majority of which are increased in concentration (458-461). These responses likely contribute to regulation of homeostasis and substrate metabolism acutely during and after exercise (116, 457, 463), but the causal role for exercise factors in exercise training-induced adaptations in skeletal muscle remains largely speculative and circumstantial (464, 483, 484, 895, 896). For an exercise factor to potentially have a causal role in adaptation, intuitively it would exhibit a change in circulating concentration during or after acute exercise, have a defined mechanisms of action (e.g. cellular receptor, signaling pathway), and exert effects on skeletal muscle when its activity/presence is manipulated by either pharmacological blockage or administration via exogenous means at physiologically-relevant concentrations. Few, if any, studies of exercise factors have taken this approach because, as recently reviewed, the focus on adaptations to exercise training, rather than metabolic regulation, is in its infancy, and many exercise factors have yet to be adequately investigated (484). Rather than there being evidence against their role in adaptation, there is simply little evidence either way. In the case of IL-6, pharmacological blockade with the IL-6 receptor antagonist tocilizumab has been applied for studies of acute exercise (45 minutes, HIIE) and exercise training (12 weeks, HIIT) albeit in obese sedentary individuals (897-900). These data demonstrate IL-6-dependent regulation of acute exercise effects on immune cell mobilization (899), and GLP-1 secretion (900), as well as exercise training effects on epicardial fat mass and left ventricular mass (897), and visceral fat loss (898). However, effects on skeletal muscle remain to be explored, and at a whole body level, exercise training-induced improvements in $\dot{V}O_{2max}$ or $W_{max}$ were not impacted by tocilizumab (898). Of the other exercise factors best-studied to date, apelin (901), mitochondrial ORF of the 12S ribosomal RNA type-c (MOTS-c) (902), IL-13 (903), IGF-I (904), myostatin (905), and follistatin (906), each demonstrate promising results for their physiological effects being consistent with adaptive processes in skeletal muscle. Additionally, we have recently reviewed the effect of exercise training on the resting concentration and cargo profile of circulating small extracellular vesicles (EVs), and their associated bioactivities (896). There are preliminary data to suggest that small EVs are released from myofibers in vivo (907), and that small EV preparations from exercise-trained samples exert bioactivities that are relevant to the beneficial effects of exercise in organs beyond skeletal muscle (908-911). Such effects remain to be demonstrated on skeletal muscle itself.

Lactate could also be classified as an exercise factor due to the marked increase in intramuscular production, and appearance in the circulation with moderate-to-heavy intensity aerobic or resistance exercise (362, 384). Lactate can act as a ligand for GPR81, a G-protein coupled receptor also known as hydroxy-carboxylic acid receptor 1 (HCAR1), whose activation downregulates cAMP-PKA-mediated signaling predominantly in adipose tissue

(912). Via MCT-mediated transport (913) and receptor-mediated signaling e.g. $EC_{50}$ ~5 mM (914), lactate is proposed to have pleiotropic effects on metabolism (362), can influence gene expression by an epigenetic mechanism involving lactate-derived lactylation of histone lysine residues (915), and is potentially a signaling molecule for skeletal muscle adaptation (92). In the context of skeletal muscle, treatment of muscle cells with lactate (10 and 20 mM) induces molecular responses analogous to aerobic (916) and resistance (917) exercise outcomes. Moreover, *in vivo* administration of lactate (~10 to 20 mM, 30 minutes) acutely increased phosphorylation of ERK1/2, Akt, S6K1, and rpS6 in mouse quadriceps muscle (918), consistent the observation of an ability to induce myogenesis and hypertrophy in cells (917). Similarly, mRNA abundance in skeletal muscle of many genes associated with mitochondrial function and biogenesis were increased 3 hours after an intraperitoneal injection of lactate that produced a ~5 to 20 mM blood lactate concentration over 60 minutes (919). The mechanism(s) underpinning these effects remains to be confirmed as muscle-specific activation of GPR81/HCAR1 via lactate has not been clearly demonstrated, and the expression of GPR81/HCAR1 may in fact be negligible (914, 920).

### *J. Specificity and interference in signal transduction pathways*

The pathways of signal transduction are clearly complex, with some pathways being complimentary, and others potentially attenuating each other's activity (interference effect). This complexity probably reflects that a multiple signal transduction control system with inherent redundancy (i.e., not all pathways are always active or necessary) has the potential advantage of allowing for fine-tuning of adaptive responses to multiple metabolic and physiological stimuli as is required in the context of exercise training. Redundancy and compensatory regulation are indeed key characteristics of biological systems that act to preserve physiological responses and adaptations to a variety of challenges to homeostasis. While both aerobic and resistance exercise share the same fundamental characteristics of exercise in muscular contraction, elevated energy expenditure and disruption to homeostasis, the neuronal, mechanical, metabolic, and hormonal responses to different types of exercise are often divergent (Section 3), and are reflected to a certain extent in the nature of adaptations to prolonged aerobic exercise training compared to resistance exercise training when performed in isolation (Section 1.D). Therefore, one postulate has been that the type of exercise stimulus and the contemporaneous environmental conditions are reflected in the specificity and divergence of the molecular signatures that are induced. In other words, specificity of adaptation may be conferred by the relative activation/repression, contribution, and magnitude of the described pathways and downstream targets being largely dependent on the intensity, duration, and type of the exercise stimulus (344, 345, 921-924), and on imposed environmental variables such as nutrient availability and oxygen availability (128).

Divergent molecular signatures produced in response to broad categorizations of exercise type at the level of number, force and duration of contraction has been best illustrated using rat skeletal muscle subjected to low frequency (LFES) or high frequency (HFES) electrical stimulation to mimic single sessions of aerobic and resistance exercise, respectively (344, 345). For example, MAPK signaling was elevated for 6 hours after running exercise, but not LFES or HFES, whereas S6K1 phosphorylation was elevated and sustained to a greater extent after HFES than LFES (344). The responses to HFES thereby being indicative of the hypertrophy response this model produces (76). Similarly, LFES (10 millisecond pulse duration at 10 Hz for 180 minutes) and intermittent HFES (6 sets of 10x3 second contractions at 100 Hz over ~20 minutes) produced a selective activation of pathways associated with mitochondrial adaptation (AMPK/PGC-1$\alpha$ pathways) and selective activation of pathways associated with anabolic signaling and muscle growth (Akt/mTOR signaling), respectively (345). Moreover, aerobic exercise-like stimulation inhibited Akt/mTOR signaling and related downstream targets TSC2 and eEF2, mostly likely via the established inhibitory effects of AMPK on TSC2 (925), and on eEF2 via eEF2 kinase (926, 927), and whose net effect would be to attenuate protein translation (114). These data, and the observation of little difference between contraction models on MAPK signaling, led to the adoption of the “AMPK/Akt master switch” concept (925), which in an exercise context was proposed as the selective activation of either AMPK/PGC-1$\alpha$ or Akt/mTOR could explain the divergent adaptations associated with aerobic and resistance exercise training (345). However as described in Sections 5.B and 5.C, more recent evidence suggests divergence between exercise types in terms of MAPK activation, mechanosensory regulation, and mTORC1-independent signaling, which may also contribute to divergent adaptations.

Throughout this Section, several examples have described the influence of features of an exercise session on magnitude and nature of the signaling response such as intensity-dependent activation of AMPK (78, 287, 295, 649, 824, 830), volume-dependent phosphorylation of MAPKs (644, 928, 929) and S6K1 (290, 698), and the degree to which eccentric and concentric contractions influence S6K1 phosphorylation (930, 931), i.e. different profiles of muscle contraction can produce divergent signaling responses. A corollary of the “AMPK/Akt master switch” concept is there being potential for “interference” between signal transduction pathways. As first proposed, the activation of AMPK by aerobic exercise antagonizes the activation of mTORC-dependent pathways, attenuating pathways that dominate in the adaptive response to resistance exercise, and such that the conflicting molecular reprogramming that underlies the respective adaptations could produce an interference effect (135, 136, 932). This hypothesis was a molecular basis to explain a phenomenon known as the “concurrent training” or ‘‘interference” effect that had first been

observed in the early 1980s (132). Simply stated, training for both endurance and strength outcomes through concurrent aerobic and resistance exercise training can result in a compromised adaptation to resistance exercise training in terms of strength, power and hypertrophy when compared with training using resistance exercise alone. However, in practice, the concurrent training effect is not widely observed in exercise training studies, or only tends to be evident in athletes undertaking large training volumes (933). For example, data from elite decathletes suggests that it is possible to excel at disciplines that require predominantly endurance or predominantly strength/power, but not both (934). The concurrent training effect had been proposed to be explained by the specificity in signal transduction pathways in response to aerobic compared to resistance exercise. Yet many studies have *failed* to observe divergent molecular signatures in response to aerobic and resistance exercise, nor does the inhibition of mTOR signaling by activation of AMPK provide a complete explanation of the phenomenon when it is observed (136).

For example, there are many studies in which no interference effect is observed in acute signaling and protein synthetic responses to concurrent exercise models (240, 256, 297, 935-940). Moreover, there is ample evidence that AMPK phosphorylation is increased in response to acute resistance exercise (249, 285, 289, 290, 393, 668, 941), and mTOR phosphorylation is increased in response to acute aerobic exercise (249, 258, 268, 298, 667, 839, 942-944). Study design limitations and confounding issues have included parallel group designs largely being employed, aerobic and resistance exercise sessions not being matched for work performed, variations in the time points after exercise at which muscle biopsies are obtained, and the influence of prior training status on the molecular response to acute exercise. Yet whether employing parallel groups designs (645, 667), or direct comparisons of the exercise types in crossover designs (249, 256, 668), there are largely similar signaling responses for AMPK- and Akt-related pathways, and therefore little evidence of specificity being conferred by an AMPK/Akt switch. Indeed, even when subtle differences in signaling have been observed for distal proteins in Akt-mTOR signaling pathways, these are not sufficient to explain differences in rates of myoPS and mitoPS when they have been observed (249, 256).

An alternative view is that training history (668), or a short period of exercise training (249, 645) is an important determinant of the specificity of the signaling response i.e. that after training exclusively for aerobic or resistance exercise, the signaling response is more refined to reflect adaptive needs of the specific training type, rather than a generalized response to unfamiliar exercise (298, 645). This point is discussed in more detail in Sections 7.C and 7.D, but for example, mitoPS is increased after aerobic or resistance exercise in the untrained state, but only after aerobic exercise in the trained state, whereas myoPS is increased only

after resistance exercise in both the untrained and trained state (249). Similarly, in response to an acute session of exercise after 10 weeks of exercise training, activation of several components of mTOR signaling (mTORC1, S6K, GSK3$\alpha$, eIF4E) was exclusive to resistance exercise, and unchanged in response to aerobic exercise (645). Therefore, studying responses in untrained individuals, or not employing exercise sessions with characteristics on the extremes of the aerobic and resistance exercise continuum as comparators, may result in masking of divergence in signaling effects (645). Indeed, the electrical stimulation models encompassed by HFES and LFES and the subsequent molecular response observed in rodent skeletal muscle may be more representative of the extreme exercise training regimens that are undertaken by (and perhaps only possible in) highly trained athletes, as opposed to untrained individuals undertaking low or moderate exercise, or mixed types of exercise (136).

Overall the evidence remains equivocal, and the concept somewhat theoretical, that these divergent signaling responses confer specificity and are coupled to a functional outcome i.e. that the specific molecular responses are contiguous with divergent physiological and functional adaptations to the respective exercise modalities as indicated by the endurance and hypertrophy phenotypes. However, as stated above, the specificity of exercise adaptation to divergent types of exercise remains likely to reside at the level of differential responses of different protein fractions (myofibrillar, sarcoplasmic and mitochondrial) (249, 256-260) as well as individual proteins (259, 261, 263). In addition to the issues of training status and characteristics of the exercise session highlighted above, the discordance between observed signaling responses and fraction-specific rates of MPS may simply reflect that the methods used to date investigation signal transduction are not sensitive enough to detect subtle differences, or alternatively, it may be that there are other signaling proteins and pathways that are important to confer specificity. Elucidating these potentially novel regulators of adaptations in skeletal muscle will require employing unbiased, large scale multi-omics analyses for which several proteomics investigations have already shown promise.

### *K. Emerging insights from proteomics approaches*

Many of the signal transduction pathways described thus far (and downstream targets described later) rely heavily on the data from the interrogation of ‘known’ pathways, especially those with well-established canonical roles in cell physiology, in the context of the molecular response to acute exercise. The alternative approach is to measure many proteins in an unbiased approach, and therefore specifically in relation to signal transduction, proteomics and phosphoproteomics have emerged as the key approach to study acute responses to exercise (945). Much progress continues to be made especially with proteome mining techniques to catalogue all proteins that can be detected by mass spectrometry. For example, just over a decade ago over 2000 proteins were identified from biopsy samples of human

skeletal muscle, which in addition to abundant myofibrillar proteins and metabolic enzymes, included signaling proteins such as AMPK and CaMKs as well as numerous eukaryotic initiation factors, proteasome subunits and E3 ligases (946), whereas a new approach with deeper coverage of the skeletal muscle proteome than other studies to date identified over 4000 proteins, and facilitates fiber type-specific analysis (146).

Innovations in the detection of posttranslational modifications coupled to declining costs of omics technologies and their more widespread application has resulted in these unbiased approaches being applied to understanding the molecular networks that are acutely responsive to exercise (947). Several studies have therefore explored the skeletal muscle phosphoproteome in response to single sessions of exercise (293, 665, 666, 948-950). In human skeletal muscle after an acute session of aerobic exercise (9 to 11 minutes at 85-92%$W_{max}$ in untrained men), 1004 phosphosites on 562 proteins were identified as being exercise-responsive (948). This number represented ~12% of the skeletal muscle phosphoproteome, and while some of these phosphosites were targets of known exercise-responsive protein kinases including AMPK, CaMK, and mTOR, the majority of kinases and substrate phosphosites had not previously been associated with acute exercise-induced signal transduction (948).

An analogous investigation of resistance exercise was performed using electrically-evoked maximal intensity contractions in mouse tibialis anterior muscle consisting of 10 sets of six contractions lasting 3 seconds with 10 seconds between contractions and a 1 minute rest period between sets (665). The analyses identified 5983 unique phosphosites, of which 663 were found to be regulated by the exercise session. Again, known phosphosites of exercise-responsive protein kinases including p38 MAPK, CaMK and mTOR were identified, but in contrast to aerobic exercise, a high proportion of the regulated phosphosites were found on proteins that are associated with the Z-disc, with ~75% of Z-disc proteins experiencing robust changes in phosphorylation (665). The phosphorylation state of two Z-disc kinases, namely striated muscle-specific serine/threonine protein kinase (SPEG) and obscurin, was dramatically altered by the maximal intensity contractions, and were proposed as novel kinases potentially playing a role in mechanotransduction in skeletal muscle (665).

These findings highlight the possibility that much remains to be discovered about acute exercise-induced signal transduction in skeletal muscle, although the functional relevance of these novel kinases and substrates remains to be established, especially in the context of having a permissive role in the adaptive response to exercise. To this end, an extension to the latter study (665) by the same research group resulted in the novel identification of TRIM28 and its Ser473 phosphorylation site as being activated by exercise and being a potential regulator of muscle size and function (albeit in non-physiological expression models) (666).

Another notable finding was that the majority of contraction-induced phosphorylation events were rapamycin-insensitive (666), which is interpreted as further evidence for the emerging concept described above of mTORC-independent signal transduction pathways as being important for regulation of skeletal muscle hypertrophy (113). Similarly, another phosphoproteome analysis of acute exercise in human skeletal muscle identified C18ORF25 as an exercise-responsive AMPK substrate potentially involved in skeletal muscle function and the signaling response to acute exercise (293). That study identified >5700 unique phosphosites that were regulated either during or after at least one type of exercise by comparing responses to an acute session of aerobic (90 minutes at 60%$\dot{V}O_{2max}$), sprint (3 x 30 seconds all-out cycling), and resistance (6 sets x 10 repetition maximum knee extensions) exercise (293). This dataset represents the largest compendium of exercise-regulated phosphorylation sites in human skeletal muscle to date. A canonical acute exercise phosphoproteome was therefore proposed based on identification of 420 phosphosites on 218 proteins identified as being regulated immediately after exercise in common to all types of exercise (293), although some caution is warranted when interpreting the response to a single session of exercise in untrained individuals due to a generalized stress response (described in further detail in Section 7).

More broadly than phosphorylation events, posttranslational modifications are central to the regulation of an array of cellular processes from signal transduction to metabolic enzyme activity. The emergence of proteomics techniques to study protein acetylation (the acetylome) (951), and ubiquitination (the ubiquitome) (952), amongst others (945), suggests that many opportunities and unknowns remain in the acute regulation of signal transduction in skeletal muscle, and its role in adaptation to exercise training.

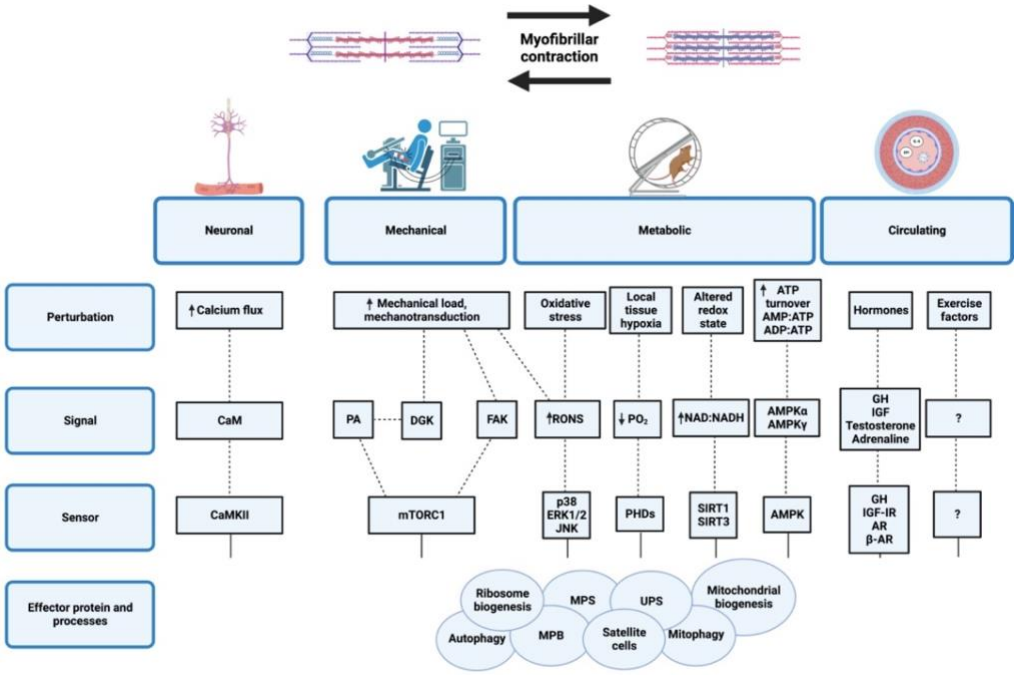


**FIGURE 9. Overview of major exercise-induced signal transduction pathways in skeletal muscle that arise from factors intrinsic and extrinsic to the exercising muscle.**

The onset of myofibrillar activity via shortening (concentric) and lengthening (eccentric) contractions during exercise results in a milieu of biochemical and biophysical stimuli localized within the contracting muscle cells, and elicit changes in circulating factors extrinsic to the exercise muscle. These perturbations in whole body and skeletal muscle homeostasis lead the activation of networks of signaling molecules including protein kinases, phosphatases, and deacetylases, which are integrated into physiological processes by downstream targets, including transcription factors, coregulators and repressors that in turn regulate a myriad of pre- and post-transcriptional and pre- and post-translational processes The relative activation, contribution, and magnitude of the described pathways and downstream targets are dependent on the intensity, duration, and mode of the exercise stimulus, and on imposed environmental variables. Here, linear pathways are depicted, but in fact, these pathways demonstrate some degree of dependence, crosstalk, interference, and redundancy in their regulation, making the exact contribution of each signaling pathway to measured changes in gene expression difficult to isolate.

## 6. EFFECTOR PROTEINS AND PROCESSES INVOLVED IN ADAPTATIONS IN SKELETAL MUSCLE TO EXERCISE

The activation and/or repression of the signal transduction pathways described above are ultimately coupled to a myriad of downstream effector proteins involved in the regulation of the transcriptional and translational processes as part of the acute response to exercise. These responses are proposed as the basis for the chronic changes in abundance, regulation and/or maximal activity of key proteins involved in energy provision, the remodeling of cellular components such as contractile proteins and the extracellular matrix, and the biogenesis of organelles such as ribosomes and mitochondria that underpin adaptations in skeletal muscle to exercise training (70, 86, 88, 92, 101). Here we will provide an overview of the regulation of transcription and translation as *acute* responses to exercise downstream of the signal

transduction pathways described so far. A selected, but not exhaustive, list of these regulators that have been associated with acute exercise- and exercise training-induced changes in skeletal muscle are provided in Table 2.

### *A. Regulation of changes in mRNA abundance in skeletal muscle*

Exercise training-induced changes in tissue form and function as an adaptive process are driven by acute and chronic changes in mRNA abundance prior to changes in proteins that provoke gradual structural remodeling and long-term functional adjustments by providing the message for the instruction of muscle tissue remodeling via translation and assembly of the encoded proteins (86). These transcriptional events are important to both aerobic and resistance exercise training adaptations (101, 953), such that the activity of transcription factors, co-activators and repressors are the subject of much interest (104, 105). As described in Section 2, most, if not all, aspects of the control of transcription have been demonstrated to be altered in skeletal muscle by acute exercise including the accessibility of chromatin, the DNA binding activity of transcription factors, and the protein stability and subcellular localization of transcriptional regulators. Epigenetic regulation by DNA methylation and histone acetylation status (Section 6.B) and RNA-mediated regulation by such as miRNA and small interfering RNA (siRNA) (Section 6.C), small non-coding RNA (such as piwiRNA) and long non-coding RNA (lncRNA) are some of the other pre- and post-transcriptional regulators ultimately dictating whether or not transcripts are translated into proteins. Because mRNA abundance reflects the sum of the rates of transcription, mRNA processing and mRNA stability, it is important to acknowledge that a change in mRNA abundance does not always only reflect a change in transcription. Nor can it be assumed that measurement of a change in mRNA abundance at some timepoint after exercise always manifests latterly as a change in physiology or phenotype (282).

Nevertheless, the majority of work in skeletal muscle has focused on transcription factors and coregulator proteins that are activated and/or repressed in response to exercise-induced signal transduction pathways for which many are primary and secondary downstream targets of those described in Section 5 (Figure 9). Therefore, these transcription factors, nuclear receptors, repressors and their transcriptional coregulators integrate contractile stimuli into molecular reprogramming by their response to specific cellular signals, co-complex with a variety of other factors, and/or demonstrate selective activation of specific gene promoters containing binding sites for the given transcription factor (Figure 10). This network is becoming increasingly well-defined (Table 2), and several illustrative examples are provided in the remainder of this section as they relate to epigenetic regulation, posttranscriptional regulation, mitochondrial and ribosomal biogenesis, protein degradation and skeletal muscle satellite cells.

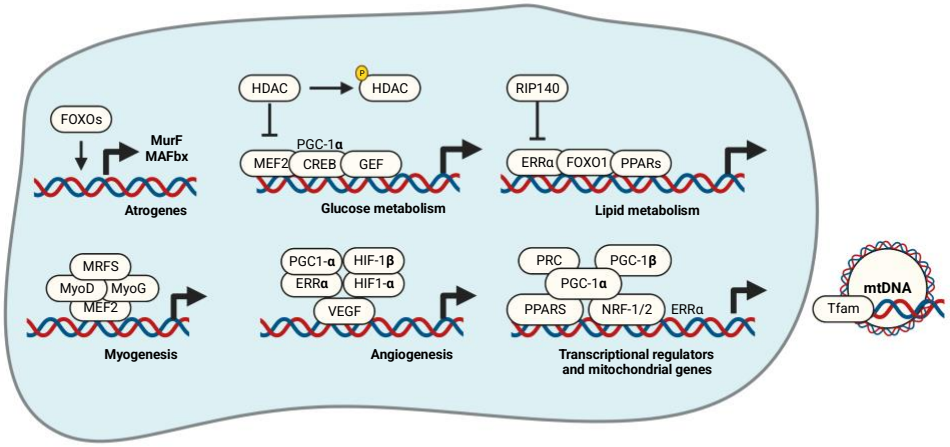


**FIGURE 10. Acute exercise-responsive transcriptional regulators of metabolism and adaptation in skeletal muscle.**

Transcription factors, coregulators and repressors can be activated or repressed in response to specific cellular signals, co-complex with a variety of other factors, and/or demonstrate selective activation of specific gene promoters containing binding sites for the given transcription factor. Many of these proteins are established as primary targets and downstream effectors of contraction-induced signal transduction pathways. Regulation of transcriptional activity occurs via change in the protein content, subcellular localization, and/or activity (e.g., posttranslational modifications), thereby integrating contractile stimuli into molecular reprogramming. Indicative interactions of transcription factors, coregulators and repressors with their gene targets are shown as being linked to specific adaptive outcomes, but these interactions are not exclusive, and other factors are likely to contribute to a given adaptive program.

### *B. Epigenetic regulation of transcription in skeletal muscle: transient alterations in DNA methylation and histone acetylation status in response to acute exercise*

Epigenetic regulation of gene expression focusses on the importance of modifications to our DNA itself (by methylation) or by modifications to proteins making up chromatin, such as histones that can surround DNA and affect the accessibility for ensuing gene transcription. Histone modifications involve either adding or removing acetyl ($CH_3CO$, acetyl group) or methyl groups ($CH_3$, methyl group) causing acetylation or methylation of histone proteins respectively. The phosphorylation of histone proteins, especially in the tail regions of histones H3 and H4 (65, 67), are also important epigenetic modifications. Other modifications include SUMOylation and ubiquitination, but methylation (of DNA and histones) and acetylation of histones after acute exercise are the most studied to date (65, 67). The enzymes that catalyze these modifications include DNA methyltransferases (DNMTs), which increase DNA methylation (hypermethylation) (Figure 11), with DNMT 3a and 3b involved in *de novo* methylation, and DNMT1 responsible for maintaining methylation, whereas the ten-eleven translocation (TET) enzymes TET1, 2 and 3 remove methyl groups (hypomethylation). For histone modifications, important enzymes adding acetyl groups are the histone

acetyltransferases or HATs (e.g. GNAT, MYST and P300/CREB families) and those responsible for removing acetyl groups are the histone deacetylases or HDACs (including class I, II, II and IV HDACs). Increased methylation to DNA, especially for example in CpG islands within promoter regions (and/or enhancers), is generally associated with reduced gene expression due to the recruitment of CpG methyl binding proteins that inhibit binding of the transcriptional apparatus (i.e., RNA polymerase and associated transcription factors), as well as a tightening of the adjacent chromatin (954, 955). With reduced DNA methylation, an increase in gene expression is more likely due to the permission of transcriptional apparatus binding and a loosening of the adjacent chromatin, but if this occurs in silencing regions, the opposite pattern is likely to occur. Intragenic methylation can affect gene expression (956-958), but this is not well-described within molecular responses to acute exercise.

The effects of histone modifications are more varied in terms of their impact on gene expression. For example, trimethylation of lysines 9 and 27 on histone H3 (H3K9me3 and H3K27me3) and lysine 20 on histone H4 (H4K20me3) are involved in reduced gene expression, whereas methylation of histones, such as lysine 4 on histone H3 (H3K4me3) and acetylation of numerous lysine residues on histones H3 and H4 (acetyl H3 and acetyl H4) are associated with increased gene expression (959, 960).

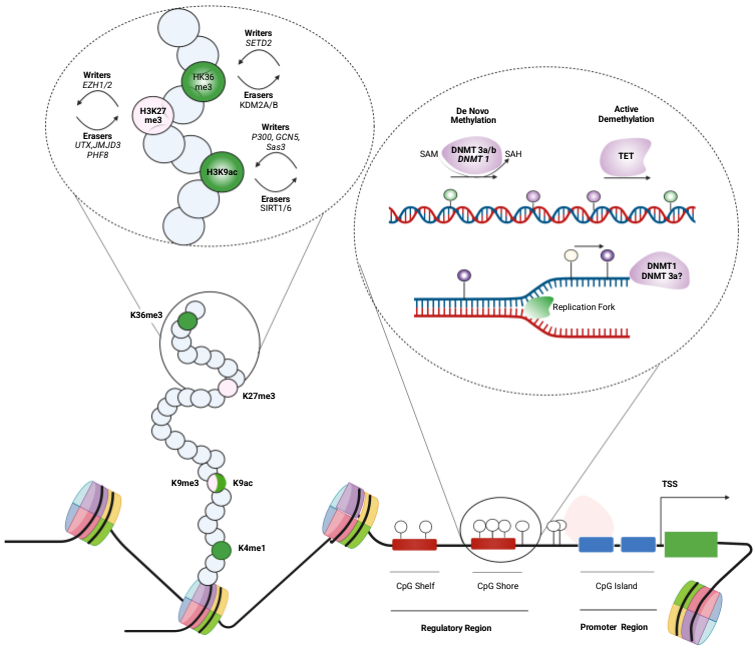


**FIGURE 11. Overview of epigenetic modifications as pre-transcriptional regulation of gene expression**

Lysine residues protruding from histone proteins are prone to a number of epigenetic modifications including methylation and acetylation. These are created via a set of “writers and erasers”. DNA methylation occurs in the CpG context, catalyzed via the de novo methyltransferases (DNMT) 3a/b, and copied through DNA replication via DNMT1 and possibly DNMT3a. Ten-eleven-translocase

(TET) enzymes convert methylation to hydroxymethylation, 5-formylcytosine, and 5-carboxylcytosine before undergoing passive or active removal causing demethylation. This figure for illustration purposes does not capture that methylation and demethylation can occur in other regulatory regions, promoter regions, and even intragenic (which is less studied). Further modifications exist to regulate transcriptional competency of the cell, including SUMOylation, phosphorylation, and ubiquitination (not depicted). Adapted from (67).

Specifically in relation to acute aerobic exercise, transient changes in histone acetylation status (214), and gene-specific changes in DNA methylation status (216), are rapidly induced, and precede changes in gene expression in the post-exercise period (214, 216). Deacetylation via the enzymatic activity of HDACs is a posttranslational modification of lysine residues on target histone proteins, and largely results in transcriptional repression (961). Class IIa HDACs can also exert transcriptional repression by direct interaction and inactivation of specific target transcription factors, in addition to recruitment of several distinct corepressors and/or protein-modifying enzymes that switch target transcription factors to their inactive form (961, 962). Phosphorylation of three serine residues ($Ser^{246}$, $Ser^{467}$ and $Ser^{632}$) plays a key role in modulating HDAC4 translocation by increasing 14-3-3 binding and leading to nuclear export and the de-repression of gene transcription (963). Serine phosphorylation of class IIa HDACs is regulated by upstream kinases such as AMPK and CaMKII (337, 858, 964-967). Class IIa HDAC phosphorylation increased in response to an acute session of MICE (214, 287, 645, 968) and repeated sprint exercise (570), but not low intensity aerobic exercise (287), or resistance exercise (645). Beyond phosphorylation events, an acute session of MICE increases nuclear export of HDACs, increases histone H3 acetylation, and reduces HDAC-mediated repression of exercise-responsive genes such as GLUT-4 and PGC-1$\alpha$ by reducing HDAC-MEF2 protein interactions (214, 231, 857, 858, 968). Therefore, the modulation of class IIa HDAC activity is proposed as a central component in the regulation of transcriptional processes in response to acute exercise (65).

Activation of transcription occurs with hypomethylation of GC-rich consensus binding sequences of DNA in specific genes, and has been observed for various exercise-responsive genes (216). More recently, we have observed the same pattern of hypomethylation induced by acute exercise in response to both resistance exercise (217, 218), and change-of-direction repeated sprint exercise (219). Our work extended these initial observations to a genome-wide analysis, and again, there was considerable overlap between genes that were transiently hypomethylated soon after exercise, and those that increased in mRNA abundance in the several hours of recovery that followed. Importantly, many of the genes identified as being hypomethylated are those that are established as important for the response and adaptation to different types of exercise. For example, with resistance exercise, gene pathways demonstrating a hypomethylated and upregulated expression profile includes those within

actin cytoskeletal, extracellular matrix and other growth-related pathways (218), whereas for SIE hypomethylation and upregulation of gene expression occurs in pathways such as MAPK, AMPK and insulin pathways (219). These studies also identified uncharacterized genes associated with the exercise response and the epigenetic memory of skeletal muscle in response to training, detraining and retraining (217-219). Pertinent examples included the gene UBR5 that was found to have enhanced hypomethylation and gene expression during retraining following an earlier period of resistance training (217). While previously uncharacterized in skeletal muscle, UBR5 has now been identified to be mechanically sensitive to load (666), and associated with muscle hypertrophy and recovery from atrophy/injury (603), whereas muscle-specific UBR5 knock-down evokes atrophy (969). These studies also demonstrated that epigenetic signatures can be retained over several weeks after both acute and chronic resistance exercise, and that these methylation signatures could be retained on some genes even during detraining when exercise had completely ceased, or further hypomethylated and gene expression increased during retraining if the exercise had been undertaken in the past (217). These observations suggest that these genes possess an epigenetic memory ('epi-memory') at the DNA level (507), and previous exposures to both acute and chronic exercise impact the response in skeletal muscle at the pre- and post-transcriptional level to future sessions of exercise.

Also noteworthy is that pre-transcriptional epigenetic modifications, at least to DNA methylation, that can be extremely rapid after exercise, detected e.g., 30 minutes after exercise and these precede the later changes in gene expression that are observed at 3 to 6 hours (217-219) (Figure 5). A possible mechanism is via metabolic perturbations regulating epigenetic modifications and influencing gene expression given the almost immediate changes in metabolite concentrations with exercise including $\alpha$-ketoglutarate, succinate, and fumarate, which are known to regulate lysine and DNA methylation activity via the DNMTs, TETs and HDAC enzymes (67).

Lastly, another recent observation is DNA hypomethylation occurring in response to acute exercise in mitochondrial DNA after resistance exercise in untrained, aged skeletal muscle (970). These changes somewhat restored the DNA methylation signature in the skeletal muscle mitochondria to profiles observed in trained younger adults (970). Studies investigating aerobic exercise and mitochondrial DNA methylation are warranted, where hypomethylation may be expected to occur in both trained and untrained muscle. Furthermore, resistance exercise, but not endurance exercise, can evoke hypomethylation of ribosomal DNA (rDNA) and several other ribosomal genes (646).

In summary, the molecular networks that link exercise to epigenetic regulation are presently a matter of intense research interest, with many metabolites and enzymes potentially

regulating acetylation/deacetylation and methylation/demethylation. Many of these enzymes are linked to metabolic pathways and signal transduction pathways that are established as being exercise-responsive (e.g. AMPK, CaMKII) and again therefore links acute exercise-induced signal transduction in skeletal muscle to regulators of epigenetic modifications (65, 67).

### *C. Post-transcriptional regulation of skeletal muscle gene expression via non-coding RNAs*

Given the discrepancies between mRNA and protein abundance described earlier, the relevance of post-transcriptional regulation by small RNA species, especially siRNA and miRNA, have become increasingly apparent. After their synthesis, miRNA form part of the RNA-inducing silencer complex (RISC), and confer specificity to the RISC complex by binding complementary sequences in their target mRNA, which results in either the degradation of the target mRNA or the inhibition of mRNA translation that ultimately results in changes in protein abundance (971). More than 2000 miRNA transcripts have been so far identified, it is estimated that up to 30% of human gene transcripts may be affected by this type of regulation. miRNA-induced alterations in protein abundance are subtle (often less than 10% change in abundance), but a single miRNA can alter the abundance of tens to hundreds of diverse proteins, meaning that changes in miRNA in response to stimuli such as exercise can have important implications when it comes to overall adaptive changes. To add further complexity, while one miRNA can have many targets, simultaneously one transcript may be targeted by several miRNAs.

Essential roles for miRNA in skeletal muscle for differentiation and development (972, 973), and the determination of fiber type (974, 975), coupled to the observations of changes in miRNA abundance during muscle atrophy (976, 977) and load-induced hypertrophy (978) has stimulated much interest in the potential contribution of miRNA to exercise training-induced adaptations in skeletal muscle (68, 69), and other tissues (979). Changes in miRNA abundance in human skeletal muscle occur in response to both acute aerobic (236, 237, 242, 980-982) and acute resistance (238-242, 983) exercise, and similarly in response to aerobic (53, 236, 237, 984) and resistance (985, 986) exercise training. For those miRNAs with altered expression after acute exercise, and their predicted target genes, pathways such inflammation, angiogenesis, and mitochondrial turnover have been implicated, but have rarely been directly investigated (68, 69). Conversely, exercise training studies have suggested that increased miR-19-3b abundance as an important regulator of skeletal muscle glucose metabolism (984), and divergent responses in miRNA abundance can be associated with the high responder classification for muscle hypertrophy (985, 986). At present, the complexity of miRNA-mediated regulation of target proteins and processes, and lack of mechanistic studies

of skeletal muscle miRNA in response to divergent types of acute exercise mean that despite these well-established effects of acute exercise and training to alter miRNA abundance, it remains unclear as to the exact role that acute changes in miRNA abundance subsequently have on changes in mRNA and protein abundance in the post-exercise period.

### *D. Coordination and control of dynamic mitochondrial turnover through biogenesis and mitophagy*

An increase in muscle mitochondrial number and volume termed mitochondrial biogenesis, as well as concomitant changes in organelle ultrastructure and composition, is recognized as a hallmark of MICT, HIIT and SIT (101, 182), but has also been observed in response to resistance exercise training (987, 988). In response to increased contractile activity, half-lives of approximately one week are observed for mitochondrial proteins and enzymatic activity (989, 990). For example when previously-sedentary humans undertook six weeks of MICT (5 days per week for 30 minutes at ~85% of maximal heart rate), increases of ~14% in $\dot{V}O_{2max}$ and ~40% in total mitochondrial volume density were observed (991), and are typical of results observed (255). Changes occur in all three fiber types with the difference being somewhat greater in type IIa than in type I and type IIx fibers (165).

The process of mitochondrial biogenesis is complex and highly-regulated, requiring the coordination and co-expression of both the nuclear and mitochondrial genomes for the assembly and expansion of the reticulum, and the generation of a dynamic mitochondrial network (101). Early work focused on the regulators of gene expression and centered on the role of PGC-1$\alpha$ as transcriptional coactivator and consequent recruitment and co-regulation of multiple transcription factors including MEF-2, NRF-1, NRF-2, estrogen-related receptor $\alpha$ (ERR$\alpha$) and mitochondrial transcription factor A (Tfam) as determinants of the expression of metabolic and mitochondrial genes (992-998). An essential facet to mitochondrial plasticity that most proteins that comprise the mitochondrial proteome are transcribed from nuclear-encoded genes prior to translation and translocation into the respective mitochondrial sub-compartments. For instance, nuclear-encoded mitochondrial proteins are chaperoned to the mitochondrion, imported into the different organelle compartments, and assembled with mtDNA-encoded proteins to form multi-subunit enzyme complexes required for oxygen consumption and ATP synthesis. Appropriate synchronization includes the transcription of nuclear genes, translation of newly-formed mRNAs and import of proteins into mitochondria, replication of mtDNA, transcription and translation of mitochondrial genes, biosynthesis of mitochondrial membrane phospholipids, and assembly of the enzyme complexes (101).

The last decade has seen a greater emphasis on mitochondria as a dynamic reticulum (999, 1000), and its regulation by mitochondrial fission-fusion dynamics (101), the

mitochondrial unfolded protein response, and mitochondrial quality control through mitophagy, the specific degradation, and recycling of dysfunctional mitochondria through the autophagosome-lysosomal system (107). Exercise training increases the ratio of fusion-to-fission proteins, promoting the fusion of mitochondria to form a reticular network. Mitofusin-1 (Mfn1) and -2 (Mfn2), and optic atrophy factor 1 (Opa1), are mitochondrial membrane proteins regulating the fusion process resulting in the generation of networks with continuous membranes and a matrix lumen, whereas dynamin-related protein 1 (Drp1) together with fission protein 1 (Fis1) mediate mitochondrial membrane fission. Fission events can be localized to specifically damaged areas of the reticulum resulting in the selective removal of damaged regions of the network, and therefore precedes apoptosis and is also coupled with autophagy/mitophagy.

Autophagy occurs through various mechanisms that differ in the way they capture proteins or organelles and deliver them to the lysosome for degradation (1001, 1002) (Table 2). TFEB has been viewed as a master regulator of lysosome biogenesis and therefore central to the regulation of autophagy through its role in determining lysosomal pool size and activity (1003-1005). In a series of muscle-specific overexpression and knockout experiments, TFEB was observed to influence lysosomal content and autophagy, mitochondrial biogenesis, and glucose metabolism, as well as TFEB overexpression augmenting mitochondrial gene expression in response to an acute aerobic exercise session (1006). Acute exercise and chronic contractile activity increase TFEB expression, subcellular localization and activity (1006-1008), which has been proposed to be under the influence of signaling pathways including AMPK, mTORC1 and calcineurin (1009). Altogether these data indicate that autophagic processes and lysosome plasticity are evident with exercise (71, 110, 1009), and may in fact be required for adaptation to aerobic exercise training (1010-1012). Several roles for the lysosome in anabolism in skeletal muscle have been observed, including the requirement for autophagy in the maintenance of skeletal muscle mass, the lysosome as the site of mTORC1 activation, and the importance of TFEB/lysosomal biogenesis-related signaling in mTORC1 activity and MPS (71).

Acute exercise, both aerobic and resistance, also impacts pathways associated with mitophagy in skeletal muscle, but whether the net effect is acutely increased or decreased mitophagy remains unclear and is likely to be time course-dependent (234, 292, 298, 748, 1013-1021). Like the investigation of many molecular pathways in human skeletal muscle, studying mitophagy with static measures of mRNA and protein markers is probably insufficient to quantify such a dynamic process given that changes observed could indicate either an increase in their synthesis or a decrease in their degradation and whole-cell lysate measurements do not allow for mitochondria-specific conclusions to be made (101). When

measured in resting samples, the capacity for autophagy and mitophagy regulation is increased in human skeletal muscle by exercise training (1017, 1020, 1022). Therefore, given the immediate and transient changes in these processes in the post-exercise period, the current model suggests that in addition to transcriptional regulation, acute exercise-induced signal transduction pathways initiate turnover of the mitochondrial pool within skeletal muscle in a coordinated process of removal of dysfunctional mitochondria, in collaboration with the activation of biogenesis (101).

### *E. Translational efficiency, translational capacity, ribosome biogenesis and satellite cell accretion*

Rates of cellular protein synthesis depend on translational *efficiency* (protein synthesis per unit amount of mRNA) and on translational *capacity* (i.e., ribosomal content per unit of tissue) (1023). While the former, as described by MPS, was the major focus of Section 2.E, both mechanisms contribute to coordinated changes in abundance of individual proteins in response to exercise (70, 114). Much of the focus on translational capacity has been on ribosome biogenesis, which in this context refers to the *de novo* synthesis of ribosomes, a process that involves the transcription and processing of ribosomal RNA (rRNA) and the assembly of several ribosomal proteins, as reviewed in detail elsewhere (1023, 1024). Several lines of evidence point to increased translational capacity because of exercise training-induced ribosome biogenesis as an important regulator of the adaptive response to exercise (70, 1023).

Repeated sessions of resistance exercise training result in an increase in total cellular RNA concentration due to the accumulation of mature rRNAs (244)(250, 326, 1025, 1026). A time course analysis revealed that these increases are greatest early (first four sessions) in a training intervention before plateauing somewhat up to session 12 and declining markedly when training was ceased for 8 days (326). The change in muscle mass in response to resistance exercise training is in turn positively correlated with the increase in translational capacity (250, 326, 879, 1025) and an increase in resting rates of MPS (250). Indeed, the often-observed increase in resting rates of MPS after resistance exercise training (246-251) is proposed to reflect an increase in translational capacity (245, 1023), given the relationships between RNA synthesis and content, and MPS and muscle hypertrophy (1027-1030). Lastly, changes in indicators of *de novo* ribosome synthesis are correlated with the magnitude of hypertrophic response and/or the identification of low or high responders to resistance exercise training in both rodents (1031-1033) and humans (326, 872, 879, 1025, 1026, 1034).

*De novo* ribosome synthesis in response to acute exercise is often interpreted from 45S pre-rRNA abundance, and phosphorylation of TIF-IA and UBF. These factors are

sensitive to regulation by energy and nutrient availability, hormones and growth factors, effects that are largely mediated by the AMPK, MAPK, and mTORC1 signaling pathways (1035). Acute resistance exercise robustly increases 45S pre-rRNA abundance (57, 250, 646, 1025, 1031, 1036-1038), with a time course of increase evident from 2 to 48 hours after exercise (646, 1036). Phosphorylation of UBF and TIF-IA was observed to be transiently increased after acute resistance exercise (1025, 1036, 1037), in both the untrained and trained state (1025). However, an acute session of concurrent aerobic and resistance exercise did not result in an increase in UBF or TIF-IA phosphorylation despite observing increases in total RNA content and abundance of some mature rRNA species with exercise training (1037). While studies of aerobic exercise are limited to date, two studies have suggested that signaling to ribosome biogenesis is unchanged or decreased in the post-exercise period (646, 722), which would be unsurprising given the proposed negative regulation of ribosome biogenesis by AMPK (1035). Time course studies will be required to further explore this effect given that even resistance exercise leads to a transient decrease in markers of ribosome biogenesis in the first hour after exercise (1025), and potentially divergent early and late responses between acute aerobic and resistance exercise (646). A recent speculation is therefore that acute increases in rDNA transcription is a feature specific to hypertrophic adaptations, and is positively related to the available rDNA template, which itself may have a genetic predisposition that influences hypertrophic potential (646).

Other nodes exist in the regulation of muscle protein turnover including regulation of protein degradation (117), myonuclear domain size and myonuclei number (70, 1023) and satellite cell function (activation, proliferation, differentiation, survival). While the role of skeletal muscle satellite cells in post-natal growth and regeneration is well-established, the role of satellite cells in hypertrophy and exercise adaptations in adult skeletal muscle continues to be debated (70, 1039-1041). Their proposed role in muscle hypertrophy revolves around the concept of a myonuclear domain – a theoretical volume of cytoplasm associated with and transcriptionally governed by a single myonucleus – and each myofiber being composed of many myonuclear domains (1042). Satellite cells provide a source for new myonuclei at a rate sufficient to maintain a constant myonuclear domain size during normal skeletal muscle growth, thereby increasing the total number of myonuclei, and thus the total amount of genetic machinery available for mRNA and protein production with little alteration in the kinetics of protein synthesis for each nucleus.

Under such a framework, satellite cell-dependent myonuclear accretion would be obligatory for adaptive hypertrophy of skeletal muscle fibers. Nevertheless, in various experimental models, including satellite cell depletion (1043-1045) and myostatin inhibition (1046), skeletal muscle fiber hypertrophy is observed without the incorporation of satellite

cells. For example, in skeletal muscle with 90% of the satellite cell population depleted, overload-induced muscle hypertrophy is normal (1043), as is regrowth after disuse atrophy (1047), whereas regeneration from acute muscle injury is impaired (1048), suggesting that the role of satellite cells is markedly different between the various growth and repair paradigms (1041). For adaptive hypertrophy, at least at the onset of a new contractile stimulus in rodent models, existing myonuclei possess the capacity to meet the new transcriptional demands and also to support ribosome biogenesis (1044, 1049).

Whether these models of adaptive hypertrophy producing supra-physiological gains in muscle size in a short timeframe adequately represent human adaptation is again worthy of a caveat (Section 3). Other considerations are whether hypertrophy over a longer timeframe and/or in later life would require the incorporation of satellite cells (1050-1052), and whether hypertrophy can be *optimal* in the absence of this effect (1053-1055). Again using the high responder approach, large ranges of inter-individual variability in the magnitude of hypertrophic response to resistance exercise training in human skeletal muscle are explained by the relative ability to mobilize satellite cells and add myonuclei to existing muscle fibers (1053, 1054). While the number of myonuclei per muscle fiber at baseline may be an important determinant of that phenomenon and may reflect training status (1056), myonuclei addition is generally greater in proportion to the degree of muscle hypertrophy (1057). Like acute resistance exercise, aerobic exercise does increase the activity and number of satellite cells (1058), which may contribute to aerobic adaptations in skeletal muscle to exercise training (1059), but overall this effect remains unclear and requires future investigation (1041).

### *F. Regulation and role of muscle protein degradation*

Despite the heavy emphasis to date on the regulation of MPS in the context of exercise adaptations, protein degradation is a central feature of proteostasis (117) (Figure 12). As an overarching model, acute exercise activates signal transduction pathways that increase the turnover of skeletal muscle proteins (i.e. activation of both degradation and synthesis (259, 261-263, 272, 273), and this increase in turnover is essential to remodeling of cellular components such as contractile proteins and the extracellular matrix that occurs with exercise training. One critical role for muscle protein degradation in this context is proposed as better clearance of damaged and misfolded proteins (117). Muscle protein degradation can occur via at least three processes, namely: (i) autophagy-lysosomal pathways already briefly described in Section 6.D, (ii) cytosolic proteolytic systems and (iii) the ubiquitin-proteasome system (UPS). The cytosolic proteolytic systems include the caspase-3 (1060), and calpain proteases (1061), which are associated with apoptotic cell death and $Ca^{2+}$-activated proteolysis, respectively. These systems are not strongly implicated in exercise-induced protein turnover *per se*, but may instead have roles in muscle remodeling in response to

muscle damage (1061). The majority of intracellular proteins are degraded by the UPS (1002)(1062)(1063) (Table 2).

Two muscle-specific E3 ligases are muscle RING finger 1 (MuRF1) and muscle atrophy F-box (atrogin-1/MAFbx), which were first discovered in models of muscle atrophy (1064, 1065), and are key regulators of skeletal muscle proteolysis under catabolic conditions (1063). The role of the UPS in skeletal muscle atrophy is well-established, but whether this pathway has an important role in the adaptive response to exercise is presently unknown. For example, expression of MuRF1 and MAFbx specifically has been observed to be either increased, unaffected or decreased in response to various forms of acute exercise (102). However, the general trend that MuRF-1 mRNA abundance is induced by acute sessions of both aerobic and resistance exercise suggests that proteolysis is involved in skeletal muscle remodeling during exercise training (71, 1060). One challenge in human skeletal muscle has been the lack of accessible and reliable methods to measure the physiological consequence of these pathways i.e. muscle protein degradation (117), but improving and emerging methods to explore such outcomes should provide greater insights (117, 945, 1030, 1066).

The regulation of MuRF1 and MAFbx at a transcriptional level occurs via a number of factors with putative binding sites in their respective gene promoters include FOXO1, FOXO3a, NF-κB, CCAAT/enhancer-binding protein-β (C/EBPβ) and Smad3 (1063), but presently it remains unclear which of these pathways may be responsive to acute exercise-induced signal transduction beyond the general point that mTORC1 signaling coordinately regulates UPS and autophagy (106, 1002). More broadly, activation of the 26S proteasome has been demonstrated to be mediated by cAMP-dependent PKA-mediated phosphorylation of the proteasome subunit PSMD11/Rpn6 (1067, 1068). Short-duration (~9 to 11 minutes) severe intensity (cycling at ~85 to 92%$W_{max}$) aerobic exercise increases PSMD11/Rpn6 phosphorylation (948, 1068), and activates proteasomal activity resulting in a concomitant, but transient, reduction in global protein ubiquitination (952, 1068). To add further complexity, this exercise session also increased the modification of NEDD8 E1/E2 ligases resulting in the rapid activation of protein NEDDylation (952), and suggested a role for protein NEDDylation in acute activation of the UPS during acute exercise in the severe intensity domain. The working hypothesis is that the rapid degradation and elimination of either pre-existing or damaged/misfolded proteins during and after exercise could synergize with translational activation to promote cellular adaptation (1068).

Other E3 ligases potentially also regulate muscle protein degradation given that there are more than 600 different E3 ligases encoded by the human genome. Interestingly, our recent work has identified the E3 ligase UBR5 as a novel regulator of skeletal muscle mass (217, 603, 969). Of particular note is that UBR5 activity is in contrast to MuRF1 and MAFbx in

that its expression is associated with anabolism and hypertrophy, is correlated with skeletal muscle mass, and muscle-specific UBR5 knockout attenuates anabolic signaling resulting in muscle atrophy (217, 603, 969). These findings suggest that the role of the UPS in the regulation of skeletal muscle adaptation is more complex than simply being a protein degradation pathway leading only to atrophy. Instead, dual roles are likely to exist for the E3 ligases via degradation of either positive and negative regulators of muscle mass resulting in muscle atrophy and hypertrophy, respectively.

On a related point, the importance of protein degradation in the control of overall protein abundance has been demonstrated using proteomic analysis of extensor digitorum longus muscle isolated after unilateral chronic low frequency electrical stimulation (10 Hz, 24 h/d) of the rat hindlimb (262). Contraction-induced changes in protein degradation contributed to the adaptation of the proteome equally as much as MPS, and varied across the 30 day time course when analyzed on a protein-specific basis. One striking example was that during the first 10 days of stimulation, a decrease in degradation rate accounted for 82% of the increase in the abundance of ATP synthase $\beta$ (262). The further exploration of such mechanisms of protein turnover, and whether these manifest in interventions that more closely mimic human exercise training will be of considerable interest.

Lastly, another aspect of proteostatic control is endoplasmic reticulum stress and the unfolded protein response ($UPR^{ER}$) (1069), which has been implicated in the regulation of skeletal muscle adaptation (1070). The initial observations in pre-clinical models were that the $UPR^{ER}$ was activated in skeletal muscle of mice by a single session of treadmill running, and this response was attenuated, but not absent, after a period of aerobic exercise training (1071). Recovery from a single session of aerobic exercise was impaired in ATF6$\alpha$ KO mice, whereas attenuating ER stress through deletion of CHOP partially rescued the exercise intolerance exhibited by PGC-1$\alpha$ mKO mice (1071). In human skeletal muscle, studies are limited but a variety of forms of acute exercise have been observed to transiently increase markers of $UPR^{ER}$ in the post-exercise period after ultra-endurance exercise (1072), and unaccustomed resistance exercise (719, 1073, 1074). Although data are presently limited and further mechanistic studies are required, because the acute increase markers of $UPR^{ER}$ is attenuated after a period of exercise training, and changes in these markers do not correlate with muscle hypertrophy (719), $UPR^{ER}$ may be more important for maintaining skeletal muscle homoeostasis than regulating adaptations (1070).

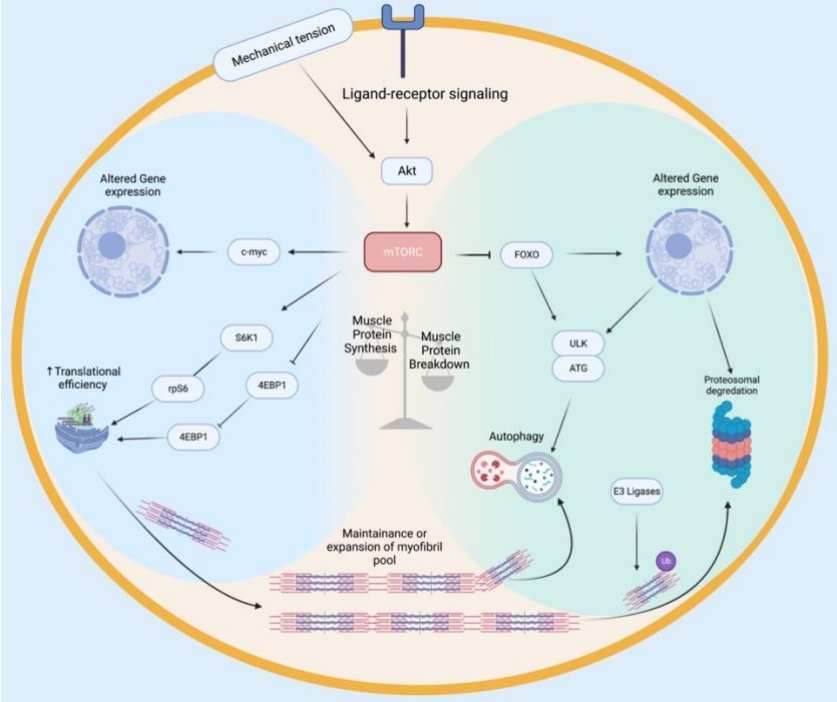


**FIGURE 12. Overview of pathways of muscle protein synthesis and muscle protein degradation in the regulation of proteostasis**

Acute exercise activates signal transduction pathways that increase the turnover (both synthesis and degradation) of skeletal muscle proteins that is essential to remodeling of cellular components. Rates of cellular protein synthesis largely depend on translational efficiency and on translational capacity. Muscle protein degradation occurs via autophagy-lysosomal pathways, cytosolic proteolytic systems, and the ubiquitin-proteasome system. See text for further details.

## 7. EFFECT OF TRAINING STATUS OR A PERIOD OF EXERCISE TRAINING ON THE MOLECULAR RESPONSE TO ACUTE EXERCISE

The molecular responses described up to this point in the review have largely been measured in untrained participants, or training status has not been an independent variable in these studies. However, given that neuronal, mechanical, metabolic, and hormonal responses to acute exercise are influenced by training status and are modulated by a period of exercise training, it is unsurprising that the molecular response to acute exercise is also markedly influenced by the same. If perturbations to homeostasis are essential stimuli and signals to activate and/or repress the molecular pathways that induce adaptive responses to exercise, then as “fitness” improves, by definition, the body is better able to maintain homeostasis in the face of the challenge provided by exercise. Therefore, over time with repeated exercise sessions, perturbations to homeostasis are less pronounced (e.g. changes in [AMP], [ADP] and [lactate], depletion of muscle glycogen, degree of muscle damage), and therefore, the molecular responses to acute exercise are likely to be attenuated.

### *A. Exercise training attenuates the metabolic stress of acute exercise*

Considering the metabolic response to acute exercise, exercise training-induced increases in mitochondrial density, and enzyme activities involved in lipid oxidation result in enhanced respiratory control sensitivity such that a lower [ADP] is required to achieve the same level of oxygen consumption per gram of skeletal muscle (142, 1075). Thus, the same cellular rate of oxidative metabolism is attained with less perturbation of adenine nucleotides and a lower rate of oxidative phosphorylation per mitochondrion. After a period of exercise training, smaller declines in [ATP] and [PCr], and smaller increases in free [AMP] and [ADP] are observed at the same absolute power output in skeletal muscle with increased mitochondrial density (1075, 1076), but are also evident with short-term training interventions in which a change in mitochondrial density would not be expected (741, 822, 1077). These changes attenuate the increase in [AMP]/[ATP], and reduce the formation of IMP, $P_i$ and ammonia, and thereby attenuate the allosteric regulation of the rates of glycogenolysis and glycolysis (and associated lactate production) (142, 143). Similarly, the [$NAD^+$]/[NADH] ratio is increased at rest, and the decline during exercise is attenuated, after a period of exercise training (740-742). A reduced accumulation of metabolites that would otherwise activate PHOS and PFK thereby reduces reliance on glycolytic pathways concomitant with an increase in the proportion of ATP provision from oxidative metabolism (142, 143).

While most studies of this nature have focused on the metabolic response to aerobic exercise after aerobic exercise training, similar metabolic adjustments (reduced PCr degradation and glycogenolysis, and an attenuated increase in muscle lactate concentration) have been observed after 4 weeks of resistance exercise training (194). SIT is a potent stimulus to increase skeletal muscle oxidative capacity as indicated by the maximal activities and protein content of various mitochondrial enzymes including citrate synthase, cytochrome oxidase and succinate dehydrogenase (196-198), which can increase by as much as ~30% after two weeks of SIT (157, 199). Consequently, metabolic adjustments during exercise resemble those associated with traditional MICT such as reduced PCr and glycogen utilization during 60 minutes of MICE at 65%$\dot{V}O_{2max}$ (158), and a higher active fraction of PDH and reduced muscle lactate accumulation during MICE (1078). Enhanced respiratory control sensitivity results in a reduction of carbohydrate utilization in the trained state, in addition to the overall capacity for lipid oxidation being markedly increased (151, 152, 160). The reduction in carbohydrate utilization during exercise in the trained state is therefore compensated for by a proportional increase in rates of lipid oxidation at the same absolute and relative intensity (142, 143, 1079). Thus, increased endurance performance observed after a period of exercise training is attributed to enhanced fatigue-resistance by virtue of reduced muscle glycogen

depletion, tighter coupling of ATP supply and demand, and thereby, smaller disturbances to homeostasis combined with a consequent reduction in metabolic by-products.

Similarly, the general pattern in the trained state or after a period of exercise training is attenuation for most of the exercise-induced increases in circulating hormones (370, 385, 408), which again largely reflects the attenuated physiological demands of exercise after a period of exercise training. The effect of exercise training on the nature of $[Ca^{2+}]_i$ transients is not well-described owing to the difficultly of measuring these parameters in human skeletal muscle *in vivo*. However, the potential for exercise training-induced changes in intracellular $Ca^{2+}$ handling is evidenced by changes in SERCA isoforms and the release and uptake of $[Ca^{2+}]_i$ by SR vesicles in trained skeletal muscle, which together are consistent with reduced $Ca^{2+}$ cycling kinetics (145, 1080-1083). The net effect of these changes in $Ca^{2+}$ handling is proposed to result in increased efficiency of contractile activity and better preservation of cellular energy homeostasis due to a more favorable balance between the utilization of the ATP by SERCA and synthesis of ATP (145).

### *B. Longitudinal studies using exercise training interventions and cross-sectional studies of individuals with divergent training status*

The effect of exercise training on neuronal, mechanical, metabolic, and hormonal responses to acute exercise, and therefore the consequent molecular responses, are typically explored by comparing responses between groups of trained and untrained individuals (cross-sectional designs), or by studying the same group of individuals before and after a defined exercise training intervention (pre-post designs). A critical consideration that arises is the difference between the *absolute* and *relative* exercise intensities at which the acute exercise session is performed. For pre-post designs comparing acute exercise responses before and after a period of exercise training, the post-training exercise session performed at the same absolute intensity (i.e. the same power output or load lifted as the session performed before commencing training) will result in a relative exercise intensity (as % of pre-training $\dot{V}O_{2max}$ or 1RM) that is less than the pre-training exercise session. In this scenario, neuronal and mechanical stimuli may be relatively well-matched, but metabolic and hormonal stimuli will differ considerably between pre- and post-training timepoints due to the post-training response being assessed at that lower relative intensity compared to *pre*-training. Alternatively, the training effect on $\dot{V}O_{2max}$ or 1RM can be assessed at the end of the exercise training intervention, and the post-training exercise session can be prescribed based on % of *post*-training $\dot{V}O_{2max}$ or 1RM. In this scenario, acute responses before and after a period of exercise training are compared at the same relative intensity.

A number of studies have compared the molecular response to acute exercise before and after a period of exercise training, with the post-training exercise session performed at the same absolute intensity (822, 943, 1084-1090), or the same relative intensity (51, 55, 60, 61, 317, 641, 1091-1095), as the pre-training exercise session, whereas others have used maximal efforts in the acute exercise session (570, 1096, 1097). The general trend from these studies is that that the magnitude of response is attenuated after a period of exercise training for outcomes such as activation of signal transduction pathways and/or changes in mRNA abundance. For example, the acute exercise-induced AMPK activity/phosphorylation (822, 943, 1093) or increase in PGC-1$\alpha$ mRNA abundance (317, 641, 1086, 1087, 1089, 1095) have been repeatedly shown to be attenuated whether the post-training exercise session is performed at the same absolute or relative intensity as pre-training. However, not all molecular responses are attenuated e.g. the increase in phosphorylation of CaMKII, p38 MAPK and mTOR were similar before and after 10 days of intensified aerobic training despite the acute exercise session being performed at a lower relative intensity (~72% vs. ~64% pre-training $\dot{V}O_{2max}$) (943). Similarly, 12 weeks of resistance exercise training did not result in the attenuation of either increases in STAT3 phosphorylation and mRNA abundance of c-MYC, c-FOS or SOCS3 (1096), or the increases in markers of satellite cell activation and myogenesis (1094), in response to acute resistance exercise. On a broader scale, the skeletal muscle transcriptome response to either acute aerobic (51, 61) or resistance (60) exercise at the same relative intensity before and after a period of exercise training generally displays an altered response as either attenuation or complete ablation of changes in acutely-responsive genes.

An alternative approach is a cross-sectional design in which it is most common to assess responses based on comparisons made at the same *relative* intensity i.e. groups of untrained and trained (or well-trained) individuals performing exercise sessions that are matched on the basis of %$\dot{V}O_{2max}$, %$W_{max}$ or %1RM (80, 213, 832, 1098, 1099). However, due to the effects of long-term exercise training, this often means that the absolute intensity (e.g. power output or load lifted) is much greater in the exercise session of the trained group, and therefore the neuronal and mechanical stimuli may still differ considerably between the groups. Another issue is that even when exercise sessions performed by different individuals/groups are matched for %$\dot{V}O_{2max}$ or %$W_{max}$ as a broad measure of physical fitness, there can remain considerable heterogeneity in the metabolic response to exercise performed at the same relative intensity (1100, 1101). Specifically, carbohydrate utilization and cardiovascular stress are greater in individuals who have a lactate threshold occurring at a lower %$\dot{V}O_{2max}$ (1100, 1101), but these responses are more similar when the relative exercise intensity is matched as a percentage of lactate threshold (1101). In fact, the case for using

lactate threshold as a means to better match exercise sessions for intensity has recently been elegantly made (120). With lactate threshold being influenced by a myriad of factors including fiber type composition, and abundance of glycolytic and oxidative enzymes, it is logical that perturbations to homeostasis will also be heterogenous when individuals with divergent training status are matched for $\%\dot{V}O_{2max}$ or $\%W_{max}$.

A related study design is the unilateral exercise model, where one leg or arm is trained for a period of time, and after which a single session of exercise is performed in both limbs and the acute responses in the trained limb compared to the untrained limb is analogous to a cross-sectional design (1102). Again, an experimental design dilemma exists in how to match the limbs for intensity, with examples of matching by both absolute (300, 605) and relative (54, 57, 936, 1103) intensity being evident. These design considerations notwithstanding, like the data from the longitudinal studies described above, the general pattern is the attenuation in trained individuals or trained limbs of the acute exercise-induced changes in signal transduction and/or mRNA abundance (54, 57, 80, 213, 605, 832, 1098).

Lastly, an innovative approach previously used was to recruit individuals from diverse training backgrounds (i.e. well-trained endurance and strength athletes) and have them complete acute exercise sessions of aerobic or resistance exercise on separate occasions i.e. a session each of their habitual or an unaccustomed exercise type (668, 1104). The aerobic and resistance exercise sessions were 1 hour cycling at $70\%\dot{V}O_{2max}$, and eight sets of five maximum effort repetitions of isokinetic leg extensions, respectively, with the power output during aerobic exercise being much greater (242±11 vs. 168±10 W) in the endurance athletes whereas the force output during resistance exercise was much greater (263±10 vs. 190±4 N) in the strength athletes. Overall the data demonstrated that the abundance of selected metabolic genes responded similarly to exercise regardless of type or training background (1104), whereas an ~8.5- to 10-fold increase in PGC-1$\alpha$ mRNA abundance occurred with aerobic, but not resistance, exercise regardless of training background (668). Abundance of selected myogenic genes were increased to a greater extent in the endurance athletes (1104). However, it should be noted that resting mRNA abundance in these different athletes does differ considerably e.g. increased expression of gene clusters related to mitochondrial and oxidative capacity in endurance athletes (1105). For signal transduction, AMPK and p38 MAPK phosphorylation increased only in response to each group's unaccustomed exercise type (668), so the suggestion was that a degree of "response plasticity" remains even after many years of training in a specific type of exercise (668).

### *C. Insights from the effect of exercise training interventions or training status on the molecular response to acute exercise*

While the various methodological issues described preclude firm conclusions to be made, the interpretation of these studies in this Section can be summarized as follows. The general pattern for the attenuation of the molecular response to acute exercise is consistent with a reduction in the metabolic stress of acute exercise after training, and consistent with the model linking perturbations to homeostasis and the nature of the molecular response to acute exercise. Through a metabolic lens, in addition to enhanced respiratory control sensitivity, the increase in resting muscle glycogen concentration in response to exercise training is another candidate in this response given the inverse relationship between muscle glycogen concentration and the activation of signal transduction and transcriptional responses (128). Through a broader phenotypic lens, an attenuation in the molecular response is typically viewed as consistent with the observation that plateaus in the adaptation to exercise training occur with parameters as they reach their physiological limits, which has been referred to as a “reduced scope of plasticity” (51). In other words, at the same relative level of effort, trained skeletal muscle requires a greater stimulus to activate adaptive processes via these molecular pathways. Therefore, features of the molecular response that are not attenuated by exercise training may be important for maintaining the necessary restorative responses after individual exercise sessions, but are not necessarily (or only partly) involved in the regulation of adaptation to exercise training. These short-term regulatory adjustments have been referred to as “accommodation”, as opposed to the longer term adjustments referred to as training adaptations (1106).

One explanation initially proposed for the attenuated transcriptional response to acute exercise was that there was an increase in resting mRNA abundance of acutely responsive genes (51). While there are many genes whose resting mRNA abundance is increased after a period of exercise training (49-53, 58, 61), and many genes have elevated resting mRNA abundance in the skeletal muscle of trained athletes (322, 1105, 1107-1109), there are obvious cases of acutely-responsive genes that show no evidence of accumulation of resting mRNA abundance (317, 318). Another explanation may be that diminished molecular responses after training are also reflective of better efficiency of signal transduction and gene expression processes. One example is that increases after a period of training in the resting phosphorylation status of a variety of signaling proteins (249, 1110) has been proposed as indicating a heightened state of responsiveness of anabolic signaling (249). The net effect would be more rapid and/or efficient activation and deactivation of anabolic processes in the trained state. Similarly, a trained muscle can retain “muscle memory” through epigenetic changes including DNA hypomethylation, with a change in gene expression then occurring

more efficiently, and sometimes to an even greater magnitude, when returning to exercise training after a period of detraining (217, 218). Ultimately it may be that acute exercise-induced changes in mRNA abundance are multidimensional and evade broad summary statements (Figure 6) e.g. some, but not all, genes respond to acute exercise, and some genes show either an augmented or attenuated response following a period of exercise training, whereas some genes may be acutely-responsive only in the trained state.

### *D. First bout effects, repeated bout effects and their implications for understanding adaptive processes in response to exercise training*

Lastly, an alternative explanation is that acute molecular response to a single exercise session in the untrained state produces a generalized response in many molecular pathways, which is largely indicative of a stress response in untrained skeletal muscle, but that over time, this response is blunted and/or refined as the muscle adapts to regular exercise (51, 54, 57, 60, 61, 320, 1090, 1097, 1111, 1112). This principle can be illustrated with as little as two resistance exercise sessions performed 48 hours apart resulting in differential expression of many genes in the post-exercise period when comparing the first and second session (320, 1111). Another example is from short-term MICT wherein a ~28% decrease in mtDNA/nDNA ratio, which is indicative of acute stress and a signal for mitochondrial adaptation, was present after one session of 1 hour of cycling at ~80%$\dot{V}O_{2max}$, but was absent after the third exercise session onwards in the training intervention (318). A final examples is that despite the relative intensity of training sessions being maintained throughout a seven session HIIT intervention, an attenuation of the acute PGC-1$\alpha$ mRNA response was observed to be progressive with each subsequent session throughout the 14 day period (317).

A phenomenon that may partially explain these observations has been long-established in exercise science as the “repeated bout effect”, which refers to the adaptation to a single session of unaccustomed eccentrically-biased exercise that confers protection against muscle damage from a subsequent session of similar activity (1113). This protection is evident within seven days (1114), and can last for up to six months (1115). The mechanistic underpinnings are stated as neural, mechanical and cellular in nature (1113), the latter of which is proposed to reflect inflammatory adaptations and attenuated myofibrillar disruptions (1116). Therefore, it is unsurprising that resistance exercise training-induced attenuation of acutely-responsive genes has consistently been observed in relation to gene clusters associated with the stress response and immune activation (54, 57, 60, 1097), and can be observed after just two exercise training sessions (320). A similar observation has been made when comparing the acute response to MICE that was either eccentrically- or concentrically biased (downhill versus uphill running, respectively), and performed before and after two weeks of exercise training in the respective types of exercise (1090). The overall acute

transcriptional response was attenuated in both types of running after the training intervention and suggestive of a habituation effect, yet the response in terms of molecular functional profile remained divergent between the types of running and suggestive of important regulatory responses being maintained and specific to exercise type (1090).

Given that many exercise studies employ cycling exercise, which has a very low contribution from eccentric contractions, the repeated bout effect is admittedly an incomplete explanation of attenuated molecular responses to MICT, HIIT and SIT. An analogous term may be to refer to this phenomenon as the “first bout effect” (182). This can be conceptualized as a broad, non-specific molecular response to an acute (first) exercise session in individuals who are unaccustomed to performing exercise in general, or unaccustomed to performing exercise of a certain type, but this response is then attenuated, or becomes more specific or refined, with subsequent exercise training sessions. Therefore, some caution is warranted when extrapolating long term adaptive responses from responses to acute exercise i.e. molecular responses to a single session of exercise in an untrained individual, or early in a training intervention may not necessarily reflect or predict the actual adaptive response taking place during the subsequent period of training (1117, 1118). A correlation or continuity between the acute molecular response and the subsequent training outcome or expected phenotype change is often assumed, and while this is observed in some cases (317, 1119, 1120), there are obvious exceptions to this assumption depending on the parameter of interest (298, 317, 695, 804, 1097, 1121).

This observation is perhaps most evident when resistance exercise-induced increases in MPS acutely over a few hours after a single exercise session do not always correlate with eventual changes in muscle hypertrophy with prolonged exercise training (1122-1124). This discordance has been the subject of some debate (1125-1128), and may be explained by an acute (first) session provoking a major perturbation to muscle homeostasis resulting in muscle damage and inflammation that is captured in the overall mixed MPS response (1124), but with subsequent training, there is a dampening of the increase in synthesis of non-myofibrillar proteins while maintaining the responsive synthesis of myofibrillar proteins (i.e. myoPS) (248). In other words, the attenuated acute exercise-induced increase in MPS in the trained state (248, 249, 1124, 1129), and early in a training intervention (243), reflects a more refined response and is better correlated with eventual muscle hypertrophy than the increase in MPS after the first session (1124, 1130). These observations are also consistent with a greater acute response of MPS to unaccustomed eccentric-based exercise (greater muscle damage) compared to a work-matched session of concentric exercise (1131, 1132), and the aforementioned exaggerated response of gene clusters involved in structural protein

remodeling, the UPS, stress responses and inflammation to an initial exercise session that are attenuated with further exercise training (54, 57, 60, 1090, 1097).

Similarly, it may be that when previously-sedentary or recreationally-active individuals commence *any* intensive exercise training intervention, the early adaptive responses are largely generic, and the true specificity of the training effect, and its molecular regulation, would only be revealed later in the intervention. If investigation of acute responses in untrained individuals is perhaps leading to erroneous interpretations, then one recommendation would be that short period of habituation to exercise training is warranted in future study designs in order to better capture molecular responses to acute exercise relevant to adaptation rather than a generalized stress response (182). For example, such an approach has been recently implemented as a four week "normal volume training" block prior to undertaking an acute exercise session and a 20 day, twice-a-day HIIT intervention (1089).

These points suggest that the generally poor correlation between acute responses and chronic adaptive changes has a physiological basis (1117, 1118), and that it is pertinent to also acknowledge several methodological issues that may influence the interpretation of these data such as limited number of sampling time points and lack of adequate control of confounding variables (Section 4). Another issue is the utility of using acute or chronic changes in mRNA abundance as outcome variables given the variability in mRNA changes to predict changes in biological function, or to directly inform molecular mechanisms of regulation (182, 1118, 1133-1135). Although changes in mRNA abundance provide important insights into physiological responses, the multiples levels of regulation of protein degradation and translation, mean that changes in protein abundance are not easily predicted from these changes, much less changes in phenotype in the longer term (275, 277, 279-283).

Moreover, a recent study has suggested a large degree of intra-individual variability exists in baseline and acute exercise-induced changes in mRNA abundance in skeletal muscle (1136). Although group mean values for baseline abundance and acute changes in PGC-1$\alpha$, PDK4, VEGF-A, HSP72 and p53 mRNA abundance induced by MICE (30 minutes at ~65%$W_{max}$) were repeatable between two identical exercise sessions undertaken between two and four weeks apart, individual changes in mRNA abundance were not repeatable (ICCs <0.22; CVs >20%), and baseline values also demonstrated high variability (CVs ~11-33%). Little of this variability was attributable to technical error in qPCR analysis, although differences in fiber type profile of the respective biopsies may have contributed somewhat to technical error (1136), i.e. inherent to variations in muscle biopsy methodology as described in Section 4.D. The majority of the intra-individual variability was attributed to random error present due to day-to-day biological variability and inherent stochastic nature of gene expression (1137), and suggests that the use of mRNA abundance as a biomarker of adaptive

potential and/or as an indicator of individual responsiveness to a specific exercise protocol is perhaps limited (1136). However, as described in Section 6.B, epigenetic mechanisms, specifically DNA methylation, can be acutely responsive to exercise and even after a single session some of these changes are retained several weeks later, which may subsequent impact gene expression when acute exercise is repeated (217). This may be another contributor to the lack of repeatability in gene expression between sessions of exercise performed a few weeks apart.

## 8. APPRAISAL OF EXISTING KNOWLEDGE AND FUTURE DIRECTIONS

### *A. Appraisal of existing knowledge on the mechanistic basis for exercise training-induced adaptations in skeletal muscle*

Throughout this review, we have attempted to describe in detail what is presently known about the molecular mechanisms that regulate changes in transcription and translation, and how these mechanisms can be activated and/or repressed by a single session of exercise, which over time with repeated exercise can result in adaptive changes known as training effects. However, there are clearly many caveats to what is known and how it might be interpreted, while there are now hundreds of proteins implicated in the regulation of adaptation in skeletal muscle to exercise, it is probable that many more remain to be discovered, all of which serves to illustrate the incompleteness of our knowledge as a field.

In an appraisal of the state of existing knowledge, it is worth revisiting some key challenges described throughout this review. The first is that acute exercise represents a complex stimulus in that there are many stressors, most of which are transient, resulting in many downstream events, some of which are simultaneous, and others that are sequential. Ultimately, there is an amazingly complicated and interlinked network of regulatory proteins and pathways contributing to transcriptional and translational events in response to acute exercise. Depicting these networks as comprising of pathways that are linear and complete, and thereby allows for deduction of cause-and-effect relationships, is common. However, these assumed features are unlikely to be truly representative of biological networks, and in many cases complete knowledge of pathways and their interactomes is improbable (1138).

Interpretation of pathways and networks of adaptation is further confounded by the fact that molecular responses to exercise are likely to predominantly represent two purposes: the acute restoration of homeostasis, and the chronic modification of skeletal muscle tissue as an adaptive response to better defend perturbations to homeostasis during future sessions of exercise. These purposes may be complimentary for some pathways, but not so for every pathway, and therefore, the caveat is that not every molecular response to acute exercise is

mechanistically involved in the molecular regulation of the adaptive response to exercise training. With the wider application of omics analyses, distinguishing between acute responses that do or do not play a role in adaptive changes will be paramount. However, Section 4 described some key methodological issues to acknowledge in the translatability of *in vitro* cell culture and animal models, as well as the limitations and confounders in the study of human muscle biopsies. Application of these more sophisticated and advanced analytical methodologies could prove largely fruitless if as a field we continue to apply suboptimal study designs, or at least fail to acknowledge the limitations of our models.

One of the most important acknowledgments must be that techniques such as knock-outs, knock-ins, and pharmacological blockade or activation of specific genes and proteins provide information on the structure of signaling pathways, the modification of downstream targets, and the potential to exert phenotypical changes. These approaches do allow for the manipulation of genes and testing of preliminary compounds that are not possible in humans due to ethical and legal reasons, but they do not always account for the transient, integrative nature of exercise. Therefore, these molecular data need interpreting with that caveat in mind in terms of how this relates to *in vivo* human exercise physiology, and this reflects that the perennial challenge is to place such experimental observations into a physiological context. Nevertheless, *in vitro*, animal, and genetic models can help inform how change in a single gene interacts with its molecular 'colleagues' in ways that were previously unknown, and thus the wider molecular pathways and phenotypes that the gene is connected to. These models have therefore been important drivers in stimulating further research into these molecular networks in human exercise studies.

However challenges in, or lack of, translation of such experiments should not be surprising when consideration is given to the biological levels of organization i.e. atoms to explain molecular events, proteins and organelles to explain cell function, cells to explain tissue and organ function, tissues and organs to explain system function, and systems to explain organismal function (282). By studying the function of a target or process at one of the lower levels of organization, a challenge arises in the ability to translate findings to explain phenomena at higher levels of organization e.g. the manipulation of a single target gene to explain whole tissue phenomena such as adaptation in skeletal muscle to exercise. Moreover, given the complexity of networks and their interactions, compensatory responses to manipulation of a single target are unpredictable, perhaps especially so when the manipulation is constitutive rather than transient. Together these considerations limit the interpretation of the importance of individual factors in exercise adaptations, necessitate caution in the extrapolation to explanation of mechanisms of exercise training-induced adaptations, and highlight the incompleteness of knowledge despite the progress to date.

Yet this reflection should not discourage the use of these methods, nor the exploration of insights from basic cell biology into human exercise contexts. Despite the issues identified in Section 4 for *in vitro* and animal experiments with gain- or loss- of function, almost all of the known important genes and pathways in molecular exercise physiology have been established from such basic discoveries i.e. in animal models of exercise, gene knockout or knock-in, or pharmacological intervention. For example, seminal discoveries of roles of AMPK (816, 1139), calcium (557, 568), mTORC (76, 566), and PGC-1$\alpha$ (992, 1140) were, in most instances, made in reductionist models that pre-dated, and consequently were foundational evidence for, the hypothesis-led exercise studies in humans. Therefore, again the use of these experimental models is important in complimenting human exercise studies for the investigation of the molecular mechanisms underpinning adaptation.

### *B. Translational perspectives: Practical utility of a deeper understanding of the molecular response to exercise*

While molecular responses cannot yet fully explain the specificity of adaptation to divergent types of exercise, there is ample evidence that using these methods, existing exercise training and co-intervention strategies can be fine-tuned, and new ideas can be explored. Examples of co-intervention strategies i.e. strategies that enhance the adaptive response to exercise with a view to optimizing adaptation and performance, include reduced carbohydrate availability, altitude training and heat exposure, timing of exercise, blood flow restriction, and essential aminoacidemia (128-130, 1141, 1142). Similarly, there is increasing interest in using molecular methods to inform optimal exercise prescription, be that in the context of concurrent exercise training (135) as described above, or understanding the dose-response relationships from MICT to SIT (21).

There is also much interest in the fundamental science of characterizing the human response to exercise depending on age, biological sex, body composition, fitness level, and previous exposure to exercise training. One of the questions that might be addressed with such approaches is whether resting (basal) expression patterns and/or the acute molecular response to exercise can explain some of the contributors to the vast heterogeneity in response to exercise (489, 1143). Our own work examining gene expression and DNA methylation responses to resistance exercise training, detraining and retraining identified several genes that were hypomethylated after a single session of acute resistance exercise, which were then maintained as hypomethylated during training and detraining, but importantly also demonstrated an enhanced gene expression after later retraining (217). One interpretation is that in addition to skeletal muscle possessing an epigenetic memory of anabolic stimuli, it may be possible to identify biomarkers of the acute response that predict later adaptation to exercise. Therefore, an attractive hypothesis is that the exercise response

(e.g. aerobic fitness, strength, insulin sensitivity) can be predicted and understood through combined molecular and physiological classifications as a function of the individual and the exercise model. Such approaches would represent personalized exercise medicine in which the exercise prescription is matched to the individual and the condition or desired outcome, as opposed to the currently broad public health guidelines.

The recently-established Molecular Transducers of Physical Activity Consortium (MoTrPAC), a $170 million project funded by multiple agencies at the National Institutes of Health (USA), aims to elucidate how exercise improves health and ameliorates diseases by building a map of the molecular responses to acute and chronic exercise in preclinical rodent and clinical human studies using multi-omic and bioinformatic analyses (556). The overarching themes echo the personalized exercise medicine concept in that a better understanding of these biological processes and pathways would allow for the development of targeted exercise interventions and prescriptions. In their overview of the program (556), the MoTrPAC investigators also stated an aim of providing a foundation for developing pharmacologic interventions, which are broadly termed “exercise mimetics” or “exercise-in-a-pill”.

Interest in exercise mimetics has increased dramatically over the past two decades with the observations of exercise training-like phenotypes in transgenic and/or KO mice and/or pharmacological treatments. For example, chronic activation of AMPK in the skeletal muscle of mice via overexpression the γ3-subunit of AMPK with an R225Q polymorphism (849), or via consumption by rats of a diet enriched with 1% β-guanadinopropionic acid (β-GPA) (817), both induce mitochondrial biogenesis and an endurance-like phenotype. Similarly, disruption of myostatin activity (a member of the transforming growth factor β (TGF-β) superfamily of growth and differentiation factors and an established inhibitor of muscle growth), either through gene knockout (1144) or pharmacological inhibition (1046), produces a muscle hypertrophy phenotype. As a result, potential applications of pharmacotherapy are to replicate the metabolic effects of aerobic exercise through targeting of SIRT1, AMPK, PPARδ and HDACs (545, 553, 727), and to address age-related declines in skeletal muscle mass and function through targeting of canonical pathways involved in protein translation and muscle hypertrophy (873).

However, thought-leaders with decades of experience in the field of molecular exercise physiology have been largely skeptical of the promise of exercise mimetics from when they were first proposed right up to the present day (496, 497). The complexities of the molecular response and systemic multi-organ effects of exercise training are well-beyond the capability of currently-available monotherapeutic approaches. Thus, the inability to reproduce the systemic multi-organ effects of exercise has been a major criticism of the exercise mimetic

concept, especially due to current efforts being largely skeletal muscle-centric, and therefore not addressing impacts of exercise on, for example, atherosclerosis and the cardiovascular system (496). Another example concerns pharmacotherapies targeting of increased muscle mass in frailty and sarcopenia. Because the molecular regulation of muscle hypertrophy has been reasonably well-described, and a variety of the molecular candidates are “druggable” targets, many pharmacotherapies have been developed to target sarcopenia and have proceeded to Phase I and II clinical trials (873). These compounds have included myostatin inhibitors, antagonists of the activin receptor, and selective androgen receptor modulators (SARMs). Results from many trials have shown impressive effects of measurable muscle hypertrophy, but in most cases have shown limited success for improving muscle strength or physical function of patients (873). The interpretation is that muscle hypertrophy in the absence of an exercise stimulus does not necessarily result in a better functioning muscle. However, combining such therapies with even very small quantities of exercise could provide marked synergistic benefits to patients compared to either approach alone (1145-1147). Our natural history and evolutionary development has had physical activity at its core, and it has been argued that in the absence of physical activity, there is little chance that any pharmacological approach will be fully successful (496). In that context, the value of pharmacotherapy in support of modified or bespoke exercise interventions should not be discounted, i.e. compounds serving as adjunct treatments to exercise that potentiate or augment the acute benefit or adaptive response to exercise (1147-1149).

Similarly, it would be unwise to discount the potential for future discoveries with translational application(s) despite the limited evidence to date. For example, the prominence of small EVs in circulation as a response to acute exercise and exercise training (895, 896) and their evident bioactivity (458)(908-911) suggests an important role as mediators of tissue crosstalk during and in response to exercise. This role is notable given the promise of developing EV-based drug delivery systems (1150), whereas the metabolic effects of IL-6 established in the exercise literature was central to the development of the designer cytokine IC7Fc that had positive therapeutic indications for type 2 diabetes in pre-clinical models (1151). Therefore, rather than aiming to wholly mimic exercise effects at a multi-system level, a positive clinical impact may be achieved recapitulating only partial effects of exercise depending on the condition. As described elsewhere in this review, progress in the omics technology promises to deliver a better understanding of the molecular mechanisms underpinning the benefits of exercise. With appropriate study designs, these approaches may ultimately distinguish between factors that are associated (correlates) with the response to acute exercise, and factors that are causal in the adaptive response to exercise, and therefore better therapeutic targets.

### *C. Future directions*

Notwithstanding the sometimes critical view of the field presented throughout this review, the last quarter century of investigation using molecular methods has provided enormous insight into molecular mechanisms of adaptation in skeletal muscle to exercise. Yet much remains to learned as implied throughout this review, and we also propose the following questions explicitly as important future directions:

1. *How repeatable is the molecular response to an acute session of exercise, and how would this influence interpretation of existing and future data?*

Several important methodological issues were emphasized in earlier sections, including the first bout effect, the importance of training status, limitations of muscle biopsy sampling, and the absence of non-exercising control conditions. Separately or combined these issues are likely to be important factors determining the repeatability of the molecular response to acute exercise. Based on recent evidence (1136), an important consideration is the extent of random error that is present in data describing acute changes in mRNA abundance. A related consideration is the value obtained from studying molecular responses in untrained individuals if the acute response to the first session of exercise contains a large contribution from a generalized stress response. These considerations should inform the design of future studies and the cautious interpretation of the existing literature.

2. *How is the specificity of adaptation in skeletal muscle to exercise training conferred at a molecular level, and what is the continuity between molecular responses to acute exercise and the molecular mechanisms of adaptation?*

An important premise that underpins the study of acute responses in exercise contexts is that there is continuity between acute molecular responses and adaptive effects of long term exercise training, yet we have highlighted several examples of discordance and a lack of continuity between the respective responses. Therefore, while a broad understanding of the molecular mechanisms of adaptation to exercise training is established, how specificity of adaptation is conferred remains to be fully elucidated. To do so, methodological limitations of studies performed to date must be acknowledged and mitigated in future work. In particular, when previously sedentary or recreationally-active individuals commence *any* exercise training intervention, it is likely that there is initially a period where generic adaptations to exercise training take place prior to a true specificity of training effect occurring later in the intervention. If true, the molecular mechanisms that confer specificity of adaptation in skeletal muscle to exercise training may not be discernible in acute exercise studies in such individuals. Accordingly, we echo the recommendation by others that a period of familiarization or habituation should be implemented before conducting acute experimental trials (182, 1089).

Incorporation of fiber type-specific analyses should also be central to this work in future studies (146, 658).

*3. What further insights can be revealed by large scale integrated omics analyses?*

The "streetlight effect" describes a form of observational bias that results in people searching for something only where it is easiest to look (1152). The analogy in present context would be to continue to pursue the investigation of canonical pathways and established regulators despite evidence emerging of alternative candidates and the likelihood of yet-to-be-discovered regulators. A recent example of discovery is the interrogation of MetaMEx database, which uses meta-analytical methods on transcriptome data collected from human studies of aerobic and resistance exercise, including acute exercise sessions and chronic exercise training (48). Using gene ontology and pathway analyses on the expression profiles, a transcription factor NR4A3 (nuclear receptor 4A3; also known as NOR-1) was identified as one of the most exercise- and inactivity-responsive genes, with the mRNA abundance of this transcription factor being induced by both aerobic and resistance exercise (48). Notably, NR4A3 had not been previously identified by authors of the published datasets, despite this transcription factor having regulatory roles in skeletal muscle consistent with producing an endurance phenotype (1153). This example illustrates the potential for new discoveries that is a goal of unbiased omic approaches. An independent study subsequently demonstrated marked changes in NR4A3 mRNA abundance during recovery from change-of-direction repeated sprint running, with these changes coinciding with hypomethylation of the NR4A family of genes (219).

Of course, resources to achieve such discoveries remain a limitation for many laboratories, but with the declining costs of omics analyses and development of sophisticated analytical methods for large scale integrated data sets including emerging computational strategies (i.e., machine learning and artificial intelligence) (1154, 1155), future research that incorporates unbiased, integrated multi-omics approaches are likely to provide the most comprehensive picture of molecular networks that are acutely responsive to exercise, and therefore promise to reveal novel regulators of adaptation in skeletal muscle to exercise training. Central to these approaches is the assumption that triangulation of data from multiple sources that point to the relevance of the same gene, pathway or network is less likely to result in false positives (1154). Notably the aforementioned MoTrPAC initiative will incorporate genomics, transcriptomics, DNA methylomics, targeted and untargeted proteomics, and targeted and untargeted metabolomics with systems biology methods and network algorithms to focus on regulatory processes and build predictive models of the effects of exercise *per se*, but also the influence of age, biological sex, race, and exercise type on these responses (556). Such an approach has been recently demonstrated in principle as the "molecular

choreography" of the acute response to aerobic exercise, which included predictions on resting blood biomarkers and biological processes influencing $\dot{V}O_{2max}$, and insights into the pathophysiology of insulin resistance (459).

4. *What are the separate and/or synergistic roles for acute transient changes and longer term stable changes in epigenetic modifications such as gene-specific DNA hypomethylation in muscle memory and the adaptive response to exercise?*

Epigenetic modifications of some genes in skeletal muscle in response to exercise have been demonstrated to be transient and others altered and retained for longer periods, suggestive of an epigenetic memory of exercise that resides in skeletal muscle (217, 218). The retention, or earlier alterations in DNA methylation following training can then impact how responsive gene expression is to a later period of exercise retraining. However, it is currently not known how long epigenetic memory of exercise in skeletal muscle lasts past a few weeks, and this question is likely to be the focus of future research in this area. Other epigenetic modifications such as those that occur on histone proteins and other factors such as the three-dimensional chromatin configuration could also be retained after exercise, and lead to an enhanced gene expression response to future exercise. Emergence of omic technologies such as ChIP-Seq (Chromatin ImmunoPrecipitation assays with Sequencing) to map histone modifications and ATAC-Seq (Assay for Transposase-Accessible Chromatin using Sequencing) to map chromatin accessibility, together with Bisulfite-Seq for DNA methylation (RRBS or WGBS), will come to the fore in understanding muscle memory within molecular exercise physiology. Recent data after weighted wheel running in mice, like earlier human studies, demonstrated that there were retained DNA methylation signatures into detraining following an earlier training period, indicative of an epigenetic memory in skeletal muscle after exercise (1156). Importantly, comparison of DNA methylation signatures specifically in myonuclei with other interstitial nuclei demonstrated that an epigenetic memory retained from training into detraining resides in both myonuclei and other resident nuclei in muscle tissue, and observed some distinct epigenetic imprints in myonuclei versus other nuclei (1156). Given that the key mechanism of muscle memory has been attributed to retained myonuclei following earlier periods of hypertrophy (1157), bisulfite sequencing of nuclei from satellite cells that could contribute to increases in myonuclei during exercise training will determine if a memory of exercise resides in this regenerative stem cell in skeletal muscle (1158, 1159). Finally, an applied implication of muscle memory research is whether altering the exercise frequency, intensity, or timing of training lead to longer lasting epigenetic memory of exercise. If athletes and coaches could optimize exercise to produce a longer lasting memory, it may have implications for training prescription, periodization, and recovery as individuals may be able to

exercise less frequently but at a higher intensity or volume, and still have a similar adaptations to exercise.

5. *Do exercise factors, including the heterogeneous cargo present in small EVs, play a causal role in the adaptive response to exercise in skeletal muscle and/or other tissues?*

Hundreds of exercise factors in the form of metabolites, several RNA species, and peptides/proteins are changed in response to acute exercise (458-461). Many of these exercise factors display potent bioactivity in cell and animal experiments, and exercising skeletal muscle can communicate with other distal tissues through such means, but little is known about their specific role(s) in exercise training-induced adaptations in skeletal muscle (464, 483, 484). There are also several important methodological limitations with the identification and analysis of cargo present in small EVs that precludes firm conclusions on their mechanistic role in adaptive responses to exercise training (895, 896). *In vitro* contraction models demonstrate the presence of a muscle *fiber* secretome (1160), but important questions also remain as to the source of circulating exercise factors in response to acute exercise i.e. muscle fibers specifically, other resident cells (466, 467), or sources independent of skeletal muscle e.g. hepatokines.

6. *Does the apparent influence of the molecular clock and circadian rhythms on acute exercise-induced molecular responses manifest as practically-meaningful effects on adaptive responses to exercise training in terms of health and/or performance outcomes?*

Circadian influences on exercise performance (1161), and the molecular response to acute exercise (637) are both long-established. With the re-emergence of interest in circadian physiology by investigation using molecular biology (1162-1164), an influence of the molecular clock and time of day on metabolic and molecular responses to acute exercise has been demonstrated (1165-1169). For example, the induction of HIF-1$\alpha$ in mouse skeletal muscle in response to acute aerobic exercise is greatest during the active phase, and unresponsive in the rest phase (1167). One translational application of this work has been proposed as the potential to optimize exercise prescription for metabolic health, and for which there is reasonable *in vivo* human evidence (1141). On the other hand, whether observations such as the effect of acute exercise to influence the skeletal muscle molecular clock (637, 1170, 1171), or for time of day-dependent differences in molecular responses to exercise (1166, 1167, 1172), ultimately manifest as practically-meaningful effects on adaptive responses to exercise training remains to be determined in human contexts. One example is that resistance exercise-induced S6K1 phosphorylation observed after morning exercise, but not afternoon

exercise, did not manifest as between-group differences in strength or muscle hypertrophy after 11 weeks of either early morning versus afternoon resistance training (1172).

7. *Are there sex-specific differences in the molecular response to acute exercise and the molecular mechanisms of adaptation in skeletal muscle to exercise training?*

Biological sex is another mediating influence on responses to acute exercise and long term exercise training, and studying sex as a biological variable is increasingly emphasized (1173-1176). Phenotypical sex differences exist in skeletal muscle in terms of gene expression (1109, 1177-1180), satellite cell (1181-1183), and metabolic and mitochondrial characteristics (1184-1189). Additionally, given the influence of endogenous and exogenous sex hormones (1190-1193), and that the responses of several genetic, metabolic and physiological parameters to exercise differs between males and females (1194-1196), sex differences at the level of molecular responses to acute exercise and exercise training seem likely (1176). Yet to date, there are relatively few studies that have directly investigated between-sex differences in the molecular responses to acute exercise (968, 1178, 1184, 1185, 1191, 1197-1202). The role of neuronal, mechanical, metabolic and hormonal factors, and the influence of absolute and relative exercise intensity on these factors suggests that a methodological challenge for such studies remains how to best “match” males and females for phenotypical characteristics when investigating sex-specific molecular responses to acute exercise.

8. *Do gut microbiota influence the molecular response to acute exercise and the molecular mechanisms of adaptation in skeletal muscle to exercise training?*

Physical activity and exercise training are associated with changes to the gut microbiome (1203), and several studies report different composition and functional capacity of the gut microbiota of athletes compared to control participants (1204-1207). There is increasing interest in the potential influence of gut microbiota on exercise performance and the adaptive response to exercise (485, 1203), with the implication that any influence on the latter may in turn be a consequence of host-microbiome interactions influencing the molecular response to acute exercise (1207). One study employing multi-omic data in endurance athletes observed an increase in *Veillonella spp*. after a marathon, which was also higher in abundance at rest compared to controls (1207). This bacterium is responsible for fermenting lactate to the short-chain fatty acid propionate. Fecal transplants of *Veillonella atypica* and supplementation with propionate in mice then resulted in improved endurance capacity in treadmill testing (1207). The working hypothesis is that *Veillonella spp*. convert circulating lactate produced during exercise to propionate in the gut, which may in turn initiate signal transduction in skeletal muscle via AMPK activation (1207). This mechanism whereby short chain fatty acids such as acetate, butyrate, and propionate produced by the gut microbiota of

athletes, and in turn influence performance and adaptation, is further detailed elsewhere (1208), and at present is the most likely explanation for a bidirectional relationship in which exercise impacts the composition and functional capacity of the gut microbiota while the microbiota influences performance and adaptation. Further work will be required to elucidate the molecular mechanisms of these potential interactions between the microbiome, molecular responses to acute exercise, and exercise training adaptations.

## 9. CONCLUDING REMARKS

The remarkable plasticity of skeletal muscle in response to exercise training has been long known and remains an area of intensive research efforts. The mechanistic basis for adaptations to skeletal muscle in response to exercise training is understood as the activation and/or repression of molecular pathways and processes induced in response to each individual acute exercise session, the consequences of which accumulate over days, weeks, and months of frequent and progressive exercise training stimuli. These molecular pathways include the transduction of signals arising from neuronal, mechanical, metabolic, and hormonal stimuli through complex signal transduction networks, which are linked to a myriad of effector proteins involved in the regulation of pre- and post-transcriptional processes, and protein translation and degradation processes. While much insight has been gleaned over the past few decades, there remains much still to be learned, and in particular, the fundamental question about the degree of continuity between molecular responses to acute exercise and the molecular mechanisms of adaptation in skeletal muscle to exercise training, given the limitations to available experimental models and existing data. Relatedly is the requirement for a more complete picture of proteins, pathways, and networks that confer the *specificity* of exercise training effects. Emerging areas of interest are the influences of biological sex, circadian rhythms, and the gut microbiome on acute responses and chronic adaptations to exercise. The judicious combination of reductionist and *in vivo* human exercise investigations in a complementary manner, when combined with the more widespread application of omic technologies and machine learning approaches, should result in many more novel insights in the coming years.

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## DISCLOSURES

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