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Exercise metabolism and adaptation in skeletal muscle

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Abstract

Viewing metabolism through the lens of exercise biology has proven an accessible and practical strategy to gain new insights into local and systemic metabolic regulation. Recent methodological developments have advanced understanding of the central role of skeletal muscle in many exercise-associated health benefits and have uncovered the molecular underpinnings driving adaptive responses to training regimens. In this Review, we provide a contemporary view of the metabolic flexibility and functional plasticity of skeletal muscle in response to exercise. First, we provide background on the macrostructure and ultrastructure of skeletal muscle fibres, highlighting the current understanding of sarcomeric networks and mitochondrial subpopulations. Next, we discuss acute exercise skeletal muscle metabolism and the signalling, transcriptional and epigenetic regulation of adaptations to exercise training. We address knowledge gaps throughout and propose future directions for the field. This Review contextualizes recent research of skeletal muscle exercise metabolism, framing further advances and translation into practice.

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Author contributions

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Introduction

As a primary site of nutrient storage, energy use and locomotion, skeletal muscle is central to the impact of physical activity on human health. Periods of inactivity reduce skeletal muscle insulin sensitivity and oxidative capacity[1], contributing towards impaired systemic metabolic flexibility[2] and an increased risk for cardiometabolic disease[3]. Physical activity offers a degree of protection against the deleterious effects of sedentary behaviour on whole-body metabolism[4]: larger volumes[4] and more vigorous types[5] of physical activity, such as formal exercise, probably convey additional benefit.

Bouts of exercise rapidly sensitize skeletal muscle to hormones (Supplementary Table 1) and nutrients. A single (or 'acute') exercise session directly increases skeletal muscle transport of amino acids[6] and glucose[7]. These effects appear somewhat specific to the contracted musculature, and they enhance postprandial muscle protein synthesis[8] and insulin-stimulated glucose disposal[9,10] in the recovery period after exercise. Consistent exercise training (over weeks, months and years) further augments skeletal muscle mass[11–13], peripheral insulin-sensitivity[12], maximal oxygen consumption ($\dot{V}O_{2max}$) [12–15] and strength[12,13]. $\dot{V}O_{2max}$ (ref. 16) and strength[17] are well-known predictors of mortality, and training for their improvement through endurance and resistance exercise (Box 1) reduces mortality risk in a manner that is most effective when both training modalities are performed within the same exercise programme[18].

Although the association between regular physical activity and health span has been realized since antiquity, recent methodological advances have allowed the field of exercise physiology to progress towards comprehensive systems-level profiling of the complex molecular interplay that occurs with exercise[19,20]. These advancements have enabled better mechanistic understanding of the cause-and-effect relationships underlying exercise adaptation and associated health benefits.

In this Review, we provide a contemporary summary of the role of skeletal muscle in response to exercise. First, we address the major cellular makeup of skeletal muscle, highlighting fibre type properties, and the importance of sarcomeric and mitochondrial networks. Next, we discuss skeletal muscle metabolism during acute exercise and the influence of select modifiers such as intensity and timing on this biology. Finally, we address underlying mechanisms of exercise-induced skeletal muscle adaptation and consider differences between training modalities that may facilitate distinct and complementary responses beneficial for human performance and health.

Skeletal muscle fibre types and subcellular characteristics

The human body contains >600 skeletal muscles mainly comprising long contractile cells called muscle fibres. The complex architecture of these fibres (Fig. 1a) provides clues into the intricacies of muscle function and homeostatic control. In this section we focus on the contractile and metabolic properties of different muscle fibre types and examine how these attributes are supported by interacting networks of sarcomeres and mitochondria.

Contractile, metabolic and myonuclear properties of muscle fibre types

Human muscles of the torso and limbs express three main fibre types, with slow-to-fast contractile properties in the following order: slow-oxidative myosin heavy chain (MyHC) type I (encoded by *MYH7*), fast oxidative-glycolytic (intermediate) MyHC type IIA (encoded by *MYH2*) and fast-glycolytic MyHC type IIX (formerly known as type IID; encoded by *MYH1*)[21]. Muscle fibres have classically been 'typed' according to metabolic — oxidative versus glycolytic enzyme — profiles[22,23] and the predominant abundance[22,24] or ATPase activity[23] of MyHC isoforms. MyHC are the motor proteins of myofibril thick filaments and determine important aspects of muscle function, such as maximum shortening velocity[25]. Yet, the full extent of fibre type characteristics (Supplementary Fig. 1) depends upon matching the excitation–contraction coupling machinery and ATP provision to MyHC activity[14,21]. This coordinated expression is a product of α-motor unit innervation and the transcriptional synchrony of resident myonuclei[26,27].

During differentiation (myogenesis) (Supplementary Box 1), a combination of intracellular forces 'squeeze' centrally located myonuclei to the fibre periphery[28], where they reside in a generally ordered pattern of 'domains' in mature muscle. Discrete populations of myonuclei serve specialized roles within fibres, including those governing the neuromuscular junction, myotendinous junction[26,29,30] (the interface between muscle and tendon) and proprioceptive muscle spindles[30]. Likewise, the function of 'body'[26] or 'canonical'[29] myonuclei (constituting ≥90% of total myonuclei)[26] is to direct fibre type specificity in muscle, and they can be identified in mouse muscle by their *Myh* isoform signature[26,29,30]. *Myh*-positive myonuclei from type I versus type II fibres possess unique chromatin accessibility and transcription factor motif enrichment profiles that are distinct from one another and from myonuclei in other cellular compartments, including the myotendinous junction[26]. This could underlie specific transcription factor-driven myonuclear programmes[26,30] that are partially responsible for regionalized gene expression in muscle cells[26,30,31] and for the co-expression of specific calcium (Ca^{2+})-handling, sarcomeric and metabolic apparatus[14,21,26,31] so that fibre type contractile (also known as 'twitch') and metabolic properties generally align (Supplementary Fig. 1b).

The myonuclei in fibres from endurance-trained younger and older individuals are more spherical, contain greater lamin A (LMNA) deposition and are stiffer and less deformable than myonuclei in untrained counterparts[32]. These structural and mechanical modifications may facilitate the transduction of cytoskeletal forces towards the nucleus and improve myonuclear resilience against contractile damage[32]. As such, exercise training-induced myonuclear remodelling could have important implications for muscle adaptation and integrity across the lifespan.

In general, human muscles often express a greater proportion of slower-twitch fibres than those of other species[22,24], and human fibres are slower-contracting than orthologous fibre types in most mammals, including rats and mice[24]. Similarly, type IIA fibres are the most oxidative fibre type in rodent muscle, whereas type I fibres are most oxidative in humans[22] (Supplementary Fig. 1). Such differences might contribute to the lack of conformity between the transcriptomes of human and mouse muscle after acute or chronic

resistance exercise[33] and should be considered when inferring human relevance from animal physiology. Moreover, the properties of muscle can vary markedly among individuals[23,34], biological sexes[23] (Supplementary Box 2), anatomical locations[35] and during ageing[34,36] (Box 2). For example, discrete spatial metabolomic differences within fibre types have been observed in mouse muscle[37], and age-associated mitochondrial impairments may induce a glycolytic shift in human fibres without a corresponding change in MyHC[36]. Collectively, this cautions against the use of MyHC as a strict marker of metabolism and vice versa. Future inquiry should better define how covariates — including biological sex, social gender, biological versus chronological age, metabolic health and training status — interact to determine the full spectrum of muscle characteristics.

Hybrid fibre types

Most canonical myonuclei within the same fibre display coordinated transcription of a single *Myh* isoform in mouse muscle[26,27]. However, a minority of fibres are hybrid[26,27] (reviewed in ref. 38), containing myonuclei that express two or more *Myh* pre-mRNA genes in the same nucleus[26,29] and/or in different nuclei across the fibre length[26]. Thus, the regional distribution of MyHC can vary between muscle biopsy sites[39] and along a single fibre[26,40].

In human vastus lateralis muscle, <10%[22,41] to 40%[38] of fibres can be hybrid types. 'True' non-transitioning or non-regenerating hybrid fibres have metabolic enzyme[22] and single-fibre contractile (force-velocity producing)[25,42] properties between those of their co-expressed MyHC isoforms, providing further functional nuance to the slow-oxidative to fast-glycolytic continuum in the following order of slowest and most oxidative to fastest and most glycolytic: type I → I/IIA → IIA → IIA/IIX → IIX[22,25,42]. In adult mice, hybrid fibres are most common in the slow-twitch soleus[26,27], and the abundance of hybrid fibres is not altered by denervation in this muscle group[26]. By contrast, sciatic nerve transection or deletion of *Six1* — a gene encoding the transcription factor homeobox protein SIX1 driving the fast-glycolytic phenotype in muscle[43] — increases hybrid fibre content in the typically fast-twitch extensor digitorum longus[26,27]. Hence, anatomical position[27], innervation[26,27] and transcription factor profiles[26] might coalesce to coordinate *Myh* expression in muscle.

For a given muscle group, a greater proportion of hybrid and pure type IIX fibres seems indicative of sedentary behaviour[41]. Alternatively, exercise tends to reduce hybrid fibre content[41,44,45], promoting a shift away from type IIX fibres towards slower myosin types[13,39,41]. Consequently, pure type IIX fibres are rare in humans[22,24,39,41] and account for <1% of the vastus lateralis fibre pool in healthy individuals[40]. As discussed in Supplementary Box 1, *Myh* expression in mouse muscle is regulated by competitive promoter–enhancer interactions[27,46]. Exercise epigenetically modifies chromatin accessibility (see the section 'Skeletal muscle responses to acute exercise'), and resistance exercise increases MyHC-specific protein synthesis[47]. Yet, it is still unclear how established regulators of fibre type switching, such as peroxisome proliferator-activated receptor-γ coactivator 1α (PGC1α, also known as PPARGC1α)[48] and nuclear factor of activated T cells, cytoplasmic 1 (NFATc1)[49], combine with *Myh*-promoter–enhancer dynamics to confer physical activity-dependent fibre type transitions. Further single-myonuclei RNA and chromatin profiling, alongside isolated fibre spatial transcriptomic and

proteomic approaches, should extend the understanding of how gene expression and cellular phenotype are regulated among the many nuclei of a syncytial muscle fibre.

The myofibrillar matrix

The sarcomere is the basic contractile unit of skeletal muscle, and in-series assembly of sarcomeres forms myofibrils. Although myofibrils were initially viewed as single, tube-like structures organized in parallel within fibres, later evidence suggested that myofibrils instead form branching networks that could act in tandem with the cytoskeleton to facilitate lateral-force transmission[50]. Using focused ion beam-scanning electron microscopy, recent studies have built upon this hypothesis showing that myofibrils indeed form a nonlinear lattice of sarcomeres[51–53] connected across the width and length of the muscle cell through three branching subtypes[51] (Supplementary Fig. 2a). In mice, the frequency of sarcomere branching decreases during early-to-late postnatal development, but increases again in adult slow-oxidative soleus fibres[51]. In comparison to soleus muscle, fast-glycolytic gastrocnemius fibres exhibit a different branching morphology (preferring myofilament transfer over sarcomere splitting) and utilize fewer total sarcomeric connections[51]. Inducing sarcomere connections, such as through gene manipulation in *Drosophila*, reduces the myofibril cross-sectional area[52]. Thus, myofibrillar matrix assembly appears specific to functional demand, and its organization in fast-twitch muscle may support the greater size[22] and power[24] of these fibres.

The relevance of a nonlinear network of sarcomeres is implied by its structure — providing an elegant mechanism for both longitudinal and lateral force transmission from muscles to bones through tendons[50,51]. A linked configuration of myofibrils could also minimize the impact of localized sarcomere damage in muscle and thereby increase the robustness of the contractile machinery across an entire fibre. Further interrogation of the function and regulation of the myofibrillar matrix in health, disease and exercise adaptation will be enlightening.

Mitochondrial complexity in muscle

Sustained muscle contraction requires a continuous supply of ATP, which is mostly derived from mitochondrial oxidative phosphorylation (OXPHOS) during submaximal[54] or longer-duration high-intensity exercise[55] (see below; Fig. 2). Depending on fibre type, around 2–10%[56] of muscle volume is filled by distinct subpopulations of subsarcolemmal (also known as peripheral) and intermyofibrillar mitochondria, differing in structure, function and localization[56–59] (Fig. 1).

Subsarcolemmal mitochondria often cluster together in the sarcoplasmic space between myofibrils and the sarcolemma[56–59], and they dedicate more of their volume to cristae (folds of the inner membrane) and matrix[57,58]. These globular mitochondria extend deep into the myofibrillar space and physically join with adjacent intermyofibrillar mitochondria through electron-dense intermitochondrial junctions[57,58], forming the mitochondrial reticulum[56–59] (Fig. 1b,c). Compared to subsarcolemmal mitochondria, intermyofibrillar mitochondria are more complex in morphology[59] and physically interact with the myofibrillar matrix[53,56,57], sarcoplasmic reticulum (SR) and intermyofibrillar lipid droplets[53,56,58] (Fig. 1b).

In humans[53,59,60] and mice[53,56], intermyofibrillar mitochondrial (sub)networks are uniform, but their organization varies between muscle types[56,60]. In fast-glycolytic extensor digitorum longus fibres of mice, intermyofibrillar mitochondria wrap around the I-bands of sarcomeres, perpendicular to the contraction axis, whereas slow-oxidative soleus fibres contain larger subnetworks of connected intermyofibrillar mitochondria, arranged in grids that surround myosin like a cage[56]. The positioning of mitochondria in the intermyofibrillar space also directly impacts the structure of adjacent sarcomeres[53]. A greater proportion of mitochondria at the Z-disc reduces the cross-sectional area of sarcomeres in this region by bending (or 'curving') peripheral myosin filaments, causing heterogenous myosin–myosin spacing along the sarcomere length[53]. However, any detrimental impact on fibre contractility might be offset by the relative preservation of cross-sectional area and myofilament spacing towards the sarcomere centre (M-line)[53] (the structure of an individual sarcomere is shown in Supplementary Fig. 2d).

Subsarcolemmal mitochondria are proximal to nutrient-delivering capillaries[56–59] and have a greater abundance of proton-motive force driving electron transport chain (ETC) complex IV[57]. As such, this subpopulation is thought to specialize in membrane potential generation for subsequent transfer through the reticulum, into the intermyofibrillar mitochondrial network[57]. Here, intermyofibrillar mitochondria, possessing higher ATP synthase (ETC complex V) expression[57] and higher surface area-to-volume ratios[59], could utilize this potential energy to support rapid ATP production and diffusion to myofibrillar ATPases[56]. Hence, much like an electrical power grid, the mitochondrial reticulum may serve to efficiently disperse energy across the fibre[56–58].

Within mitochondria, ETC complexes form higher-order structures termed 'supercomplexes'. The maximal oxygen consumption of permeabilized fibres was positively correlated with the preferential redistribution of complexes III and IV into supercomplexes in the muscle of elderly individuals after 4 months of endurance training[61]. Alternatively, 6 weeks of high-intensity exercise improved muscle mitochondrial respiration and ETC enzymatic activity in the absence of supercomplex alterations in young adults[15]. Supercomplexes are clearly important structural features of the ETC, but whether they support exercise adaptation beyond their stoichiometric relationship with mitochondrial abundance is yet to be determined. Furthermore, the assembly and stability of supercomplexes depends on the integrity of mitochondrial cristae[62,63], which can be regulated by SR stress-induced signalling based on eukaryotic translation initiation factor 2α (EIF2α) kinase 3 (PERK, also known as EIF1AK3), EIF2α and activating transcription factor 4 (ATF4) (PERK–EIF2α–ATF4)[63]. Endurance-trained athletes have greater cristae density in mitochondria of type I fibres[64], and PERK and ATF4 proteins are increased in muscle ~48 h after acute resistance exercise[65]. However, cristae structure was unchanged after 10 weeks of moderate-intensity endurance training in sedentary individuals with obesity[64]. Thus, further study is required to distinguish the impact of exercise on mitochondrial cristae remodelling.

Moving forward, better understanding of mitochondrial networks, subpopulations and ETC configurations could benefit strategies to combat age-associated decline in muscle function (Box 2) and mitochondria-related diseases. Focus should be directed towards the

heterogenous mitochondrial populations in human muscle, which are smaller than those in mice[59].

Acute exercise metabolism in skeletal muscle

Relative to weight, the basal thermogenesis of muscle is lower than that of most other organs[66] because myosin is maintained in disordered relaxed and super-relaxed states characterized by slow and extremely slow ATP kinetics, respectively[67] (Supplementary Figs. 2b,c). Upon contraction, mechanosensing rapidly initiates myosin conformational change from relaxed to active[68], and muscle ATP consumption increases dramatically during short-duration exhaustive exercise[69,70]. As free ATP in muscle (~20–25 mmol per kg dry mass) is only sufficient to sustain maximal exercise for <2 s (refs. 69,70), continued contractile activity requires ATP resynthesis from a combination of intramuscular energy stores (Fig. 1a and Box 3) and circulating substrates, such as glucose and non-esterified fatty acids (NEFAs)[71,72] (Fig. 2).

In this section we detail the metabolic responses that enable muscle to match the considerable demands of acute exercise. We also address how this substrate–energy pairing facilitates the integral role of muscle in exercise-mediated inter-organ communication (Box 4) and touch upon the interaction between exercise metabolism and biological rhythms.

Muscle substrate utilization is exercise intensity-dependent

Muscle fibres are part of a functional ‘motor unit’ comprising an α-motor neuron and the muscle fibres innervated by its axon. The force generated by a muscle depends on both the number of activated motor units — and thus fibres — and the rate at which motor units discharge action potentials once recruited (known as rate coding) (reviewed in ref. 73). Stimulation of motor units conforms to the size-orderly principle of recruitment, such that smaller units are activated first, followed sequentially by larger units as contraction intensifies[73]. Consequently, the higher power outputs achieved during progressively demanding physical activity relies on the stimulation of a greater number of motor units, a larger proportion of the muscle fibre pool, and therefore the recruitment of more type II fibres.

ATP consumption per unit of time is ~2.5–4-fold higher in type II fibres than in type I fibres[74], and the maximal rate of ATP resynthesis is fastest through oxygen-independent (anaerobic) versus oxygen-dependent pathways, in the following order of descending speed: anaerobic phosphagen system (including adenylate kinase and creatine kinase (CKM)) and glycolysis reactions provide the fastest supply of ATP, followed by carbohydrate oxidation and finally NEFA oxidation. Human type II fibres are hence enriched with creatine phosphate (CrP) and glycogen energy depots[75] and contain higher levels of adenylate kinase[21], glycogenolysis and glycolysis metabolic machinery[14,21]. Conversely, type I fibres are more abundant in peroxisomes[14], mitochondria[14,21,76,77] and intramyocellular lipids (IMCLs)[76–78], consistent with their slower ATP turnover[74] (Supplementary Fig. 1b).

Together, this indicates that the contribution of specific energy systems and substrates to working muscle is mainly a function of the neuromuscular activation required to match

the intensity of the exercise being performed. This understanding is envisaged within the 'crossover concept' (reviewed in ref. 79), which describes the larger relative contribution of NEFA oxidation towards whole-body energy expenditure at low-to-moderate exercise intensities, with an incremental and necessary 'switch' towards preferential (oxygen-dependent and independent) carbohydrate utilization during exercise at higher levels of mechanical effort[71,72].

Metabolic inertia at the onset of exercise

Although the lower metabolic cost of rest[72] and light exercise[71] is mainly fuelled by the oxidation of circulating NEFAs, further upregulation of OXPHOS ATP provision is delayed at the onset of moderate-to-high intensity physical activity, and a larger oxygen-independent contribution fuels muscle in the first ≤30–60 s of exercise[54,80]. This is despite sufficient blood flow[54,80] and adequate intramuscular oxygen levels for maximal mitochondrial respiration to occur (≥0.5–2 mmHg) (reviewed in ref. 81).

The lag in oxygen-dependent metabolism at the beginning of exercise may stem from a combination of linked temporal factors, including activation of the mitochondrial matrix enzymes pyruvate dehydrogenase (PDH), 2-oxoglutarate dehydrogenase (OGDC)[82] and carnitine acetyltransferase (CRAT)[83,84]; the availability of tricarboxylic acid (TCA) cycle substrates (pyruvate and glutamate)[82] and precursors (acetyl groups)[83–85]; and sarcoplasmic buffering of the interrelated allosteric metabolites creatine and ADP[86] (Fig. 2a). Indeed, pharmaceutical activation of PDH increased muscle acetyl-carnitine availability and reduced the reliance on phosphagen and glycolytic energy pathways during acute ischaemic exercise[85]. The ability of previous sprinting intervals to prime OXPHOS in subsequent high-intensity work bouts could also occur through better coupling of oxidative substrate delivery to mitochondrial enzymatic activity, although carryover of residual fatigue probably plays an additional role in this scenario by impeding power output[69,70].

Oxygen-independent exercise metabolism

During short-duration maximal efforts, accelerated muscle ATP demand is mostly met anaerobically, through rapid stimulation of the phosphagen and glycolytic energy systems[69,70]. The simplicity and proximity of the sarcoplasmic CKM reaction (Box 3) allows CrP to supply equimolar amounts of high-energy phosphate to ATPases within milliseconds[87], which is essential for sprint performance[69,70]. CrP hydrolysis peaks at the onset of contraction but declines within less than 6 s, and CrP stores can be >90% depleted after around 30 s of intense exercise. [70] compared to only ~20% after 10 min of cycling at ~50% of $\dot{V}O_{2max}$ (ref. 88).

The initial increase in glycogen breakdown is due to posttranslational modification of glycogen phosphorylase (PYGM) from a less-active *b* form to the constitutively active *a* form by phosphorylase kinase (PHK)[70]. Transients of Ca^{2+} and AMP are probable regulators of PHK and thus the switch to PYGM*a* (reviewed in ref. 89). AMP can simultaneously increase PYGM*b* activity to support maximal rates of glycogenolysis[70], and the systemic rise of adrenaline during sprint intervals[69] or endurance exercise[71,90,91] may help to stabilize the PYGM*a* conformation[92]. Downstream of glycogen and glucose,

allosteric regulation and replenishment of nicotinamide adenine dinucleotide (NAD^+) can stimulate the rate-limiting enzymes phosphofructokinase (PFK) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), respectively, to promote continued flow through glycolysis (Fig. 2c).

Average power output drops substantially over the final half of a 30-s all-out physical effort, corresponding to the pattern of PYGM activity (reversion from *a* to *b*) and reduced substrate-level phosphorylation[70]. This muscular fatigue might be driven by alterations to the intracellular metabolic milieu. Above a critical threshold of ~15 mmol l^{-1} (ref. 93), inorganic phosphate can enter the SR and precipitate with Ca^{2+}, thereby impairing Ca^{2+} release and muscle contraction[94]. Additionally, owing to the high activity and near-equilibrium state of lactate dehydrogenase (LDH), the end product of glycolysis is always lactate[95]. After ~3.5–4 min of exhaustive cycling, muscle lactate reaches levels more than twofold higher than resting concentrations[55], more than fourfold higher following acute resistance exercise[96], and ≥8-fold[97] to 30-fold[69] higher after 10 rounds of short-duration sprinting. The accumulation of lactate (a strong anion) encourages dissociation of water to $HO^- + H^+$, and muscle pH can reach ≤6.5 during strenuous exercise bouts[55]. Lower intramuscular pH could diminish PYGM activity[70], and H^+ may act on group III (mainly mechanosensitive) and IV (mainly metabosensitive) muscle afferents in the interstitial space[93]. Once activated, these sensory neurons can feed back, potentially through inhibitory γ-aminobutyric acid type B ($GABA_B$) receptors[98], to reduce motor cortex excitability and suppress motoneuronal output[93]. The severity of peripheral fatigue correlated with the extent of quadriceps activation during self-paced time-trial cycling[99]. However, mechanisms of exercise-induced peripheral and central fatigue are complex, and the major contributory factors are probably specific to modality, intensity and duration (reviewed in ref. 94).

Oxygen-dependent exercise metabolism

Reactive oxygen species production during exercise.

In contrast to maximal sprinting for ≤30 s (refs. 69,70), high-intensity cycling lasting ≥3 min derives >70% of energy from OXPHOS, with a post-exercise muscle metabolome enriched for pathways using pyruvate, long-chain NEFAs and amino acids such as alanine, arginine and glutamate[55]. This upregulation of OXPHOS reduces mitochondrial superoxide (H_2O_2) emission[100,101] from ETC complexes I–III[100] and thus mitochondria contribute minimally to reactive oxygen species (ROS) generation in contracting muscle[102]. Rather, nicotinamide adenine dinucleotide phosphate (NADPH) oxidases (NOX), located in the sarcolemma and in transverse tubules (T-tubules)[102] and the sarcoplasmic reticulum[103], appear to be the predominant sources of ROS during contraction[101,102]. Specifically, NOX2 (ref. 101) and NOX4 (ref. 104) are indispensable for exercise-stimulated sarcoplasmic ROS production in mice.

ROS signalling is implicated in adaptations to endurance and resistance training (reviewed in ref. 105). High-intensity intervals augment *NOX4* mRNA in human vastus lateralis ~3–4 h after exercise, and a retrograde NOX4–ROS-axis led to activation of transcriptional regulators — such as nuclear factor erythroid 2-related factor 2 (NRF2, also known

as NFE2L2) and PGC1α — required for mitochondrial biogenesis and the endogenous antioxidant defence response to exercise training in mouse muscle[104] (Fig. 3). Accordingly, blunting oxidative stress through *Nox4* deletion in mice[104] or high-dose vitamin C and E intake in humans[106] attenuates the insulin-sensitizing effects of endurance training. Still, any potential detriment of antioxidant (such as vitamin C and/or vitamin E) supplementation to gains in $\dot{V}O_{2max}$, lean body mass or human endurance and strength performance seems relatively minor[107].

Glucose uptake and carbohydrate oxidation in exercising muscle.

When workload[71,72] and (to a lesser extent) duration[71] of moderate-intensity exercise increases, so does the contribution of blood glucose towards total carbohydrate utilization, although energy provision from intramuscular glycogen is always higher[71,72]. Muscle glucose uptake reaches ~15-fold resting levels at the cessation of non-fatiguing exercise (~50% of $\dot{V}O_{2max}$) and ~50-fold resting levels at the cessation of exhaustive endurance exercise (~100% of $\dot{V}O_{2max}$)[88]. This increased rate of blood glucose extraction is supported by greater hepatic glucose output[108], a ≥5–10-fold rise in blood flow[88], and enhanced perfusion of muscle capillaries[109]. As exercise intensifies, these circulatory and microvascular responses collectively maintain relatively constant plasma–to–interstitial glucose concentrations[110] and enlarge the area available for nutrient exchange to occur[109]. Such events further serve to promote the release of discrete biologically active molecules from muscle[20]. As discussed in Box 4, many of these muscle-derived ‘exerkines’ or exercise-induced ‘myokines’ are implicated in aspects of local and systemic exercise adaptation (reviewed in ref. 111).

The exocytosis and fusion of glucose transporter 4 (GLUT4)-containing vesicles with the sarcolemmal membrane and T-tubules is essential for contraction-stimulated glucose transport into muscle[112]. During exercise, translocation of GLUT4 is regulated by a combination of Ca^{2+}, metabolic stimuli and mechanosensitive stimuli that converge on calcium/calmodulin-dependent protein kinase II (CaMKII), 5′-AMP-activated protein kinase (AMPK) and RAS-related C3 botulinum toxin substrate 1 (RAC1) (reviewed in ref. 113), with redundancy between pathways[113,114]. The role of RAC1 in contraction-induced glucose uptake might be mediated in part by downstream NOX2 activation[101] but seems independent of $AMPK\alpha_2$ (ref. 114) during submaximal treadmill running in mice. Indeed, the catalytic α subunit of AMPK appears dispensable for exercise-stimulated glucose transport in vivo[115,116]. Instead AMPKα plays a more notable role in post-exercise substrate metabolism[117] and insulin sensitivity[116] through the upregulation of pyruvate dehydrogenase kinase 4 (PDK4)[117], the promotion of RAS-related protein RAB8A-perilipin 5 (PLIN5) lipid droplet–mitochondrial tethering[118] and the phosphorylation of TBC1 domain family member 1 (TBC1D1)[115] and TBC1D4 (also known as AS160)[116].

For glucose to be oxidized in muscle, lactate — simultaneously produced from glycolysis and taken up through sarcolemmal monocarboxylate transporters (MCT1 and MCT4)[81] — must first be converted to pyruvate by sarcoplasmic or mitochondrial LDH (reviewed in ref. 119) (Fig. 2c). Glucose oxidation is higher in men than in women (Supplementary Box 2) and rises with exercise intensity, dietary carbohydrate and muscle glycogen levels, and

peri-exercise carbohydrate intake[120]. Intra-workout consumption[121] or 'mouth-rinsing'[122] of exogenous carbohydrates can also benefit exercise performance. Whereas the ingestion of carbohydrates feasibly provides metabolizable substrate[120] and spares liver glycogen stores during bouts of longer−duration exercise[108], the potential ergogenic effect of mouth-rinsing is most probably achieved through oral receptor afferents that signal centrally to increase voluntary force production[123].

Muscle lipid metabolism during exercise.

The post-exercise serum metabolome is distinct between exercise modalities. An acute bout of endurance or resistance exercise differentially regulates discrete subclusters of metabolites across a 3-h post-exercise period, despite similar trends for metabolome recovery over time[124]. Of note, amino acid, nucleotide and carbohydrate (for example, lactate and pyruvate) signatures are prominent after resistance exercise, compared to the lipid-derivative enrichment (for example, various acyl-carnitines and the ketone body β-hydroxybutyrate) after endurance exercise[124]. This illustrates unique physiological challenges posed by specific interventions.

At intensities eliciting peak fat oxidation (~60–65% of $\dot{V}O_{2max}$), the contribution of plasma NEFAs and IMCLs is ~1:1 and roughly equal to total carbohydrate utilization[71,72]. In mouse muscle, liberation of NEFAs from lipid droplets is almost entirely dependent[125] on the redundant enzymes[125,126] adipose triacylglyceride lipase (ATGL) and hormone-sensitive lipase (HSL). The recruitment of ATGL to PLIN5 at lipid droplets could be enhanced by AMPK-regulated assembly of the RAB8A–PLIN5 tethering complex[118]. Alternatively, exercise upregulates HSL in an intensity-dependent manner[127] through additive contraction (Ca^{2+}–protein kinase C (PKC))[128] and adrenaline (cAMP–PKA)[91]-mediated pathways that promote its translocation to lipid droplets[129]. These signalling events augment rates of muscle lipolysis, and IMCL contents of type I and type II fibres can be ~45% and ~20% depleted, respectively, after ~60–180 min of cycling at ~50–75% of $\dot{V}O_{2max}$ (ref. 130). Muscle HSL activity is transient during acute moderate-intensity exercise[91] and its downregulation — through negative feedback from AMPK[91] or allosteric metabolites (for example, long-chain fatty acyl-CoA) — mirrors the greater relative contribution of circulating NEFAs towards whole-body energy expenditure[71].

Circulating NEFA availability is increased during longer bouts of physical activity at low-to-moderate intensity[71] due to greater release from adipose tissue and the improved affinity of triacylglycerol-rich lipoproteins for hydrolysis by lipoprotein lipases (LPL)[131] anchored to the endothelial surface of interstitial capillaries. The utilization of plasma NEFAs is enhanced by contraction-induced sarcolemmal enrichment of long-chain fatty acid transporters (fatty acid translocase (FAT, also known as CD36), plasma membrane-associated fatty acid binding protein (FABPpm) and fatty acid transport protein 1 (FATP1) and FATP4)[132], which have varying capacities for increasing NEFA uptake and oxidation in muscle[133]. Upon exercise, the exocytosis of CD36 (and possibly other transporters) seems independent of AMPK[134] but could involve calcium/CaMK kinase (CaMKK)[135] and MAPK/ERK kinase 1 (MEK1) and MEK2 signalling[136]. Furthermore, although FABPpm is structurally identical to mitochondrial aspartate aminotransferase (mAspAT), these proteins

serve distinct functions within their respective subcellular compartments (that is, FABPpm-mediated transport of long-chain NEFAs across the sarcolemma versus mAspAT-based delivery of reducing equivalents into mitochondria)[137].

Compared to the situation at rest, NEFAs entering exercising muscle are preferentially directed towards breakdown rather than to re-esterification and storage[138]. Before being used by downstream metabolic pathways, long-chain NEFAs must be activated by thioesterification to long-chain fatty acyl-CoA. Several fatty acid transport proteins possess intrinsic acyl-CoA synthetase activity[139], but acyl-CoA synthetase long-chain family member 1 (ACSL1) is the predominant and critical ACSL isoform in muscle[140]. As the inner mitochondrial membrane is impermeable to long-chain NEFAs, long-chain fatty acyl-CoA molecules are delivered into the mitochondrial matrix through the ‘carnitine shuttle’, where they undergo subsequent β-oxidation and OXPHOS.

Prolonged high-intensity exercise resulting in elevated rates of lactate production might directly impede IMCL lipolysis over time by downregulating cAMP–PKA signalling[141]. However, the glycolytic flux of higher-intensity exercise most probably outcompetes NEFAs for oxidation by depleting the muscle free-carnitine pool[72,142]. This limits the capacity of the carnitine shuttle and thus impairs the mitochondrial import of long-chain fatty acids (Fig. 2b). Longer exercise durations, lower intramuscular glycogen stores, higher dietary fat intakes and greater type I fibre abundance and aerobic fitness ($\dot{V}O_{2max}$) levels can reduce carbohydrate reliance and increase fat utilization during physical activity[120]. Nevertheless, although these factors can delay the transition from predominant fat usage to predominant carbohydrate usage (referred to as the substrate ‘crossover point’), the biphasic pattern of NEFA oxidation with exercise intensity[71,72] is ultimately maintained[77,143].

Biological rhythms and skeletal muscle

Normal circadian fluctuations in behaviour and physiology — such as sleep–wake cycles, nutritional state, body temperature, cardiovascular function, and tissue production and sensitivity to hormones (Supplementary Table 1) — coincide to influence acute exercise capacity[144] and response in a time-specific, tissue-specific manner[20]. Endogenously generated circadian rhythms are predominantly maintained by photic (light-dependent) cues relayed through the central pacemaker (hypothalamic suprachiasmatic nucleus)[145] in concert with cell-autonomous clocks in peripheral tissues, including skeletal muscle (reviewed in ref. 146). Muscle clocks regulate transcriptional programmes that prepare the tissue for transitions between fasting and feeding[147–149] and orchestrate 24-h rhythms in muscle glucose[147,149], lipid[148,149] and amino acid[148] metabolism. Consequently, muscle clock disruption in mice impairs muscle insulin sensitivity[147,150] and metabolic homeostasis[147,148,150].

Hormonal, metabolic and temperature-dependent changes associated with exercise shift the central pacemaker[151] and impact circadian clocks in cell types throughout the body[152]. In human vastus lateralis muscle, acute endurance[153] and resistance[153,154] exercise upregulates the core clock genes *CRY1* and *PER2*, in part through Ca^{2+}-mediated activation of cAMP response element-binding protein (CREB)[153]. Reciprocally, physical activity patterns

directly modulate the expression, phase and/or amplitude of ~15–20% of all rhythmic genes in mouse soleus and tibialis anterior muscles independently of the muscle clock[155]. This occurs through transcription factors such as NFATc1 that are stimulated by motoneuronal firing patterns and downstream Ca^{2+} dynamics[155]. Together, these studies provide a mechanistic and biochemical rationale for observed time-dependent variations in human athletic performance (reviewed in ref. 156) and the metabolic disturbances[157,158] and increased risk of cardiovascular disease[159] resulting from circadian misalignment.

A high-intensity exercise intervention in humans was shown to offset the detrimental impact of short-term sleep restriction on whole-body glucose tolerance and mitochondrial respiration in permeabilized muscle fibres[160]. Treadmill running exercise in mice also stimulated hypoxia-inducible factor-1α (HIF1α) and a broad range of muscle transcriptomic[161] and metabolic[20,161] responses in a time-dependent manner. Such findings have prompted researchers to begin investigating exercise as a therapy to promote circadian alignment and ameliorate metabolic disease. Preliminary human evidence indicates that training in the afternoon or evening might enhance the beneficial effects of exercise on aspects of insulin sensitivity[162,163] and blood glucose control[164]. Furthermore, moderate-to-vigorous physical activity imparts greater risk reductions for cardiovascular disease and all-cause mortality when ≥50% of the total activity volume is undertaken after 11:00 h (ref. 165).

Although rapidly emerging, this branch of ‘chrono-therapeutics’ is still in its infancy, and precise protocols to elicit specific metabolic outcomes are only beginning to come to fruition[20]. Additionally, there is large population-level variation in human chronotypes[166], and individual chronotype may interact with diurnal exercise timing to modify acute responses and longer-term adaptations. Each age group contains a normal distribution of early-to-late chronotypes, yet the average chronotype differs according to biological sex (Supplementary Box 2) and changes during ageing[166] (Box 2). Individuals with later chronotypes could benefit from exercise-induced phase advances irrespective of exercise timing, whereas earlier chronotypes might suffer circadian misalignment with evening exercise[151]. Moving forward, chronotype will be an important covariate to consider in exercise biology, especially for the goal of making personalized therapeutic recommendations.

Skeletal muscle responses to acute exercise

The perturbation of systemic[19,124] and local[55] homeostasis posed by acute exercise triggers an integrated series of metabolic, hormonal, growth factor-related, inflammatory and mechanosensitive events. In this section we discuss how these stimuli converge to temporally regulate the acute post-exercise adaptive landscape in muscle — from signal transduction[90] to gene expression[167–169] (Fig. 3).

Post-exercise signalling

Sprint and resistance exercise impart stronger whole-body stress responses and more robustly impact the muscle phosphoproteome than continuous endurance exercise does, although there is similarity in the immediate post-exercise signature[90]. Over 400

phosphorylation sites on >200 proteins were commonly altered among these exercise modalities[90], leading to a shared enrichment of canonical exercise regulatory kinases, including CaMK, AMPK, mammalian target of rapamycin (mTOR), mitogen-activated protein kinase (MAPK), PKA and PKC[90,170]. Much of this post-exercise pathway convergence is conserved in contracted (exercised) rat and mouse muscle, despite little overlap at specific phosphorylation sites[171].

Modality-driven divergence in the phosphoproteome becomes more apparent during the 3-h recovery period after exercise[90] and may facilitate distinct muscle adaptations with endurance, sprint interval or resistance training. Phosphorylation of rapamycin-sensitive substrates of mTOR complex 1 (mTORC1) and p38 MAPK are potentiated after resistance exercise[90]. mTORC1 is a central regulator of muscle hypertrophy that critically permits mechanical overload-induced myofibrillar accretion[172]. In murine muscle, mTORC1 activity is in part mediated by mechanostimulated diacyl glycerol kinase-ζ (DGKζ) production of phosphatidic acid[173] and raptor-dependent translocation of the catalytic component of mTORC1 to the late endosomal and lysosomal system[172]. By contrast, mTOR in human muscle already associates with the lysosome, and resistance exercise does not further augment this interaction[174]. Thus, the contraction-induced migration of the mTOR–lysosomal complex to focal adhesions[175] and microvasculature[174] at the sarcolemma may be a more important step in humans[174–176]. Here, mTOR associates with RAS homologue enriched in brain (RHEB) and eukaryotic translation initiation factor 3 (EIF3)[174], and activates downstream target ribosomal protein S6 (RPS6)[175]. The cooperation between mTOR–EIF3 (ref. 174) and Ser235 and Ser236 phosphorylation of RPS6 (ref. 175) at the fibre periphery was greater when a post-exercise protein and carbohydrate beverage was consumed, and this interplay could contribute towards the nutrient-sensitizing effects of resistance exercise on mTORC1 activation[6,177].

Complementary contraction-responsive mechanisms act in tandem with mTORC1 to promote gains in muscle mass[6,178–180]. For example, sarcomere shortening recruits the β-isoform of MAP3K20 (also known as ZAKβ) to the Z-disc, resulting in downstream stimulatory phosphorylation of p38 MAPK and JUN N-terminal kinase 1 (JNK1) and JNK2 (ref. 179). Inhibition mediated by JNK1 and JNK2 (ref. 181) and/or Notch[182]-mediated inhibition of myostatin–suppressor of mothers against decapentaplegic (SMAD) signalling is a purported molecular ‘switch’ in muscle, promoting resistance-type over endurance-type adaptations by preventing SMAD complex nuclear translocation[181]. This suppression of the myostatin–transforming growth factor-β (TGFβ) pathway might be distinct from the regulation of myostatin gene expression[182], as both resistance and endurance training downregulate myostatin mRNA levels in human muscle[183] despite contrasting hypertrophic phenotypes over time[13].

Endurance training has been suggested to impair resistance training adaptations through AMPK-mediated blunting of mTORC1. Although this biochemical reaction is known to occur, exercise induction of both AMPK and mTORC1 signalling is consistent with meta-analyses indicating little, if any, interference effect of concurrent exercise on muscle hypertrophy[184,185]. Bidirectional AMPK–mTORC1 phosphorylation[177] could coordinate

simultaneous[90] or temporally distinct[177] post-exercise activity and downstream substrate specificity of these kinases.

Physiological proteome and mitochondrial remodelling with exercise requires the turnover of select proteins, and autophagic and proteasomal trafficking are integral to these processes. Indeed, genetic disruption of proteostasis results in the accumulation of damaged proteins and impairs force production in fast-twitch extensor digitorum longus and slow-twitch soleus mouse muscle[186]. Treadmill running exercise increases the activity of an AMPK complex composed of α_1, α_2, β_2 and γ_1 isoforms located on the outer mitochondrial membrane ('mitoAMPK') that could spatially regulate quality control through mitophagy across the mitochondrial reticulum[187]. This pool of mitoAMPK is conserved in human muscle[187] and may govern mitophagy through phosphorylation of downstream substrates such as UNC-51-like autophagy activating kinase 1 (ULK1)[188] and mitochondrial fission factor (MFF)[189]. In humans, acute high-intensity[190,191] and sprint interval[90] exercise rapidly and transiently activates ubiquitin-proteasomal degradation in a cAMP–PKA-dependent manner[190,191] that requires the proteasomal subunit PSD11 (ref. 190). cAMP can then upregulate enzymatic activity of E3 ubiquitin ligases for continued removal of damaged proteins in the post-exercise recovery period[191] (Fig. 3). Coordinated proteolytic signalling is further detected 3 h after resistance exercise, as implied by co-regulated phosphorylation of proteins in the N-end rule, calpain regulation and ubiquitin receptor RAD23 pathways[90].

Many exercise-responsive phospho-sites have yet to be functionally interrogated in muscle[90,170,178]. Additionally, there are numerous other protein modifications such as arginine methylation, ubiquitin-like modification, ribosylation and acylation that remain relatively understudied in the context of physical activity despite presumably coinciding to influence muscle phenotype. Deeper coverage of the posttranslational proteome combined with the use of subcellularly targeted biosensors[187] should enhance the spatial understanding of how integrated post-exercise signalling governs unique exercise adaptations in muscle, and how these networks change with exercise training over time.

Epigenetic regulation of exercise adaptation

Histone modifications and DNA methylation.—Upstream signalling, together with an altered intramuscular metabolic milieu, can change the chromatin landscape in muscle and thus influence transcription factor-driven gene expression in response to exercise (reviewed in ref. 192). Epigenetic modifications on amino acids in histone tails or globular core domains render a permissive or repressive chromatin state depending on the type of modification and the residue and/or histone protein being targeted[192]. An acute bout of endurance exercise can increase H3K36 acetylation[193], a mark of euchromatin, and promote CaMKII-dependent phosphorylation[194,195] and nuclear exclusion of the transcriptionally repressive class II histone deacetylases (HDAC) HDAC4 and HDAC5 (ref. 193). High-intensity resistance exercise also transiently stimulates myonuclear H3S10 phosphorylation through p38 MAPK and mitogen and stress-activated protein kinase 1 (MSK1) in human muscle[196]. This same axis was necessary for the upregulation of myocardin-related transcription factor B (MRTFB)–serum response factor (SRF)-target genes and protein synthesis after in vivo contractions in mice[196].

Post-exercise histone modifications coalesce with changes in DNA methylation, typically at the 5′ end of cytosine residues at CpG sites throughout the genome. DNA methylation in *cis*-acting regulatory regions is commonly associated with gene silencing, but some transcription factors, particularly of the homeodomain POU and NFAT families, favour methylated CpG sequences[197]. Acute exercise[198–200] and exercise training[199,201] induce both hypomethylation and hypermethylation of DNA in muscle, mostly within gene bodies, intergenic regions and enhancer regions[201]. The greatest number of CpG-site methylation changes and the highest percentage of mRNA transcripts with inversed DNA methylation patterns occurred 3 h after a single session of resistance exercise, irrespective of workload (80% or 30% of 1 RM)[200]. These changes began to revert towards baseline methylation levels by 6 h[200], suggesting that the muscle methylome is dynamic and tightly regulated during recovery from exercise. Yet, select DNA hypomethylation and hypermethylation events are sustained after acute resistance exercise and resistance training, providing a plausible mechanism for a 'muscle memory' of previous exercise that facilitates future adaptation[199]. Modality-dependent DNA methylation signatures might also direct specific post-exercise transcriptional programmes in muscle, as acute bouts of resistance[199] and sprinting-type[202] exercise share enrichment for only 36 of the top 100 differentially methylated pathways.

Conceivably, exercise-induced metabolic fluxes, which stimulate glycolysis and OXPHOS, could alter acetyl and methyl group availability, cellular [NAD^+] to [NADH] redox state and the activity of 'writers' and 'erasers' of epigenetic marks in muscle[192]. However, the current understanding of exercise-regulated DNA methylation and chromatin remodelling is incomplete. For example, lactate modifies ('lactylates') H3K18 in promoters and enhancers of genes related to tissue development and metabolism in human muscle[203], but whether lactylation of histone lysine residues contributes to exercise adaptation remains to be ascertained.

Non-coding RNAs.—In addition to protein-coding genes, RNA polymerase II transcriptional activity produces microRNAs (miRNAs; ≤22 nucleotides) and long non-coding RNAs (lncRNAs; ≥200 nucleotides) that alter mRNA levels and protein abundance without influencing the genetic code. Many miRNAs[204] and lncRNAs[205] are exercise-responsive in muscle, and both classes seem involved in aspects of myogenesis. During resistance exercise, the downregulation of the muscle-enriched miRNA myomiR-1, through its contraction-mediated release in extracellular vesicles, may facilitate hypertrophy[206]. Once in circulation, myomiR-1 could act upon adipocytes to potentiate adrenergic signalling and lipolysis[206]. Still, muscle was not a major source of bloodborne extracellular vesicles upon resistance exercise in humans[207] or treadmill running exercise in mice[208]. Rather, muscle-derived extracellular vesicles — loaded with myomiRs — preferentially accumulated in the interstitial space, and treatment with these vesicles promoted differentiation of myoblasts in vitro[208]. Therefore, extracellular vesicles originating from muscle might play a more prominent role in local cellular communication (Supplementary Fig. 3). Satellite cells may signal to muscle fibres[209] and fibroadipogenic progenitor cells[210] using extracellular vesicles containing myomiR-206. In mice, this was shown to regulate the levels of extracellular matrix (ECM)-related genes (for example, *Mmp9*) in muscle

fibres[209] and *Rrbp1* in fibroadipogenic progenitor cells[210] to coordinate physiological ECM remodelling during the early stages of hypertrophy. However, the ability of extracellular vesicles to deliver biologically active miRNA cargo into recipient cells remains contentious[211] and their role in exercise adaptation warrants further interrogation.

Though the impact of lncRNAs in human muscle is less studied than that of miRNAs, lncRNAs respond to endurance, resistance, concurrent and high-intensity interval training with distinct differential expression profiles between modalities[205]. The lncRNA *CYTOR* is induced by endurance and resistance exercise in human muscle and can regulate fast-twitch fibre formation in rodents by sequestering TEA domain family member 1 (TEAD1) transcriptional activity[212]. *Cytor* mRNA is decreased upon ageing and overexpression of this lncRNA increased type IIA and type IIB fibre abundance, which was sufficient to improve grip strength and uphill running performance in aged mice[212]. RNAs putatively annotated as lncRNAs may also contain short open reading frames that encode for micropeptides (≤150 amino acids). The muscle-specific micropeptides dwarf open reading frame[213] and myoregulin[214] competitively interact with sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) to positively and negatively affect SR Ca^{2+} kinetics and muscle contractility, respectively. Other classes of non-coding RNAs, including circular RNAs and tRNA fragments, await future study in the context of human muscle exercise adaptation.

mRNA transport and myonuclear propagation—The dense myofibrillar lattice in adult muscle fibres may impede diffusion of mRNAs expressed from myonuclei. Indeed, recent evidence suggests that mRNA distribution away from the perinuclear region is almost entirely dependent on microtubule networks in muscle, whereas diffusion has a minor role[215,216]. Transport might be reliant on motor proteins, reminiscent of cardiomyocytes that use kinesin-1-mediated transport of mRNA and ribosomes to direct protein translation to specific sites within the cell[217]. Microtubule-based trafficking of mRNA facilitates transcript distribution through the crowded sarcoplasmic space, but dispersion of mRNAs from progenitor myonuclei is still potentially confined to ≤50 μm (refs. 215,216). By contrast, the movement of proteins translated from transcripts derived from a specific myonucleus could be ≥5 times more far-reaching[218].

The use of fluorescent reporters has shown that nuclear proteins can be detected in surrounding myonuclei and in those distant from the nucleus of origin[218–220], a phenomenon termed ‘nuclear propagation’[218]. Smaller proteins (≤40 kDa) that freely diffuse across the nuclear pore complex can propagate to many myonuclei throughout a myotube in vitro[218]. Conversely, larger proteins (>40–60 kDa) are limited in their propagation potential, possibly due to increasing reliance on nuclear transport receptors to traverse the nuclear pore complex[218]. Protein properties (such as size) and myonuclear characteristics (such as shape or location within the muscle fibre) probably interact to control nuclear propagation. For example, myonuclei in ex vivo fibres differ in capacity for classical versus non-classical nuclear import, resulting in a gradient of import rates between myonuclei of the same fibre[219].

The potential for myonuclear propagation casts further doubt on strict myonuclear domains in muscle and represents an additional layer of spatiotemporal control, combining fibre-

wide communication with myonuclei-autonomous processes in response to adaptive cues. Furthermore, resident myonuclei may be capable of endoreplication in mouse muscle (resulting in polyploidy), which could support efficient DNA synthesis under heightened transcriptional demand[221]. Myonuclear propagation was detected after wheel-running exercise training[220] and synergistic ablation-induced hypertrophy increased the total number (from ~0.7–1% to ~3%) of DNA-replicating myonuclei[221] in murine muscle. The extent to which these processes occur under physiological conditions in humans remains unclear.

The post-exercise transcriptome—Considerable effort has been dedicated towards characterizing the transcriptional response of human muscle to exercise, and several key studies are highlighted in Supplementary Table 2. High-powered meta-analyses have curated much of this scientific endeavour to generate deeper insight into exercise adaptation[167,183]. Acute exercise initiates temporal clusters of gene expression[167–169], including early induction of stress response genes, increased expression of muscle adaptation genes in early and middle stages following exercise[167,169], and later[167] or sustained[168] increases in immune-related signatures. These transcriptional programmes are driven by combinations of different transcription factors[167,169] and alternative transcription start-site usage[169]. Akin to the phosphoproteome[90], a single bout of resistance exercise alters more transcripts than does moderate-intensity endurance exercise[183]. However, there is considerable overlap in pathway enrichments, and both training modalities upregulate stress response genes, kinase activity and metabolism-related processes, among others[183].

NR4A3 (also known as *NOR1*)[222] and *PGC1A*[223] are genes commonly induced after endurance and resistance exercise[183] and mainly promote oxidative muscle adaptations. *NR4A3* and *PGC1A* have similar temporal expression patterns after exercise, peaking ~2–3 h into the recovery period[167,183], suggesting common upstream regulation through CREB[224,225]. The nuclear receptor subfamily 4 group A (NR4A) family (including NR4A1 (also known as NUR77) and NR4A2 (also known as NURR1)) share >90% homology in their DNA-binding domains and can interact as monomers with a single NGFI-B response element[226], or as homodimers and heterodimers (alongside other NR4A members) with the Nur response element[227]. *NR4A1* is also exercise-responsive in muscle[167,202] and may serve partial redundancy with NR4A3, as implied by the slow-oxidative phenotypes of mice expressing either *Nr4a1* (ref. 228) or *Nr4a3* (ref. 222) from skeletal muscle-specific promoters.

The PGC1α family of coactivators consists of different isoforms, which are derived from alternative promoter use or splicing events (reviewed in ref. 223). Endurance exercise preferentially targets[229] and hypomethylates[230] the proximal PGC1α promoter in muscle, increasing PGC1α isoform 1 (PGC1α_1) expression. PGC1α_1 drives mitochondrial biogenesis and oxidative fibre properties[48] at least in part through C-terminal domain RNA binding, which facilitates recruitment in transcriptionally active chromatin condensates[231]. A bout of endurance exercise also leads to promoter region hypomethylation of other key mitochondrial and metabolic genes, including *TFAM* and *PPARD*, with corresponding increases in mRNA expression[230]. The PPARδ-mediated upregulation of lipid metabolism

and suppression of glycolytic processes in muscle[232] is largely dependent on protein–protein interaction with Krüppel-like factor 15 (KLF15)[233].

Most PGC1α isoforms have overlapping roles in muscle[223]. The truncated variant PGC1α isoform 4 (PGC1α$_4$) differs in that it can be induced by intramitochondrial [Ca^{2+}][234] and is transcribed from the alternative PGC1α promoter after resistance exercise[229,235] (Fig. 3). PGC1α$_4$ facilitates muscle hypertrophy[229,234,235] and a PPARβ–AMPK-dependent upregulation of glycolytic metabolism[235], which could enhance the anaerobic capacity of muscle after resistance training. Incidentally, the accretion of muscle mass often coincides with a glycolytic switch that may support anabolism by increasing de novo synthesis of nucleotides and non-essential amino acids from intermediates in glucose metabolism (reviewed in ref. 236).

An acute bout of resistance exercise also stimulates *MYC* mRNA expression and the hypomethylation of enhancer and MYC-accessible intergenic regions of ribosomal DNA[198]. These events coalesce to augment pre-45S rRNA transcription and ribosomal biogenesis[180,198]. Whereas mTORC1 signalling promotes translational efficiency[172,180] and directs ribosomal function through Ser235 and Ser236 phosphorylation of RPS6 (ref. 237), total ribosomal content[180] and the altered composition of ribosomal proteins (for example, an increased ratio of RPL3 to RPL3L)[238] determines translational capacity in muscle. Collectively, upregulation of the sarcoplasmic and mitochondrial protein synthetic machinery (such as mito-ribosomes) enables the beneficial proteome remodelling that occurs with high-intensity interval, resistance or combined exercise training[12].

Skeletal muscle adaptations to long-term exercise

In this section, we contextualize the impact of consistent exercise (exercise training) on muscle phenotype, considering general and select adaptations to specific training interventions and the influence of muscle fibre type[14]. We then summarize how muscle-related adaptations can contribute to improvements in systemic measures of exercise performance and discuss whether altered molecular perturbations in muscle with exercise training[33,239–241] reflect an attenuated or refined adaptive state.

Transcriptome, proteome and contractile property adaptations—Repeated exposure to specific exercise modalities results in shared and divergent alterations in muscle over time[12,183] (Fig. 3). At the transcriptomic level, pathways related to ECM remodelling, angiogenesis and the TCA cycle are enriched in muscle after exercise training[167]. Whereas resistance exercise robustly effects ECM reorganization[183,242] and growth-related pathways[242], endurance training favourably impacts oxidative metabolic processes and the gene expression of mitochondrial complex subunits[183]. Crosstalk between the various cell populations in muscle appears particularly important for exercise-associated adaptations within the interstitial space (Supplementary Fig. 3).

Training-induced improvements in muscle respiration often coincide with expansion and modification of the mitochondrial proteome[12,15,143] in type I and type II fibres[14]. Resistance training can augment the respiratory and ATP-producing capacity of mitochondria[243], but

the effects on mitochondrial phenotype are typically less pronounced than those observed after high-intensity interval[12,15,143] and endurance[14] training. The total volume of endurance exercise performed during a training intervention might be the strongest stimulus for mitochondrial biogenesis in muscle (reviewed in ref. 244). This could be related to the additive effects of proximal exercise bouts on the nuclear enrichment of transcriptional regulators of mitochondrial abundance (for example, PGC1α_1 and transcription factor EB (TFEB))[245] and the upregulation of mito-ribosomes[12,14,15] and mitochondrial protein synthesis[12]. During mitochondrial biogenesis, stoichiometry of the ETC is maintained through proportional translation of ETC complex subunits from the mitochondrial and nuclear genomes[246]. Increased expression of mitochondrial transit peptides and translocase proteins of the outer and inner mitochondrial membrane following endurance training[14] facilitates the transport of nuclear-encoded precursor components into the mitochondrial matrix. However, in the early stages of mitochondrial remodelling, synthesis of fatty acid oxidation and TCA cycle enzymes may be prioritized over ETC machinery biogenesis, to enhance reducing equivalent NADH and $FADH_2$ production and electron delivery to OXPHOS[15].

High-intensity interval training at ≥90% of maximum power output ($\dot{W}_{max}$) most consistently increases normalized (mass-specific) mitochondrial respiration[244], with intensities ≥100% $\dot{W}_{max}$ (as in sprint interval training) doing so more efficiently per unit of time spent exercising[244]. The impact of intensity on mitochondrial respiration appears uncoupled from total training volume, suggesting that these variables drive complementary but somewhat distinct mitochondrial adaptations[244]. High-intensity interval training also induces lysine acetylation of TCA cycle and ETC proteins[143], although whether such posttranslational modifications contribute towards intensity-dependent changes in mitochondrial function requires additional study.

After endurance training, some discrete differences can be seen in the adaptive response of type I and type II fibres[14]. Still, most of the commonly detected exercise-regulated proteins related to mitochondrial and glucose metabolism behaved similarly between fibre types[14]. For example, training upregulated ACSL1, malate/aspartate shuttle proteins, the mitochondrial CKM isoform CKMT2, PDH, CRAT and LDHB in both type I and type II fibres[14]. *LDHB* mRNA is increased after endurance[247] and resistance[235] training, potentially through PGC1α_1 coactivation of distal myocyte enhancer factor 2 (MEF2) and proximal oestrogen-related receptor (ERR) binding sites in the *LDHB* promoter[247]. LDHB preferentially catalyses the conversion of lactate to pyruvate and, together with enhanced mitochondrial density, could improve lactate clearance in trained muscle through higher rates of conversion to pyruvate and subsequent oxidation[247] (Fig. 2c). Further refinement of substrate handling with programmed exercise is implied by greater expression of key proteins of metabolic pathways involving glycogen (for example, glycogen synthase)[11], NAD^+ (nicotinamide phosphoribosyltransferase) and branched-chain amino acid (branched-chain α-ketoacid dehydrogenase kinase) metabolism, as well as ubiquinone biosynthesis (5-demethoxyubiquinone hydroxylase)[143], after high-intensity interval training.

Muscle contractile properties are likewise affected by regular exercise in a somewhat training modality-dependent manner. Protein isoforms regulating SR Ca^{2+} release[143] and myofibrillar Ca^{2+} sensitivity[14,143] were altered after moderate-intensity endurance[14] and high-intensity interval[143] training, coincident with a reduction in fibre size-adjusted quadriceps peak-twitch torque and prolonged half-relaxation time[11] — indicative of a slower muscle phenotype. Conversely, stretch-shortening cycle (ballistics or plyometrics) training increased contractile velocity, force production and thus peak power of single type I, type IIA and type IIA/X fibres[42]. Exercise modalities commonly decrease *MYH1* mRNA[183] and protein (MyHC-IIX)[11,13] content in muscle. However, the upregulation of *MYH7* gene expression[183] and type I fibre abundance after endurance training[44] can be in contrast to the effects of resistance training, which often downregulates *MYH7* (ref. 183) with no corresponding change in the prevalence of type I fibres[45]. This is consistent with endurance[44] and resistance[45] exercise differentially shifting hybrid fibres towards pure type I and pure type IIA fibres, respectively (reviewed in ref. 38). Indeed, endurance training resulted in the modification of more proteins in type I than in type II fibres[14], and long-term exposure to a particular exercise stimulus[41] may largely explain the majority of type I or type II fibres detected in biopsies from endurance or strength athletes[13].

Training-induced adaptations impact exercise performance—Together, exercise training adaptations in muscle contribute towards. improvements in gross performance measures such as $\dot{V}O_{2max}$ (refs. 12,14,15), peak power output[11,143], ballistic power[42] and strength[12,13]. Endurance and resistance training increase muscle capillarization[248–250] and endurance-type training enhances NEFA oxidation at a given exercise intensity[77,120,143,251]. These glycogen-sparing and metabolite-buffering (for example, lactate clearing)[247] effects render muscle more robust to disturbances in metabolic homeostasis, delaying mechanisms of peripheral and central fatigue[94] and raising exercise tolerance and capacity (that is, higher velocities and percentages of $\dot{V}O_{2max}$ produced at ventilatory thresholds, and greater maximal running speeds or distances covered)[251]. Metabolomics analysis also implies that 4 weeks (13 bouts) of resistance exercise may suffice to modify the acute muscle metabolome[252]. Yet, the specific metabolic changes that occur with resistance training are not extensively characterized and warrant further interrogation.

High-intensity interval[11,12] and endurance[13] training both induce a degree of muscle hypertrophy, but gains in muscle mass and strength are less pronounced than seen with long-term resistance exercise[13]. Conversely, any beneficial effect of resistance training on $\dot{V}O_{2max}$ appears limited to individuals with lower initial aerobic fitness levels (for example, a $\dot{V}O_{2max} \leq 25$ or ≤40 mL kg^{-1} min^{-1} in adults >60 and ~20–35 years of age, respectively) (reviewed in ref. 253) and thus high-intensity interval[12] and endurance training[13,248] typically increase $\dot{V}O_{2max}$ to greater extents.

A period of aerobic pre-conditioning can potentiate the hypertrophic effects of resistance exercise[248], in part by enhancing fibre capillary density[248,250] (Supplementary Fig. 3). Alternatively, high-intensity resistance training (≥90% of 1 RM or ≤4 RM) can improve running economy (that is, reduce the metabolic or $\dot{V}O_2$ cost of running)[254]. These complementary adaptations further emphasize the utility of incorporating numerous exercise

(sub)modalities into a sustainable routine for maximum performance[12] and protective health benefits[18].

Adaptive responses in muscle are modified with exercise training— The recurring stimulus of exercise training dampens select signalling[239,240] and transcriptomic[33,241] responses to an acute bout of physical activity. This is evident after just nine sessions of high-intensity interval exercise through a modest reduction in the number (~17%) and amplitude (~30%) of altered mRNA transcripts in human muscle compared to the first exercise bout, including glycolysis pathway and HIF1α target genes[241]. The magnitude of change in the muscle transcriptome with exercise training becomes even clearer over 30 daily sessions of electrically induced mechanical overload in rat hindlimb muscle[33]. In particular, the directionality of altered genes contrasted substantially between exercise naive and trained muscle. At the 1-h sampling time point after exercise, ~70% of the (~2400) differentially expressed transcripts were upregulated on day 2 compared with ~83% (of ~3300 genes) downregulated on day 30 (ref. 33). This indicates that certain signalling mechanisms are sensitive to the modified intracellular environment after a period of regular exercise. In combination with genetic predisposition (Box 1), blunted post-exercise molecular responses probably converge to limit the adaptive potential of muscle, slowing progress with advanced training experience. Nevertheless, some alterations might reflect a refined (as opposed to impaired) response.

Pathways related to ribosomal biogenesis and protein synthesis are positively enriched in the early adaptive stages in exercised rat muscle[33] but become downregulated in favour of metabolism-related processes once hypertrophy plateaus[33]. Rates of myofibrillar protein synthesis also correlate better with measures of muscle mass accrual after 8 weeks[176] to 10 weeks[255] of resistance training in humans, corresponding to the attenuation of exercise-induced muscle damage[255]. Furthermore, although 20 days (40 sessions) of high-frequency, high-intensity interval training reduced the post-exercise upregulation of nuclear $PGC1\alpha_1$ and p53 phosphorylation at Ser15, resting levels of these same markers were greater in human muscle after the exercise intervention[239]. Phosphorylated p53 can translocate to the mitochondrial genome where it interacts with transcription factor A, mitochondrial (TFAM) to regulate mitochondrial transcription[256] (Fig. 3). Muscle-specific *Trp53*-knockout mice have reduced basal mitochondrial contents and enzymatic activities but retain the capacity for exercise-stimulated mitochondrial biogenesis[257]. This suggests that p53 may be more important for the maintenance of muscle mitochondrial integrity than exercise adaptation.

Given these diverse modifications, longer-duration trials are needed to fully elucidate the modality-dependent temporal landscape of exercise adaptation in human muscle and to highlight specific changes in the acute exercise response with consistent training.

Future directions

The complex and intricate macrostructure to ultrastructure of skeletal muscle allows for a high degree of metabolic flexibility during exercise. Further study of the regulation and interaction of key cellular components, such as myofibrillar and mitochondrial networks[53], should enable more comprehensive understanding of how muscles mount such a malleable

response. The use of fluorescent metabolite[258] and myofilament[259] biosensors could aid this effort by elucidating real-time dynamics of muscle metabolism and contraction.

Current knowledge of exercise adaptation is heavily biased towards canonical regulators such as AMPK, PGC1s and mTORC1. Although these factors are integral to muscle remodelling with exercise, aspects of adaptation are maintained in the absence of AMPKα_1 and AMPKα_2 (ref. 260) and of PGC1α and PGC1β (ref. 261), and rapamycin-insensitive pathways also contribute towards muscle hypertrophy[178,180]. Most of the exercise-induced phosphoproteome remains relatively unexplored[90,170,178], and its functional interrogation will help to identify redundancies in adaptative signalling and new players in the distinct and complementary effects of resistance, endurance and high-intensity exercise.

As exercise biology moves increasingly towards systems-level profiling, the multiplexing of new technologies could provide invaluable insight into the spatiotemporal control of gene expression[262], translation[263] and metabolism[37] in discrete muscle cell populations. If performed in tandem with commensurate functional assays, this would enable causal association of specific cellular events with their phenotypic impact.

Exercise is an effective adjunct therapy for certain neurodegenerative[264] and mental health[265] disorders and offers protection against cardiometabolic disease[266], sarcopenia (Box 2) and all-cause mortality[18]. Multicentre interventions including spectrums of chronological age, metabolic health, chronotype, ethnicity, biological sex and social gender will move the field closer to uncovering the dynamic changes that coordinate the benefits of exercise training and further define the role of muscle in the integrative exercise response. Knowledge derived from such approaches should not only inform personalized exercise prescription, but also reveal new molecular avenues of drug discovery for the improvement of human health.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Glossary

Adrenoceptor

Transmembrane G-protein-coupled adrenergic receptors (GPCRs) that mediate the actions of the endogenous catecholamines adrenaline and noradrenaline. There are nine subtypes of adrenoceptors: α_{1A}, α_{1B}, α_{1D}, α_{2A}, α_{2B}, α_{2C}, β_1, β_2 and β_3. The α_{2A} and α_{2C} adrenoceptors regulate presynaptic neurotransmitter release from central adrenergic and peripheral sympathetic nerves

Ergogenic
A performance-enhancing effect

Hypertrophy
Typically refers to an increase in the cross-sectional area (or radial growth) of muscle fibres, resulting in gains of skeletal muscle mass in response to mechanical loading activities, such as resistance exercise

Ketone body
A lipid-derived, water-soluble, organic compound produced in the liver that can be used as an alternative energy source by extra-hepatic tissues — predominantly the brain, but also heart and skeletal muscle

Maximal oxygen consumption
($\dot{V}O_{2max}$). The maximum volume of oxygen (ml kg^{-1} min^{-1}) that can be inspired and utilized during exhaustive exercise, such that the value ($\dot{V}O_2$) plateaus despite increasing workloads. $\dot{V}O_{2max}$ is a measure of aerobic or cardiorespiratory fitness and is commonly used to standardize exercise intensity for clinical trials (for example, *x*% of $\dot{V}O_{2max}$)

Mitophagy
A specific form of lysosome-dependent catabolism (autophagy), through which damaged mitochondria are selectively removed. Mitophagy of the mitochondrial reticulum has an essential role in maintaining cellular energy homeostasis

Muscle spindles
Structures embedded in most mammalian skeletal muscles that continuously relay proprioceptive information regarding muscle length and movement to the central nervous system. Muscle spindles consist of intrafusal muscle fibres enclosed within a capsule layer and are distinct from the extrafusal muscle fibres discussed in this Review

Non-esterified fatty acids
(NEFAs). A metabolic substrate utilized by muscle at rest and in an intensity-dependent manner during exercise

Peak-twitch torque
The force produced by muscle (through a moment arm) evoked by a single electrical stimulation from, for example, applied electrodes

Phosphagen system
A rapid energy-producing pathway comprising the ATP regenerating adenylate kinase (ADP+ADP ⇌ ATP + AMP) and creatine kinase (CrP+ADP ⇌ ATP + Cr) reactions. Of these reactions, creatine kinase has a greater capacity for ATP resynthesis in muscle due to the availability of creatine phosphate stores

Proprioceptive
Able to sense intrinsic information regarding bodily position and locomotion. The primary proprioceptive sensory organ of the body is the muscle spindle

Proton-motive force

The proton electrochemical gradient in mitochondria consisting of an electrical charge gradient (also known as the ‘membrane potential’) and a pH gradient. The proton-motive force is generated by the proton-pumping action of respiratory complexes across the inner mitochondrial membrane and couples substrate oxidation to ATP generation

Transverse tubules

(T-tubules). Invaginations in the sarcolemmal membrane that insert between myofibrils. T-tubules tightly associate with two terminal cisternae (calcium-releasing regions) of the sarcoplasmic reticulum, forming the ‘triads’, which are essential for excitation–contraction coupling

Voluntary force production

The conscious or ‘voluntary’ production of muscle force (in other words, not triggered by exogenous electrical stimulation)

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Box 1

Different exercise (sub)types and training response heterogeneity in humans

Exercise (sub)types

Exercise can be broadly classified as resistance, cardiorespiratory, balance and flexibility-based. Flexibility and balance are important aspects of physical fitness contributing to fall prevention in older individuals[282]. However, discussion herein is dedicated to iterations of resistance and cardiorespiratory exercise, which are the main focus of this Review.

Resistance exercise

Traditional resistance exercise entails repetitions of dynamic concentric (muscle-shortening) and eccentric (muscle-lengthening) contractions against external load and is an effective intervention to increase skeletal muscle mass and strength[249,283]. The total amount (volume), frequency and intensity of exercise are inherently linked training variables that impact adaptation and performance. For resistance exercise, volume is often reported as the number of times (or 'sets') a particular muscle group is trained per week and is the main stimulus for muscle accrual[284]. Twelve to 20 weekly sets is sufficient to maximize hypertrophy[285], and the frequency of exercise can be adjusted to disperse training volumes according to personal preference[286].

Intensity of resistance exercise is generally normalized to a percentage of the maximum load that an individual can lift (expressed as the percentage of 1 repetition maximum (RM))[287] or how close an exercise set is taken to momentary muscular failure (in other words, the inability to complete the concentric portion of a movement)[288]. Equal gains in muscle mass can be made irrespective of load-intensity (<30% 1 RM to ≥80% 1 RM)[287] when sets are taken proximal to failure[288]. Thus, increasing the number of repetitions against a fixed load or increasing the load lifted for a fixed number of repetitions are both viable progression strategies to promote muscle hypertrophy[289]. Conversely, absolute strength (1 RM)[287], tendon stiffness[290] and running economy (that is, the metabolic cost at a given velocity of submaximal running)[254] are improved more by high-load resistance training. Collectively, this implies that implementing various loading strategies could represent the best approach for attaining the breadth of muscle-related adaptations to resistance training.

The stress imposed by a single bout of resistance exercise upregulates amino acid transporters and sensors in muscle at least in part through activating transcription factor 4 (ATF4)[6]. Synergizing with the mammalian target of rapamycin (mTOR) (see the section 'Skeletal muscle responses to acute exercise') (Fig. 3), this sensitizes muscle to amino acids for ≥24–48 h after resistance exercise[6,8], and dietary protein intakes of ≥1.2–1.6 g per kg body mass daily modestly complement the hypertrophic response to resistance training[291].

Endurance and high-intensity cardiorespiratory exercise

Cardiorespiratory exercise has numerous subtypes that are associated with training-induced improvements in maximal oxygen consumption ($\dot{V}O_{2max}$)[292–294]. Approaches

used to standardize cardiorespiratory exercise are plentiful (reviewed in ref. 295) and most often include the allocation of intensity against fixed percentages of maximal power ($\dot{W}_{max}$), velocity ($\dot{V}_{max}$), heart rate (HR_{max}) or $\dot{V}O_{2max}$. However, the validity of normalizing cardiorespiratory exercise to maximal parameters has been questioned[295,296], as this can produce dissimilar physiological and metabolic perturbations between individuals[296]. Moving forward, studies should strive to establish methods of standardization that provoke homeostatic disturbances consistent with distinct exercise intensity domains.

Endurance (also known as aerobic) exercise is typically considered to be a continuous bout of formal activity performed at low (<50%), moderate (~50–79%) or high intensities (≥80%) of $\dot{V}O_{2max}$ (ref. 292). High-intensity cardiorespiratory exercise can be further divided into high-intensity interval exercise (HIE) and sprint interval exercise (SIE), both of which constitute several bursts of higher-intensity effort interspersed with periods of low-intensity active recovery. High-intensity intervals are conducted at ≥80% of $\dot{V}O_{2max}$, whereas sprint intervals are supramaximal or 'all-out'[292]. Comparison of cardiorespiratory exercise suggests that training within the high-intensity range is more effective[292,293] or equally as effective[294] at increasing $\dot{V}O_{2max}$ and requires less training volume than moderate intensities to do so[294]. HIE and SIE training also improve endothelial function to greater extents, whereas moderate intensities favour long-term glycaemic control (lower glycated haemoglobin A_{1c} (HbA_{1c}))[292]. Further intricacies are seen between interval training subtypes. Eight weeks of HIE training improved cardiac stroke volume, $\dot{V}O_{2max}$ and endurance (3 km) performance[297]. By contrast, an equivalent period of SIE training potentiated anaerobic capacity and sprinting (300 m) performance[297].

Training variables might also discretely regulate muscle mitochondria (see the section 'Skeletal muscle adaptations to long-term exercise'). Hence, combinations of endurance exercise, HIE and SIE are recommended to maximize both health and performance benefits. Consistent with this, just 1–3 h per week of moderate or high-intensity cardiorespiratory exercise could lower mortality risk, and incorporating resistance exercise confers added protection[18].

Interindividual variation in exercise adaptation

The magnitude of adaptation to resistance[298] and endurance[299,300] training differs substantially between individuals. In part, this probably stems from the aforementioned challenges regarding exercise standardization[295,296], but also from genetic[300] and environmental interactions that converge to produce the heterogenous molecular responses that are observed systemically[19] and in muscle[177] after a common exercise bout.

Interindividual difference in exercise trainability has led to the concept of 'responders' and 'non-responders'. Increasing the volume of fixed-intensity exercise[301,302] or the intensity of fixed-volume exercise[302] can somewhat attenuate exercise non-response, with larger volumes of higher-intensity exercise perhaps doing so most effectively[302]. However, lower responders still require a greater training stimulus and time commitment to achieve results comparable to those of more-responsive trainees[301,302].

As the field of sports genetics continues to grow and be refined, it should provide actionable knowledge for the efficient personalization of training programmes. In the meantime, it is important to emphasize that low responders for a given parameter, such as $\dot{V}O_{2max}$, often improve in other outcome measures[299]. Therefore, exercise remains beneficial for all who are able to partake.

Box 2

Effects of ageing on skeletal muscle and the benefits of exercise

The loss of total muscle mass[303], fibre number[34] and type II fibre size[34,36,250] becomes most evident at ≥50 years of age[34,303] and is inherently linked to perturbations in muscle metabolism. Mitochondrial content is lower in type I and type II fibres from older individuals than from younger individuals[36], and diminished muscle oxidative capacity is linked to mobility decline among adults ≥60 years of age[84,304]. Glycolytic enzymes and chaperone proteins of myofibrillar quality control are also decreased in type II versus type I fibres from individuals >65 years of age[36]. Together with an age-dependent decline of *MYH1* and *MYH2* mRNA expression (encoding myosin heavy chain IIX isoform (MyHC-IIX) and MyHC-IIA, respectively)[47], this disconnect between muscle metabolic and contractile apparatus could contribute towards the reduced type II fibre size[34,36,250] that predominantly underlies detriments in muscle force and power with advanced age[305] (reviewed in ref. 306). Older humans and aged mice can further accrue subsets of senescent muscle fibres[307] and resident mononuclear cells (for example, satellite[308], myeloid[307–309] and fibroadipogenic progenitor[307,308] cells). The number of senescent cells is typically low in resting muscle but increases after resistance exercise[310] and injury[308,309] irrespective of age. The defective clearance and potential accumulation of senescent cells during ageing[308,309] might impair muscle regeneration[308,309], hypertrophy (especially of fast-twitch glycolytic fibres)[310], strength[307,311] and maximal mitochondrial respiration[311], in part through a *Cdkn1a*-driven transcriptional programme[307,311].

Exercise can mitigate several aspects of muscle ageing and improves systemic insulin sensitivity[12]. Lifelong endurance exercisers have a greater density of muscle mitochondria[312,313] and a more complex and connected mitochondrial reticulum[313] relative to less-active elderly counterparts. This is coincident with higher protein levels of inner mitochondrial membrane fusion-factor optic atrophy 1 (OPA1)[313]. Additionally, resistance training was shown to offset age-related CpG-site methylations in the mitochondrial genome[314], and long-term mixed-modality endurance-type exercise might attenuate methylation events in the promoter regions of important cytoskeletal, sarcomeric, glycolysis, glycogen synthesis and tricarboxylic acid cycle-related genes[312]. These epigenetic events could combine to preserve muscle contractile and metabolic integrity. Furthermore, exercise training-induced changes in plasma apelin (APLN) positively correlated with chair stand, balance and walking (Short Physical Performance Battery) test scores in elderly women and men[315]. This suggests that factors produced from exercising muscle help to mediate the protective effects of physical activity against age-related functional decline (Box 4). APLN released from muscle during exercise can act in an autocrine manner to augment satellite cell-mediated repair, mitochondrial abundance and muscle oxidative capacity[315], and/or through paracrine mechanisms to stimulate endothelial cell expansion[316] that could enhance hypertrophy of type II fibres[248,250] (Supplementary Fig. 3).

Endurance[61], high-intensity interval and concurrent[12] training all increase maximal oxygen consumption ($\dot{V}O_{2max}$) and muscle mitochondrial content in older individuals.

Reciprocally, resistance training improves strength[12,240,317] and fat-free mass[12] but to a lesser extent than seen in young adults[240]. The attenuated muscle anabolic response with age could be due to blunted ribosomal biogenesis[240], reduced activity of amino acid sensors (such as leucyl tRNA synthetase (LARS))[318] and lower systemic production and local tissue sensitivity to hormones such as testosterone and insulin-like growth factor 1 (IGF1)[240] (Supplementary Table 1). Progressive resistance training programmes can further benefit hip and femur bone mineral density in people ≥65 years of age[317], and incorporating power-type resistance exercise (involving explosive concentric movements) may be superior for physical performance outcomes, including ‘get-up- and-go’ and chair stand tests[319]. Collectively, these findings emphasize the importance of maintaining a diverse physical activity profile across the lifespan.

Box 3

Energy depots in skeletal muscle

Creatine phosphate

The total concentration of muscle creatine is ~120–130 mmol per kg dry mass in young adults[320]. Approximately 66–67% of this muscle creatine pool is creatine phosphate (CrP), with the rest existing in unphosphorylated form (free creatine)[320]. Resting CrP levels are ~10–15% higher in type II fibres, which rely on CrP to a greater extent than type I fibres[75]. CrP is the main ATP substrate of the phosphagen energy system through a reversible reaction catalysed by sarcoplasmic creatine kinase (CKM). At physiological pH during exercise (~6.5–7.0)[54,55], CKM is bound to sarcomeres through association with myomesins (MYOM1 and MYOM2) at the M-line[321] and phosphofructokinase (PFK) at actin filaments of the I-band[322]—coupling CKM to contraction and glycolysis. CrP hydrolysis peaks at the onset of maximum contraction but deteriorates within ~6 s (ref. 70), and stores can be ~75–90% depleted after roughly 30s of hard exercise[70,75]. The majority of CrP resynthesis is linked to aerobic metabolism through the ‘creatine phosphate shuttle’, whereby mitochondrial CKM re-phosphorylates free creatine to CrP in the intermembrane space, using ATP from oxidative phosphorylation[323] (Fig. 2). Intramuscular creatine rarely reaches saturation by diet and de novo synthesis alone, and supplementing an additional ~3 g per day (~0.03 g per kg body mass daily) increases stores ~15% (to ~140 mmol per kg dry mass) in ~28 days, raising mostly free creatine[324]. Creatine supplementation appears safe for renal function[325] and consistently improves strength performance[326]. The role of creatine in brain health is an emerging area of interest and creatine supplementation may improve the memory function of older adults[327].

Glycogen

Muscle glycogen concentrations vary depending on nutritional state but are ~400–500 mmol per kg dry mass in the vastus lateralis of individuals following mixed macronutrient diets[328]. Glycogen granules are nonuniformly distributed between three specialized pools in muscle[329]: (1) the intermyofibrillar pool, found between myofibrils, close to mitochondria and the sarcoplasmic reticulum; (2) the subsarcolemmal pool, positioned just beneath the cell membrane; and (3) the intramyofibrillar pool, situated within the myofibril at the Z-line of the I-band (Fig. 1a). The intermyofibrillar pool is most abundant and accounts for ~77–84% of muscle glycogen, whereas intramyofibrillar and subsarcolemmal stores constitute ~8–15% and ~6–12%, respectively[35,96,97,267,268]. The relative concentration of each glycogen reserve appears unaffected by age[267], type 2 diabetes (T2D)[268] or anatomical location (triceps brachii compared to vastus lateralis)[35], but may vary depending on physical activity status[35,267,268] and fibre type[35,97]. Total glycogen content and utilization is typically higher in type II versus type I fibres[75,97], and type II fibres have ~23% more glycogen in intramyofibrillar stores[97].

Intramyofibrillar glycogen fuels myosin and sodium/potassium (Na^+/K^+)-ATPases[329] and is preferentially mobilized during strenuous endurance[35], high-intensity interval[97,330] and resistance[96] exercise. Myosin-ATPases also utilize intermyofibrillar glycogen, as does the

sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase (SERCA)[329] (Fig. 2). Thus, sufficient muscle glycogen is important for many types of exercise and a critical threshold of ~250mmol per kg dry mass has been proposed[330], below which self-perceived level of effort increases[330] and sarcoplasmic reticulum function[330,331], power output[331] and repeated-sprint ability[330] decline until glycogen stores are replenished[330,331]. Nevertheless, exercising with low glycogen availability (~100–300 mmol per kg dry mass) might augment signal transduction (for example, 5′-AMP-activated protein kinase (AMPK), p53) and gene expression (for example, *PGC1A* and *TFAM*)[332] associated with oxidative metabolism and mitochondrial biogenesis (see the main text). This has led to the concept of 'train low, compete high' or 'carbohydrate periodization' (reviewed in ref. 332). Whether carbohydrate periodization leads to improved exercise performance over time awaits confirmation, as does delineation of the potential underlying factors (for example, glycogen content per se versus hypocaloric diets that cause weight loss[333] and/or proximal glycogen-depleting exercise bouts that result in higher training volumes[245]).

Intramyocellular lipids

Intramyocellular lipids (IMCLs) are stored in the hydrophobic core of lipid droplet ellipsoids[77] at peripheral (subsarcolemmal, SS_{LD}) and central (intermyofibrillar, IMF_{LD}) regions within fibres[76–78,268].Women may have ~43% more individual lipid droplets in muscle, contributing to a greater (~84%) density of total IMCLs than in men[334]. Similarly, type I fibres utilize more IMCLs during moderate- intensity exercise[130] and have ~2–3-fold higher IMCL contents and lipid droplet numbers than type II fibres[76,78]. Although IMCLs are mostly deposited (>85%) in IMF_{LD}[76–78], the relative distribution and characteristics of lipid droplet subpopulations (particularly in type II fibres) vary depending on training status, body composition and metabolic health[78].

Endurance-trained athletes and adults with T2D have similar total IMCL concentrations in muscle[78,335] despite markedly dissimilar insulin sensitivity profiles[78,84,335]. This apparent contradiction has been termed the 'athlete paradox'[335] but could be partly explained by contrasting lipid droplet properties[77,78]. Individuals with T2D have a greater number of extremely large SS_{LD} in type II fibres[77,78], which also possess lower subsarcolemmal mitochondrial contents[77]. This results in higher relative contributions of SS_{LD} to the overall IMCL pool[78] and fewer mitochondria-to-SS_{LD} contacts[77]. Spatially, SS_{LD} could interfere with insulin signalling and the T2D-SS_{LD} phenotype negatively correlates with peripheral insulin sensitivity[78]. Conversely, endurance athletes have approximately twofold more IMCLs in type I fibres and an increased abundance of adipose triacylglyceride lipase (ATGL) and perilipin 5 (PLIN5)[78] — proteins associated with lipid droplet turnover[118]. The higher type I fibre IMCL content of trained individuals is specifically stored in smaller IMF_{LD}[78], which are favourably depleted during prolonged endurance exercise[76] (Fig. 2b).

An 8-week programme of high-intensity interval training reduced SS_{LD} size, increased the subsarcolemmal mitochondria-to-lipid droplet ratio and redistributed IMCLs into small IMF_{LD} in type II fibres[77]. Accordingly, lipid droplet profiles were similar between adults with normal weight, overweight or obesity, or T2D after the training intervention,

regardless of baseline differences[77]. Therefore, consistent exercise may alleviate muscle insulin resistance somewhat through remodelling of lipid droplets[77,78].

Box 4

Select factors produced from skeletal muscle during exercise

Enhanced blood flow during exercise not only improves delivery of nutrients and hormones (Supplementary Table 1) to muscle but also facilitates the release and transport of discrete factors from muscle[20]. These secreted molecules (known as exercise-induced 'myokines' or muscle-derived 'exerkines') can act in an autocrine, paracrine or endocrine fashion and are thought to promote many of the favourable adaptations associated with physical activity[111].

Interleukin 6 (IL-6) is perhaps the prototypical example of an exercise-stimulated, muscle-secreted factor[336]. Elevations in circulating IL-6 with endurance-type exercise[19,336,337] might contribute towards short-term energy allocation by transiently inhibiting inflammatory processes (for example, monocyte production of tumour necrosis factor (TNF)) while preferentially directing liberated non-esterified fatty acids (NEFAs) towards working muscle (reviewed in ref. 338). Accordingly, systemic pharmacological blockade of the IL-6 receptor promotes re-esterification and storage of NEFAs[337]. A ventromedial hypothalamic circuit of locally synthesized IL-6 controls NEFA oxidation after swimming exercise specifically in the soleus of mice, through sympathetic α_{2A}/α_{2C} adrenoceptor modulation of 5′-AMP-activated protein kinase (AMPK)–acetyl-CoA carboxylase (ACC)[339]. The effects of centrally produced IL-6 preceded a rise in peripheral IL-6 concentrations, and whether IL-6 from muscle can activate this same neuromuscular axis is unclear[339].

Lactate is an established signalling metabolite and its production is enhanced by glycolytic stressors. Elevated rates of muscle glycolysis during higher-intensity (for example, sprint and resistance) exercise increases plasma lactate to greater extents than moderate-intensity endurance exercise[90]. Muscle-derived lactate can initiate an adipose tissue-transforming growth factor β2 (TGFβ2) secretion axis, which mediates improvements in murine glucose tolerance after 11 days of voluntary wheel running[340]. Lactate exiting muscle can be further converted to *N*-lactoyl-phenylalanine (Lac-Phe) in cells expressing *CNDP2*, such as immune cell populations (for example, macrophages and monocytes) or epithelial cell populations[341]. Intraperitoneal Lac-Phe delivery caused appetite suppression and weight loss in obese mice[341]. However, post-exercise concentrations of Lac-Phe in human plasma were orders of magnitude lower than those administered in the murine experimental model[341]. As such, the role of Lac-Phe in the hunger-suppressing effects ~0–3 h after high-intensity exercise[342] warrants additional study.

Apelin (APLN)[315] and succinate[343] are other examples of molecules released from exercising muscle that promote crosstalk between muscle fibres and resident mononuclear cells. This retrograde signalling is particularly important for adaptive extracellular matrix remodelling and angiogenesis (Supplementary Fig. 3). Longer-duration high-intensity intervals[55] and prolonged endurance exercise[124] may increase circulating succinate more than resistance exercise does. Similarly, only endurance exercise lowered the [kynurenine (KYN)] to [kynurenic acid (KYNA)] ratio in

plasma[124]. KYN is a neurotoxic metabolite and the systemic reduction in [KYN] to [KYNA] protects against stress-induced depressive-like symptoms in mice[344]. This occurs through a muscle peroxisome proliferator-activated receptor-γ coactivator 1α (PGC1α) isoform 1 (PGC1α_1)–peroxisome proliferator-activated receptor-α/δ (PPAα/δ)–KYN aminotransferase (KAT) cascade that detoxifies KYN to KYNA[344]. KYNA subsequently released from muscle could influence energy homeostasis by activating G protein-coupled receptor 35 (GPR35) on adipocytes to stimulate lipid turnover[345]. Numerous other exercise-responsive secretory factors regulated by PGC1α can reportedly signal from muscle (reviewed in ref. 223), including neurturin (NRTN)[346]. NRTN operates through both autocrine and paracrine mechanisms to coordinate slow-oxidative muscle fibre and slow-twitch motor neuron property transitions in mice, and its mRNA is upregulated in human vastus lateralis ~72 h after sprint interval exercise[346].

Collectively, cell-to-cell and inter-organ communication appears to have an important role in the local and global effects of exercise. Although muscle is a mediator of this crosstalk, other metabolically active tissues (for example, the heart, liver, adipose, nervous, endocrine and immune systems) also contribute, as discussed in detail elsewhere (reviewed in ref. 111).

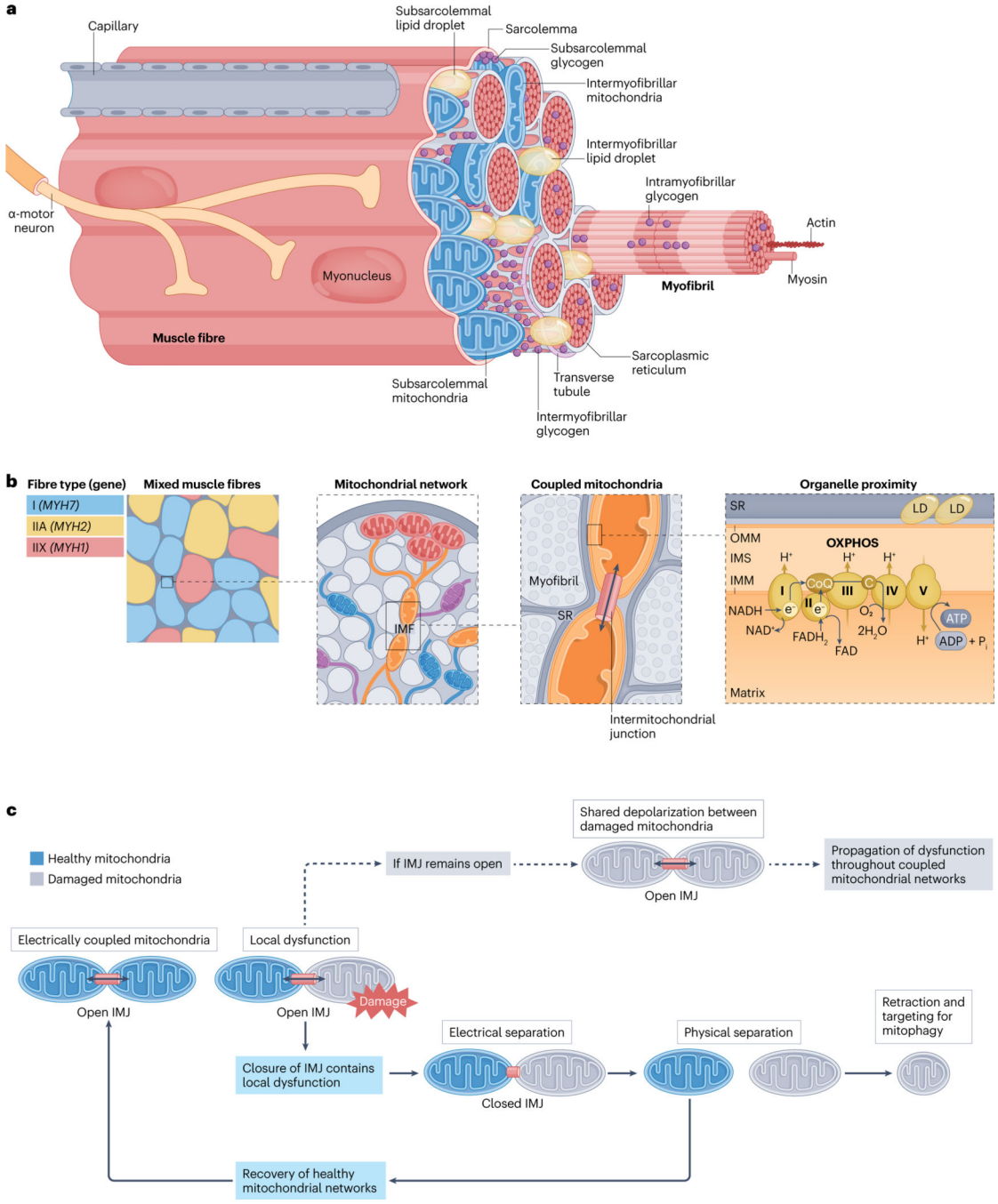

Fig. 1 |. Skeletal muscle fibre ultrastructure.

a, Location of mitochondrial subpopulations and energy stores in muscle fibres. Skeletal muscle is composed of layers of connective tissue and fascicles (also known as muscle bundles). Fascicles contain organized arrangements of individual syncytial muscle fibres, each covered by an endomysium, or basal lamina, which is anchored to the fibre membrane (also known as the sarcolemma). Muscle stem cells, termed ‘satellite cells’, reside within this sarcolemma-basal lamina ‘niche’ (Supplementary Fig. 3). Specialized components, such as sodium/potassium pumps (Na^+/K^+-ATPase), triads (consisting of

transverse tubule and sarcoplasmic reticulum (SR)) and proteins of the myofibrils (long arrangements of connected sarcomeres) enable fibre contraction through the process of excitation–contraction coupling and sliding filament theory (Supplementary Fig. 2). Free ATP in muscle is limited[69,70], and fibres possess additional energy depots to maintain contractile activity, including creatine phosphate, glycogen and intramyocellular lipids (Box 3). Glycogen granules are nonuniformly distributed between intramyofibrillar, intermyofibrillar and subsarcolemmal pools[35,267,268]. Alternatively, intramyocellular lipids are stored in lipid droplets (LDs) found predominantly at central (intermyofibrillar) but also peripheral (subsarcolemmal) regions within healthy muscle fibres[76,78]. During submaximal[54] and longer-duration high-intensity interval[55] exercise most ATP in muscle is regenerated by mitochondrial oxidative phosphorylation (OXPHOS) (see the section ‘Acute exercise muscle metabolism’) (Fig. 2). **b**, Spatial distribution of the mitochondrial reticulum within muscle fibres. Human muscle comprises three main fibre types[14,21,22,24,36], type I (marked by *MYH7* expression), type IIA (with *MYH2* expression) and type IIX (expressing *MYH1*). Differences in mitochondrial protein content[14,21] and mitochondrial network configuration[56,60] between fibre types directly impacts muscle metabolism and function. Muscle mitochondria form an interconnected reticulum[56–60] that enables swift and efficient distribution of potential energy from subsarcolemmal (also known as peripheral) mitochondria to intermyofibrillar mitochondria (IMF), deep within the fibre[56–58]. The positioning of mitochondria in the intermyofibrillar space influences the structure of adjacent sarcomeres, resulting in variable cross-sectional areas and myofilament spacing at different regions across the sarcomere length[53]. The branching morphology of IMF also accommodates functional interactions with nearby cellular components, such as the sarcoplasmic reticulum and intermyofibrillar lipid droplets[53,56,58]. In oxidative mouse muscle, ~20% of all IMF are connected to lipid droplets, which may facilitate efficient ATP production and distribution[56]. **c**, Adjacent mitochondria form networks and share energy potential through the intermitochondrial junction (IMJ). Analogous to circuit breakers, intermitochondrial junctions split the reticulum into smaller subnetworks, permitting swift separation of defective mitochondria before their removal through mitophagy[58]. In this way, intermitochondrial junctions provide a dynamic layer of quality control, rapidly rewiring the mitochondrial reticulum through healthy network components to sustain muscle function[58]. C, cytochrome *c*; CoQ, coenzyme Q; IMM, inner mitochondrial membrane; IMS, intermembrane space; OMM, outer mitochondrial membrane.

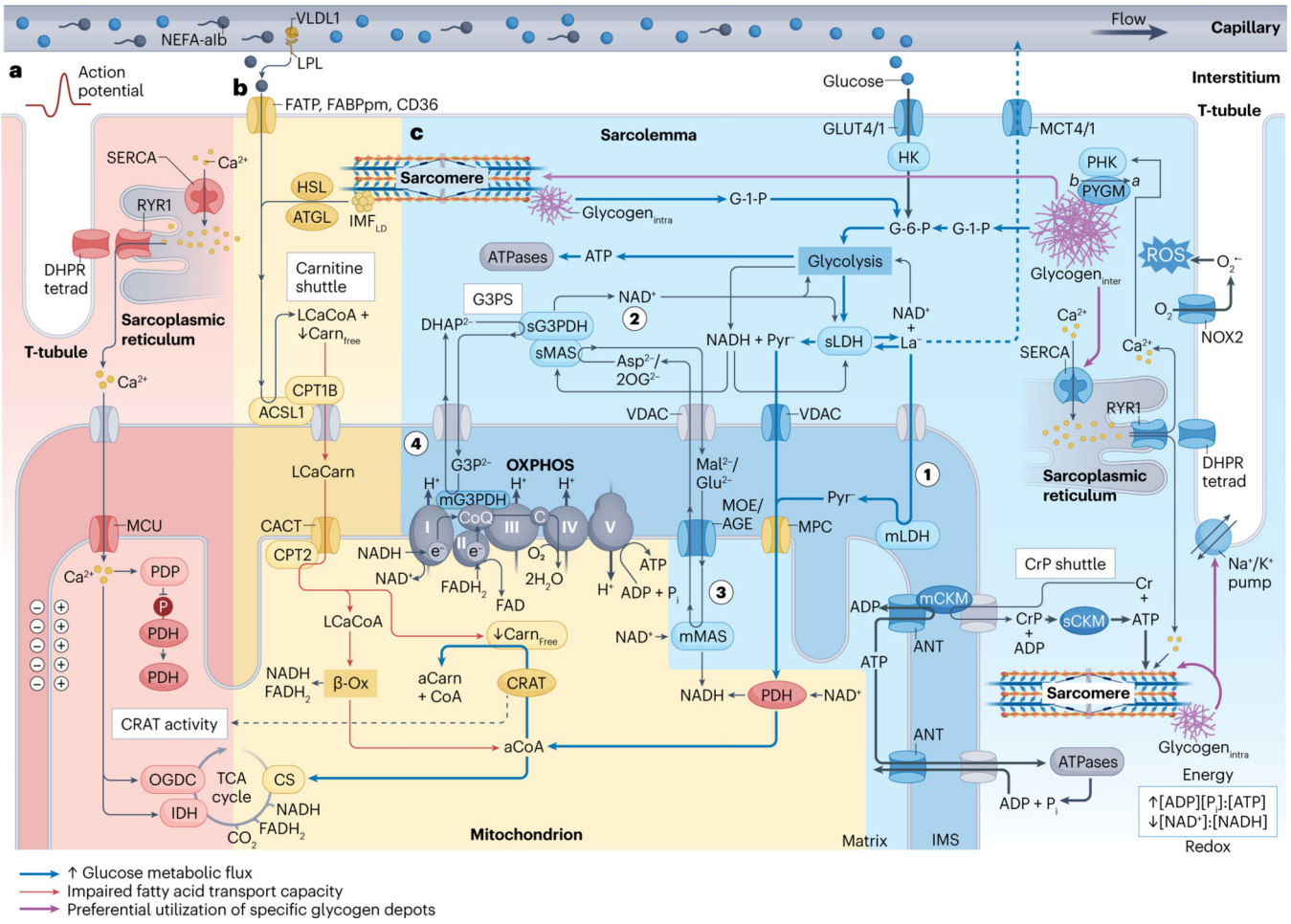

Fig. 2 |. Skeletal muscle metabolism during higher-intensity exercise.

a, Exercise-onset metabolic inertia (red area). Acetyl-carnitine (aCarn) abundance[83–85] and the acetyl coenzyme A (aCoA)-producing capacities of carnitine acetyltransferase (CRAT)[83,84] and pyruvate dehydrogenase (PDH)[85] appear rate-limiting for oxidative adenosine triphosphate (ATP) regeneration at the onset of moderate-high intensity exercise. Contraction-induced calcium (Ca^{2+}) transients promote mitochondrial Ca^{2+} uptake into the matrix space[269] through the inner-membrane mitochondrial calcium uniporter (MCU) complex[270,271]. Increased matrix Ca^{2+} can upregulate PDH[272] through activation of its phosphatase (PDP)[273], and can upregulate isocitrate dehydrogenase (IDH)[274] and 2-oxoglutarate dehydrogenase (OGDC)[272,274,275] directly to fine-tune oxidative metabolism via stimulation of the tricarboxylic acid (TCA) cycle[82]. Ca^{2+} kinetics probably precede an allosteric[86] rise in the [ADP][P_i] to [ATP] and [creatine][P_i] to [creatine phosphate] ratios in part because ADP and creatine (Cr) are buffered by the adenylate kinase (not shown) and creatine phosphate (CrP) shuttle reactions. Thus, synchrony between mechanisms of substrate provision, Ca^{2+}-feedforward and metabolite feedback regulation might underlie acute metabolic inertia. This could be particularly prominent in type II fibres, which have lower CRAT[83] and MCU abundance[21] and slower mitochondrial Ca^{2+} import rates[276]. Furthermore, metabolic inertia is more pronounced in metabolically compromised and older

untrained adults, related to the lower CRAT activity and acetyl-carnitine content of muscle in these individuals[84]. **b**, Carbohydrates outcompete non-esterified fatty acids (NEFAs) for oxidation at higher intensities (yellow area). Muscle glucose uptake[88] and carbohydrate utilization[71,72,120] increases with exercise intensity. At workloads above maximum fat oxidation (>60–65% of maximal oxygen consumption ($\dot{V}O_{2max}$)) flux of pyruvate to acetyl-CoA progressively exceeds rates of TCA cycle entry at citrate synthase (CS), leading to depletion of the muscle free-carnitine ($Carn_{free}$) pool through CRAT-dependent acetylation to acetyl-carnitine[83]. After higher-intensity submaximal exercise, acetylation of the free-carnitine pool is greatest in type I fibres[142]. Insufficient free-carnitine availability would inhibit NEFA mitochondrial import at the first step of the carnitine shuttle — that is, carnitine palmitoyl transferase 1B (CPT1B) conjugation of carnitine to long-chain acyl-CoA (LCaCoA). Reduced fat oxidation is associated with diminished free-carnitine levels at ~70% of $\dot{V}O_{2max}$ (ref. 72), whereas medium-chain NEFA metabolism bypasses carnitine shuttling and is maintained at higher submaximal workloads[277]. Therefore, free-carnitine levels appear rate-limiting for long-chain NEFA utilization at increasing exercise intensities. **c**, Lactate and pyruvate oxidation and NADH shuttles (blue area). Downstream of glycolysis, pyruvate (Pyr^-) and/or lactate (La^-) pass through voltage-dependent anion channels (VDAC), where lactate is converted to pyruvate by mitochondrial lactate dehydrogenase (mLDH) in the intermembrane space[119] (step 1). Pyruvate then enters the mitochondrial matrix through the mitochondrial pyruvate carrier (MPC)[81,119]. The glycerol-3-phosphate ($G3P^{2-}$) shuttle (G3PS) and malate (Mal^{2-})/aspartate (Asp^{2-}) shuttle enables mitochondrial oxidation of lactate and pyruvate through compartmentalized redox shuttling. G3PS and MAS recycle extra-matrix nicotinamide adenine dinucleotide (NAD^+) (step 2) and transport reducing power from glycolysis to the mitochondrial matrix. This occurs through reactions associated with Mal^{2-} and Asp^{2-} delivery into the matrix space[81,119] (step 3) and $G3P^{2-}$ donation of electrons directly to coenzyme Q (CoQ) of the electron transport chain[119] (step 4). As such, saturation of these shuttles increases lactate accumulation and upregulates the lactate-favouring *LDHA* isoform in vitro[278]. See Box 3 for discussion of CrP, intermyofibrillar and intramyofibrillar glycogen ($glycogen_{inter}$ and $glycogen_{intra}$, respectively) and intermyofibrillar lipid droplet (IMF_{LD}) stores, and section ‘Acute exercise muscle metabolism’ for details of their metabolism during acute exercise. β-Ox, β-oxidation; ACSL1, acyl-CoA synthetase long-chain family member 1; AGE, aspartate/glutamate exchanger; ANT, adenine nucleotide translocator; ATGL, adipose triacylglyceride lipase; *b* → *a* denotes posttranslational modification of PYGM from its less-active *b* form to the constitutively active *a* form by PHK; C, cytochrome *c*; CACT, carnitine acylcarnitine translocase; mCKM mitochondrial creatine kinase muscle; sCKM, sarcoplasmic CKM; $DHAP^{2-}$, dihydroxyacetone phosphate; DHPR tetrad, four dihydropyridine receptors associated with one ryanodine receptor 1 (RYR1); FABPpm, fatty acid binding protein plasma membrane; FAD, flavin AD; $FADH_2$, reduced FAD; FATP, FA transporter protein; GLUT4/1; glucose transporters 4 and 1; G-1-P, glucose-1-phosphate; G-6-P, glucose-6-phosphate; HK, hexokinase; HSL, hormone-sensitive lipase; IMS, intermembrane space; LCaCarn, long-chain acyl carnitine; LPL, lipoprotein lipase; sLDH, sarcoplasmic LDH; mMAS, mitochondrial MAS; MCT4/1, monocarboxylate transporters 4 and 1; mG3PDH, mitochondrial glycerol-3-phosphate dehydrogenase; MOE, malate/2-oxoglutarate exchanger; NADH, reduced NAD; NEFA-alb, albumin-bound

NEFA; NOX2, NAD phosphate oxidase 2; $O_2^{\bullet -}$ superoxide; $2OG^{2-}$, 2-oxoglutarate; OXPHOS, oxidative phosphorylation and associated respiratory complexes; PHK, muscle phosphorylase kinase; PYGM, glycogen phosphorylase muscle-associated; ROS, reactive oxygen species; SERCA, sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase; sG3PDH, sarcoplasmic glycerol-3-phosphate dehydrogenase; sMAS, sarcoplasmic MAS; T-tubule, transverse tubule; VLDL1, very low density lipoprotein 1.

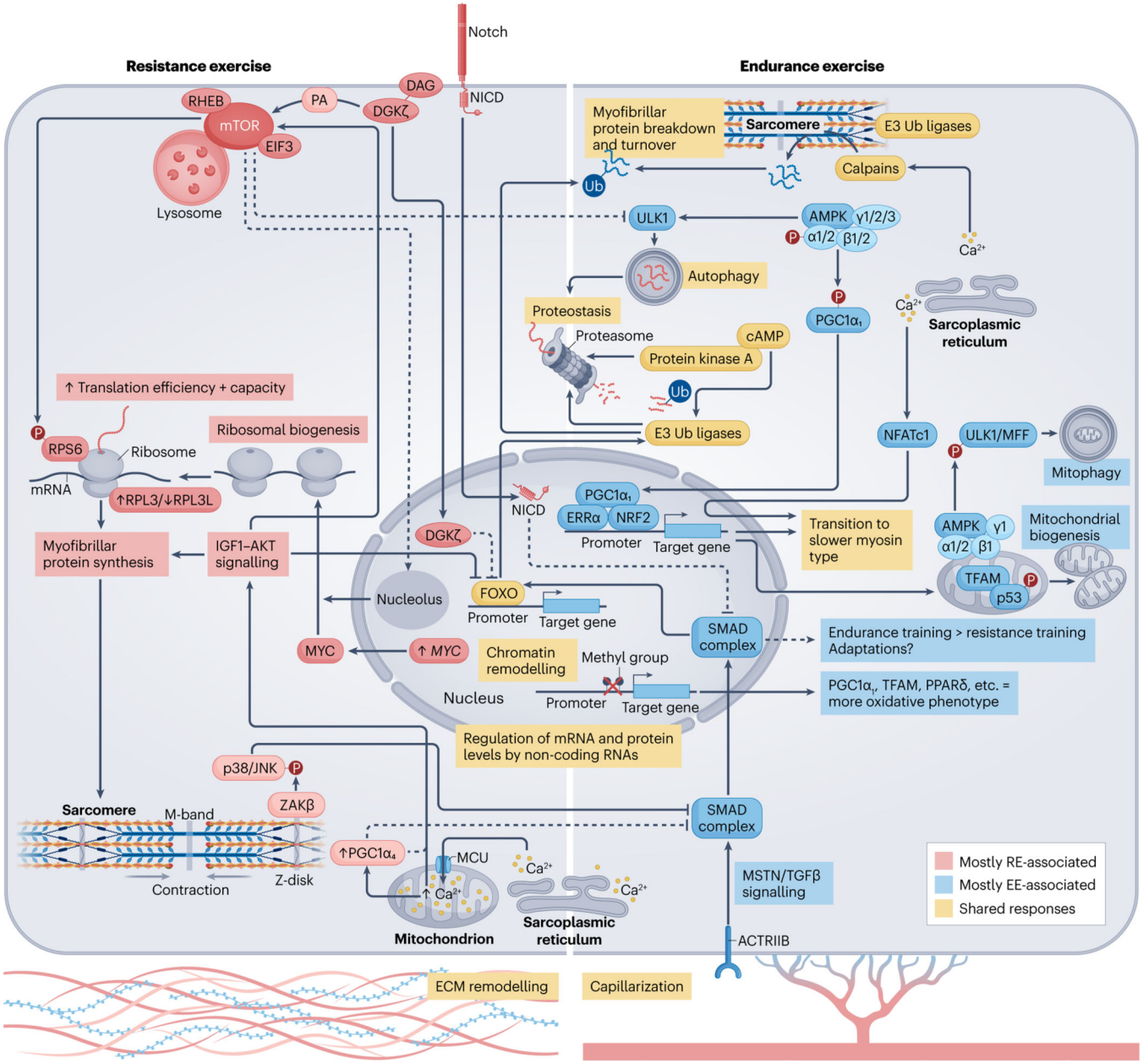

Fig. 3 |. Molecular responses to acute exercise and exercise training.

Exercise-induced alterations in circulating molecules[19,124] and the intramuscular milieu[55,101], together with mechanical tension[178], initiates a temporal series of biochemical and molecular events that lead to muscle adaptation. Activation of signalling cascades promote substantial posttranslational modification of the muscle proteome[90,170] and DNA accessibility[198,199,230]. Collectively, this drives transcription factor-dependent[169] changes in gene expression[167,183], alongside microRNAs[204] and long-non-coding RNAs[205] that are thought to ‘fine-tune’ the molecular responses to exercise. Endurance exercise (EE) and resistance exercise (RE) are often considered divergent stimuli, primarily driving oxidative versus hypertrophic muscle adaptations, respectively. However, common processes among exercise modalities can result in shared enrichment of signalling cascades[90] and

transcriptional networks[183] in the post-exercise period. For example, coordinated proteolysis is detected following acute exercise, irrespective of exercise type[90]. This may require cAMP–protein kinase A (PKA)[190,191] and ensures protein quality control and physiological muscle remodelling. 5′-AMP-activated protein kinase (AMPK) activity[90] and the expression of total *PGC1*A mRNA[183] are also increased after a single bout of either endurance or resistance exercise. AMPK phosphorylation activates peroxisome proliferator-activated receptor-γ coactivator 1α isoform 1 (PGC1α_1)[279], and both AMPK and PGC1α_1 potentiate angiogenic factors[260,280] and mitochondrial bioenergetics[48,235,260,279] in muscle. After endurance exercise, a distinct pool of mitochondrial AMPK (composed of α_1, α_2, β_2 and γ_1 isoforms) regulates mitophagy[187–189] and promoter hypomethylation facilitates the transcription of peroxisome proliferator-activated receptor-δ (PPARδ) and mitochondrial transcription factor A (TFAM)[230]. Whether resistance exercise elicits these same effects is unclear. Despite similarities, there are more distinct than overlapping post-exercise responses between modalities[90,183]. Rapamycin-sensitive substrates of mammalian target of rapamycin (mTOR) complex 1 (mTORC1) are phosphorylated to a greater extent after resistance exercise[90]. Mechanical overload initiates translocation of the mTOR–lysosomal complex[174] and diacylglycerol (DAG) kinase-ζ (DGKζ)[173] to the sarcolemma. Here, mTOR colocalization with RAS homologue enriched in brain (RHEB) and eukaryotic initiation factor 3 (EIF3)[174] and phosphatidic acid (PA) produced by DGKζ[173] might coalesce to fully stimulate mTORC1 (ref. 175) and the translation of contraction-associated mRNAs. Acute attenuation of UNC-51-like autophagy activating kinase 1 (ULK1) autophagic signalling after resistance exercise[65] and nuclear DGKζ-mediated suppression of forkhead box protein O (FOXO)-dependent proteasomal degradation could also support muscle mass by moderating the breakdown of myofibrillar proteins[173]. At the sarcomere, contraction recruits ZAKβ to the Z-disc[179] where it acts through JUN N-terminal kinase 1 (JNK1) and JNK2 (refs. 179,181), potentially alongside Notch[182], to inhibit myostatin (MSTN)/transforming growth factor-β (TGFβ) signalling. This represents one of many intracellular changes permitting resistance training-induced hypertrophy over endurance-like adaptations[181]. The upregulation of *MYC* with resistance exercise stimulates ribosomal biogenesis[180,198], and the formation of a specialized pool of ribosomes with a high ratio of ribosomal protein large 3 (RPL3) to RPL3-like (RPL3L)[238] may favour protein synthesis over translational fidelity. *MYC* expression is mTOR-independent but MYC cooperation with mTOR is necessary to successfully increase ribosomal content[180], possibly requiring an mTOR-driven reorganization of nucleoli to aid rRNA transcription[281]. Divergence between endurance and resistance exercise at the transcriptomic level is magnified after a period of training[183]. Endurance training increases electron transport chain complex expression[183], mitochondrial content and muscle oxidative capacity[12]. Conversely, growth-related pathways[183], ribosomal abundance[198] and muscle mass[12] are augmented more by resistance training. This could be mediated in part by PGC1α isoforms. Nuclear localization of PGC1α_1 and Ser15 phosphorylation of p53 are greater in resting muscle after high-intensity interval training[239], which might help to preserve mitochondrial content and function[257]. By contrast, PGC1α_1 protein is unchanged after resistance training, whereas the PGC1α isoform 4 (PGC1α_4) protein is preferentially enriched[235]. PGC1α_4 stimulates muscle hypertrophy and is associated with enhanced *Igf1* expression[229], insulin-like growth

factor 1 (IGF1)–serine/threonine protein kinase (AKT) and mTORC1 signalling[234] and downregulation of *Mstn* mRNA in mouse muscle[229]. However, unlike PGC1α$_1$ (ref. 280), PGC1α$_4$ does not coactivate oestrogen-related receptor-α (ERRα)[229] and has no effect on oxidative phosphorylation enzymes[229,235]. Appreciable overlap in the initial stages of exercise training probably underlies the degree of shared adaptation between endurance and resistance exercise (see the sections ‘Skeletal muscle responses to acute exercise’ and ‘Skeletal muscle adaptations to long-term exercise’). Depending on individual predisposition (Box 1), dedicated training of a certain exercise modality could amplify discrete differences in the adaptive response, resulting in distinct muscle adaptations and the development of specific phenotypes over time[13]. Evidence showing that combined endurance and resistance training can blunt muscle hypertrophy in humans is scarce[184,185] but concurrent training could impede gains in explosive strength[184]. Still, a combined exercise regime may offer dual benefits for most individuals[12]. ACTRIIB, activin receptor type 2B; ECM, extracellular matrix; MFF, mitochondrial fission factor; MCU, mitochondrial calcium uniporter; NFATc1, nuclear factor of activated T cells, cytoplasmic 1; NICD, Notch intracellular domain; NRF2, nuclear factor erythroid 2-related factor 2; p38, p38 mitogen-activated protein kinase; RPS6, ribosomal protein S6; SMAD, mothers against decapentaplegic homologue; Ub, ubiquitin; ZAKβ, MAP3K20 isoform-β.