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Review

Regulation of skeletal muscle mitochondrial fuel utilization during exercise

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Skeletal muscle exhibits remarkable metabolic plasticity, with mitochondria playing a central role in adapting to energy demands during exercise. These organelles form a dynamic and specialized system capable of remodeling to meet metabolic challenges. Recent studies demonstrate that exercise not only stimulates mitochondrial biogenesis but also engages finely tuned quality-control mechanisms to sustain energy efficiency and performance. A key adaptation is mitochondrial fuel flexibility, the capacity to switch between lipid and carbohydrate oxidation, which underlies endurance and metabolic health. Importantly, efficient lipid utilization, rather than low lipid content, explains why trained muscle can accumulate lipids while remaining insulin sensitive. Here, we review emerging insights into how exercise reprograms skeletal muscle mitochondria to optimize fuel use and highlight implications for metabolic disease.

Highlights

Skeletal muscle mitochondria exhibit remarkable structural and metabolic plasticity, dynamically remodeling in response to exercise to support contractile and metabolic demands.

Exercise induces coordinated mitochondrial biogenesis and quality control, regulated by the integrated actions of peroxisome proliferator-activated receptor-γ coactivator 1α, Adenosine monophosphate-activated protein kinase, and folliculin-interacting protein 1 signaling networks.

During exercise, skeletal muscle mitochondria oxidize carbohydrates and fats as major substrates, with substrate preference dynamically shifting based on exercise intensity and duration.

Mitochondria act as metabolic hubs, orchestrating fuel flexibility and enabling efficient switching between carbohydrate and fatty acid oxidation to sustain endurance performance.

Efficient mitochondrial utilization of intramuscular lipids, rather than their absolute levels, underlies the 'athlete's paradox' and promotes metabolic health in endurance-trained muscle.

The specialized skeletal muscle mitochondrial system

Skeletal muscle mitochondria are highly dynamic organelles, undergoing continuous remodeling to meet the energy and contraction demands of muscle. Mitochondria in skeletal muscle form an intricate reticulum that efficiently distributes energy across myofibers (Box 1) [1,2,6]. This unique system allows for metabolic adaptation in response to changing physiological and pathophysiological conditions [3,7–9]. Exercise stimulates **mitochondrial biogenesis** (see Glossary) and enhances oxidative enzyme activity, thereby optimizing ATP production. In parallel, endurance training promotes mitochondrial quality through dynamic remodeling processes such as fusion, which increases the formation of elongated, interconnected mitochondrial networks that improve the efficiency of **oxidative phosphorylation (OXPHOS)** [3,7,10,11]. Conversely, mitochondrial dysfunction is a hallmark of metabolic and age-related diseases [11–13].

Evidence suggests that mitochondrial biogenesis and **quality control** are coordinately regulated, ensuring both the expansion and maintenance of a high-quality mitochondrial pool essential for muscle exercise. Moreover, the ability of mitochondria to flexibly switch between lipid and carbohydrate oxidation has emerged as a defining feature of endurance and metabolic health. In this review, we synthesize current insights into how exercise reprograms skeletal muscle mitochondria to optimize fuel utilization and endurance performance. We first discuss key transcriptional and signaling pathways that govern mitochondrial biogenesis and quality control. We then examine mechanisms of fuel utilization and metabolic flexibility, with a focus on how mitochondria enable efficient switching between lipid and carbohydrate utilization during exercise. Finally, we consider how disruption of these adaptive processes contributes to metabolic disease and exercise intolerance. Together, these studies reveal that the benefits of exercise arise not simply from increased mitochondrial content, but from coordinated remodeling that optimizes mitochondrial quality, fuel utilization, and efficiency, providing a framework for understanding both endurance performance and the therapeutic potential of exercise.

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Box 1. Structural and functional heterogeneity of skeletal muscle mitochondria

Skeletal muscle mitochondria comprise multiple spatially and functionally distinct subpopulations that together form an integrated bioenergetic network. They are primarily categorized into subsarcolemmal (SS) and intermyofibrillar (IMF) mitochondria, and each subpopulation is tailored to distinct metabolic functions, ensuring an optimal energy supply for muscle contraction and endurance. SS mitochondria reside beneath the sarcolemma and are closely associated with capillaries exhibiting a more lamellar morphology with an expanded inner membrane structure, which is thought to optimize their role in OXPHOS and substrate metabolism [1–3]. In contrast, IMF mitochondria are smaller and more compact and interwoven among contractile filaments, where they directly supply ATP for muscle contraction [1–3]. Additionally, IMF mitochondria interact with the sarcoplasmic reticulum and intramuscular LDs [4]. In three-dimensional electron microscopy, additional mitochondrial subtypes have been identified, including paravascular mitochondria (PVM), I-band mitochondria (IBM), fiber-parallel mitochondria (FPM), and cross-fiber connection mitochondria (CFCM) [1,5]. These mitochondrial subtypes interconnect to form an adaptive energy network, ensuring localized ATP delivery and efficient bioenergetic responses to exercise.

Integration of exercise signaling pathways with muscle mitochondrial biogenesis

To meet the elevated energy demands of exercise, skeletal muscle enhances its oxidative metabolism through robust mitochondrial biogenesis. This is orchestrated by a transcriptional network centered on the coactivator **peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1α)**, which integrates upstream exercise-responsive signaling with **nuclear receptor (NR)** pathways to promote mitochondrial biogenesis, regulate fuel utilization, and optimize OXPHOS capacity (Figure 1, Key figure). These NRs, including members of the peroxisome proliferator-activated receptor (PPAR) and estrogen-related receptor (ERR) families, play crucial roles in adapting muscle cells to the increased energy demands during exercise [7,14–21]. They regulate mitochondrial oxidative capacity, fuel utilization, and muscle fiber-type adaptation by modulating the expression of genes involved in **fatty acid oxidation (FAO)**, glucose metabolism, and mitochondrial respiratory function [14–16]. The discovery of PGC-1α as a transcriptional coactivator that regulates mitochondrial biogenesis and oxidative metabolism marked a significant advancement in understanding how skeletal muscle adapts to exercise [7,9,22–26]. In response to acute exercise, PGC-1α expression is upregulated through β-adrenergic signaling, with cAMP acting as a key mediator [27]. The calcium-dependent pathways, including calmodulin-dependent kinase (CaMK), also activate PGC-1α following exercise [28]. Additionally, **AMP-activated protein kinase (AMPK)** activation, driven by ATP depletion during exercise, plays a crucial role in PGC-1α regulation [29,30]. AMPK can directly phosphorylate PGC-1α or activate Sirt1, which in turn promotes PGC-1α activity [29,30]. Emerging evidence suggests that **folliculin-interacting protein 1 (FNIP1)** is a critical effector of AMPK signaling in skeletal muscle, influencing mitochondrial function and fuel utilization during exercise [31]. FNIP1, a binding partner for folliculin (FLCN), regulates mitochondrial biogenesis by inhibiting the PGC-1α pathway [32–34]. Recent studies have shown that AMPK phosphorylates FNIP1 at serine 220 (S220) in mouse skeletal muscle during exercise [31]. FNIP1 acts as a direct AMPK substrate that is thought to initiate the transcriptional program of mitochondrial biogenesis via transcription factor EB (TFEB) and PGC-1α [35]. Phosphorylation of FNIP1 by AMPK contributes to maintain mitochondrial function, as nonphosphorylatable FNIP1 mutants (S220A) display defective mitochondrial activity and an exercise intolerance phenotype in mice [31]. Loss of FNIP1 in muscle-specific knockout (mKO) mice results in increased mitochondrial content and improved oxidative metabolism [31]. FNIP1 also participates in an NR–miR-499 feedback loop that regulates PGC-1α and mitochondrial biogenesis [34]. In this pathway, PPARβ/δ and ERRγ upregulate the muscle-specific microRNA (miR-499), which suppresses FNIP1 expression, thereby relieving inhibition on PGC-1α and promoting mitochondrial biogenesis and oxidative metabolism [19,33,34]. In addition to FNIP1, several other factors also converge on PGC-1α and its regulatory network, providing additional layers of regulation. For example, PGC-1 and ERR-induced regulator in muscle 1 (PERM1) is an exercise-induced protein that enhances PGC-1α and ERRα expressions, increasing mitochondrial content and oxidative capacity in skeletal muscle [36].

Glossary

Athlete's paradox: a phenomenon in endurance-trained athletes characterized by elevated IMCL content coupled with high oxidative capacity and enhanced insulin sensitivity.

AMP-activated protein kinase (AMPK): an evolutionarily conserved serine/threonine kinase that acts as a key metabolic sensor of cellular energy status, activated by a rise in intracellular AMP and ADP levels relative to ATP levels.

Electron transport chain (ETC): a system comprising protein complexes (I–IV) and electron carriers in the inner mitochondrial membrane that sequentially transfers electrons from NADH and $FADH_2$ to the terminal acceptor, oxygen.

Fatty acid oxidation (FAO): the mitochondrial breakdown of fatty acids to produce ATP occurs primarily through β-oxidation, an enzymatic process that converts fatty acids into acetyl-CoA units for the TCA cycle.

Folliculin-interacting protein 1 (FNIP1): an adaptor protein that binds FLCN and AMPK to coordinate metabolic signaling. It exerts multifaceted, context-dependent functions including the regulation of mitochondria, reductive stress, and protein stability.

Intramuscular triacylglycerol (IMTG): LDs stored within skeletal muscle fibers, primarily located near mitochondria in oxidative (Type I) fibers. These LDs serve as a critical fuel source for trained muscle.

Mitochondrial biogenesis: the process of increasing mitochondrial content within a cell. It involves the coordinated replication and expression of the mitochondrial and nuclear genomes, coupled with the synthesis and import of proteins and the expansion of mitochondrial membranes.

Mitochondrial dynamics: the processes that control mitochondrial shape and location within the cell. It combines fission, fusion, and transport to adjust the network according to cellular needs.

Mitochondrial fuel flexibility: the capacity of mitochondria to efficiently switch between oxidizing carbohydrate and fatty acids based on substrate availability and physiological demand.

Mitochondrial quality control: the mechanisms that maintain a functional mitochondrial pool. These processes

Key figure

Integration of exercise signaling pathways with skeletal muscle mitochondrial biogenesis and quality control

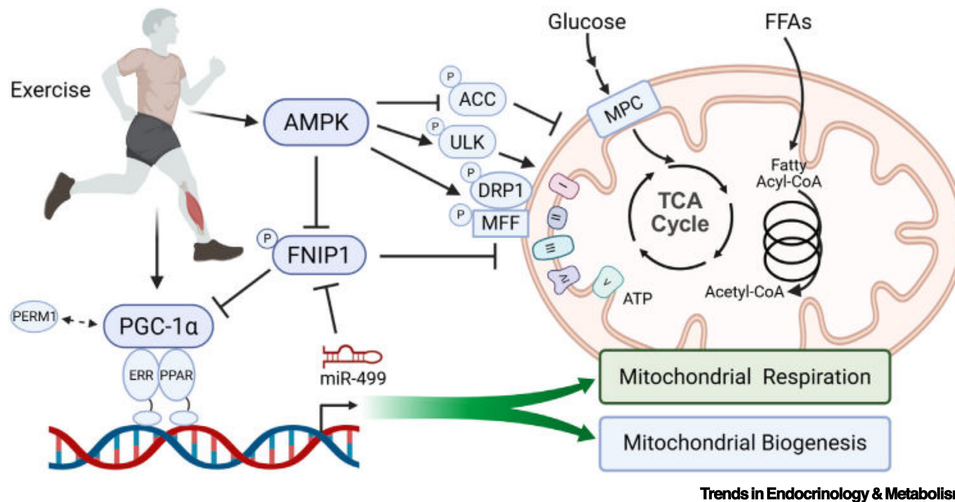

Figure 1. This schematic illustrates how exercise-induced signaling pathways coordinate skeletal muscle mitochondrial adaptations. Peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1α) serves as a master transcriptional coactivator of mitochondrial genes through interactions with nuclear receptors such as estrogen-related receptor (ERR) and peroxisome proliferator-activated receptor (PPAR), thereby promoting mitochondrial biogenesis, fatty acid oxidation, and oxidative phosphorylation. PGC-1α activation is influenced by upstream regulators including AMP-activated protein kinase (AMPK) and PGC-1 and ERR-induced regulator in muscle 1 (PERM1). Mechanical and metabolic cues stimulate AMPK, a key energy sensor, which phosphorylates multiple downstream targets including acetyl-CoA carboxylase (ACC), UNC-51 like autophagy activating kinase 1 (ULK1), dynamin-related protein 1 (DRP1), and mitochondrial fission factor (MFF) to enhance fatty acid oxidation, mitophagy, and mitochondrial fission, respectively. AMPK also phosphorylates folliculin interacting protein-1 (FNIP1), relieving its inhibitory effects on mitochondrial biogenesis and quality control. FNIP1 further participates in a nuclear receptor–miR-499–PGC-1α feedback loop that promotes mitochondrial adaptations. Glucose and fatty acids are funneled into the tricarboxylic acid (TCA) cycle via mitochondrial pyruvate carrier (MPC) or fatty acyl-CoA oxidation, respectively, enabling ATP production via the electron transport chain. This integrated regulatory network ensures optimal mitochondrial biogenesis, quality control, and fuel utilization during exercise adaptation. FFAs: free fatty acids. Figure was created using BioRender (www.biorender.com).

Orchestration of muscle mitochondrial quality control and biogenesis

Skeletal muscle mitochondria require continuous surveillance to maintain their functional integrity. Recent research has uncovered an intricate system that links mitochondrial biogenesis with quality control in muscle cells. PGC-1α plays an important role in orchestrating this integration. In mouse skeletal muscle, the deficiency of PGC-1α and PGC-1β leads to impaired mitochondrial function, characterized by a heterogeneous population of mitochondria with both fragmented and elongated forms [26]. This disruption in **mitochondrial dynamics** is associated with reduced expression of critical fusion and fission proteins such as mitofusin1 (MFN1), mitofusin2 (MFN2), and dynamin-related protein 1 (DRP1) [26]. These findings underscore the direct relationship between the transcriptional regulation of mitochondrial biogenesis and mitochondrial dynamics. Importantly, PGC-1α not only promotes mitochondrial biogenesis but also regulates **mitophagy**, further contributing to mitochondrial quality control during exercise [37]. However, muscle-specific PGC-1α/β loss-of-function mouse models demonstrate that exercise-induced mitochondrial adaptations are attenuated rather than completely abolished [38], indicating the existence of compensatory or parallel regulatory programs. Notably, transcription factors such

range from molecular protein quality control (proteostasis) to organelle-level dynamics and mitophagy for removing damaged components.
Mitophagy: the selective autophagic removal of aged, damaged, or dysfunctional mitochondria, is a key process for maintaining mitochondrial quality.
Nuclear receptors (NRs): a class of transcription factors that activate gene expression in response to specific ligand binding. A canonical example includes the PPAR family, which binds endogenous fatty acids to directly regulate networks governing lipid metabolism and energy homeostasis.
Oxidative phosphorylation (OXPHOS): the metabolic pathway that couples electron transfer through the respiratory chain with ATP synthesis. Energy released from redox reactions drives proton pumping across the inner mitochondrial membrane, generating an electrochemical gradient that powers ATP synthase to produce ATP from ADP and inorganic phosphate.
Peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1α): a master transcriptional coactivator that, through its interaction with transcription factors (e.g., PPARs and ERRs) rather than direct DNA binding, orchestrates mitochondrial biogenesis and oxidative metabolism to regulate tissue-specific physiology.

as ERRγ can partially substitute for PGC-1α/β to regulate oxidative remodeling, mitochondrial biogenesis, and endurance adaptations, particularly when combined with exercise [39].

AMPK also plays a crucial role in regulating mitochondrial biogenesis and quality control in response to exercise. As described above, once activated, AMPK promotes mitochondrial biogenesis through PGC-1α [29]. Furthermore, AMPK has been shown to promote mitochondrial fission by phosphorylating mitochondrial fission factor (MFF), which recruits DRP1 to the mitochondria, initiating the process of fission [40]. In addition to regulating mitochondrial fission, AMPK also regulates mitophagy by phosphorylating UNC-51-like autophagy activating kinase 1 (ULK1), a key kinase that initiates the autophagic cascade [41,42]. Recent studies have shown that AMPK-ULK1 signaling is crucial for exercise-induced mitophagy in skeletal muscle [42,43]. Exercise is also effective in promoting mitochondrial **electron transport chain (ETC)** respiratory supercomplex assembly [44]. More recently, FNIP1 has emerged as an additional AMPK-responsive node that links energetic stress to the coordinated regulation of mitochondrial biogenesis, ETC remodeling, and quality control. Compared with other well-established AMPK effectors and mitochondrial quality-control hubs such as TFEB/TFE3, and the PINK1–Parkin pathway, which have been reviewed in [45–47], the FNIP1 axis represents a relatively young area of investigation, with the strongest mechanistic evidence currently derived from mouse and cell models. Recent findings have revealed that both mitochondrial ETC complex remodeling and the biogenesis of mitochondria are coordinately regulated by AMPK phosphorylation of FNIP1 [31,33,35]. Moreover, FNIP1 degradation under reductive stress conditions, mediated by E3 ligase CUL2-FEM1B, sustains mitochondrial import and preserves ETC integrity by responding to changes in reactive oxygen species (ROS) levels [48–50]. Structurally, FNIP1 recognition by CUL2-FEM1B is mediated by zinc ions, which act as molecular 'glues' to facilitate the selective degradation of the reduced form of FNIP1, ensuring a precise redox-dependent regulatory mechanism [49]. Additionally, FNIP1 plays a role at the mitochondrial import gate, where it interacts with translocase of the outer mitochondrial membrane, subunit 22 (TOM22), to modulate protein import and ETC biogenesis in response to reductive stress [50]. This dynamic control of FNIP1 turnover prevents mitochondrial overload during high metabolic demand while ensuring adequate respiration under stress conditions. These findings position FNIP1 as a mitochondrial gatekeeper, linking metabolic cues to mitochondrial maintenance. However, human data on FNIP1 regulation in skeletal muscle metabolism remain limited and largely associative. Recent studies indicate that FNIP1 expression in human skeletal muscle can be altered under pathological conditions, as evidenced by its reduction in patients with limb–girdle muscular dystrophy (LGMD) [51]. In addition, FNIP1 mutations in humans are associated with multisystem disorders, including immunodeficiency, cardiomyopathy, and myopathy [52,53]. Further work is needed to define how FNIP1 expression and function are modulated across human muscle diseases and metabolic states.

The integration of AMPK, FNIP1, and PGC-1α signaling pathways provides a mechanistic axis that links exercise-induced signaling to mitochondrial remodeling and, ultimately, to fuel utilization (Figure 2). Contraction-driven AMPK activation not only initiates mitochondrial biogenesis through FNIP1–PGC-1α-dependent transcriptional programs but also enhances mitochondrial respiratory capacity and quality control, thereby increasing the efficiency with which mitochondria oxidize carbohydrates and fatty acids. This integrated metabolic network ensures efficient fuel supply and energy production in active muscle during exercise.

Skeletal muscle energy substrate utilization during exercise

Fuel mobilization and utilization during exercise

Mitochondrial oxidative metabolism of carbohydrates and fats provides almost all the required ATP during exercise. The major sources of energy for contracting skeletal muscle include

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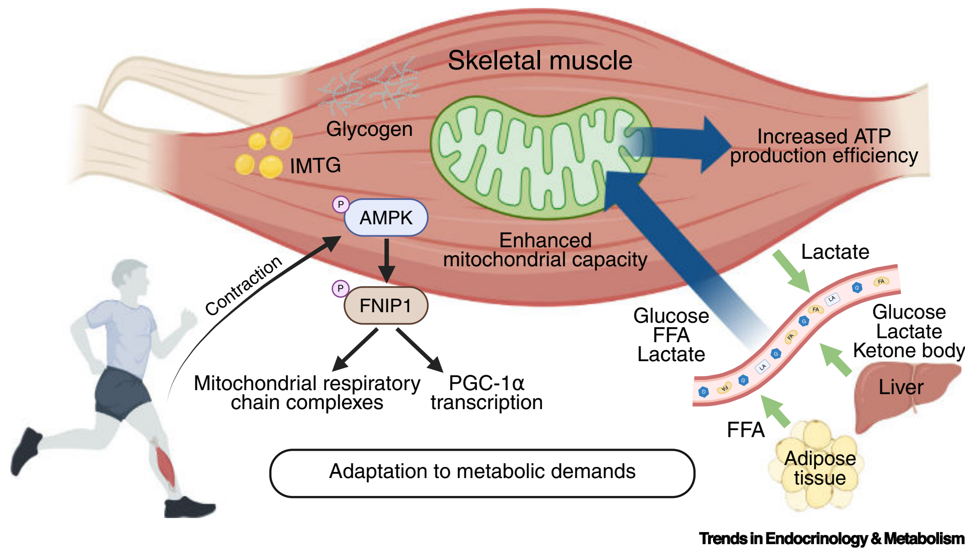

Figure 2. Integration of AMPK–FNIP1–PGC-1α signaling links exercise-induced mitochondrial remodeling to fuel utilization in skeletal muscle. Muscle contraction activates AMPK, which functions as a metabolic sensor to couple energetic stress to adaptive remodeling. AMPK signaling engages FNIP1 and promotes PGC-1α-dependent transcriptional programs that enhance mitochondrial biogenesis, respiratory chain capacity, and oxidative efficiency. In parallel, these metabolic adaptations increase the capacity of mitochondria to oxidize multiple substrates, including glucose, fatty acids, and lactate, thereby improving ATP production efficiency during exercise. Enhanced mitochondrial capacity also supports coordinated interactions with intramyocellular triglycerides (IMTG) and circulating fuels supplied by liver and adipose tissue. Collectively, the exercise-induced AMPK–FNIP1–PGC-1α signaling integrates mitochondrial remodeling with fuel utilization in skeletal muscle. Figure was created using BioRender (www.biorender.com). AMPK: AMP-activated protein kinase; folliculin-interacting protein 1 (FNIP1); peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1α); adenosine triphosphate (ATP); free fatty acid (FFA).

phosphocreatine (PCr), muscle glycogen, blood glucose (derived from liver glycogenolysis, gluconeogenesis, and dietary intake), and fatty acids sourced from both **intramuscular triacylglycerol (IMTG)** and adipose tissue triglyceride stores (Figure 2). During the initial seconds of exercise, ATP is supplied by the rapid breakdown of PCr, which provides the most immediate energy but is constrained by a very limited intramuscular PCr pool that can be largely depleted within seconds. This rapid energy supply is quickly supplemented and followed by ATP generation from muscle glycogen via anaerobic glycolysis. With increasing exercise duration, muscle shifts toward utilizing circulating carbohydrates and free fatty acids (FFAs), ensuring a continuous supply of energy. The relative contribution of carbohydrates and fats to energy metabolism is influenced by exercise intensity and duration. At low-to-moderate exercise intensities, lipolysis, fatty acid uptake, and oxidation increase significantly, reaching peak fat oxidation rates at approximately 65% VO_2 max in humans [54]. This reliance on fatty acids helps preserve glycogen stores and sustains prolonged endurance performance. By contrast, at higher exercise intensities, skeletal muscle shifts predominantly to carbohydrate oxidation, particularly from muscle glycogen, to meet the higher ATP demand [55].

As muscle glucose demand rises, hepatic glucose output increases through both glycogenolysis and gluconeogenesis, supporting systemic glucose homeostasis. Prolonged exercise can lead to a decline in liver glucose output below muscle glucose uptake, increasing the risk of hypoglycemia, which can be mitigated by carbohydrate ingestion [56]. Interestingly, a recent study indicated that lactate, glycerol, and liver glycogen all fuel exercise-induced glucose flux [57]. At the onset of exercise, lactate-derived carbons contribute nearly 50% of gluconeogenic flux [57]. As exercise progresses, however, glycerol-derived carbons increasingly support glucose production in

mice [57]. During mild-to-moderate intensity exercise, fat oxidation increases dramatically above resting levels, driven by heightened muscle energy demands and enhanced fatty acid availability. A significant portion of these fatty acids originates from adipose tissue lipolysis, which rises due to β-adrenergic stimulation, facilitating a steady supply of fuel for oxidative metabolism.

Glucose uptake and carbohydrate oxidation in exercising muscle

Exercise activates phosphorylase kinase and phosphofructokinase, enhancing glycogen breakdown and glycolysis. Pyruvate generated from glycolysis enters the tricarboxylic acid (TCA) cycle to maximize ATP production (Figure 3). Glucose uptake during exercise is driven by the

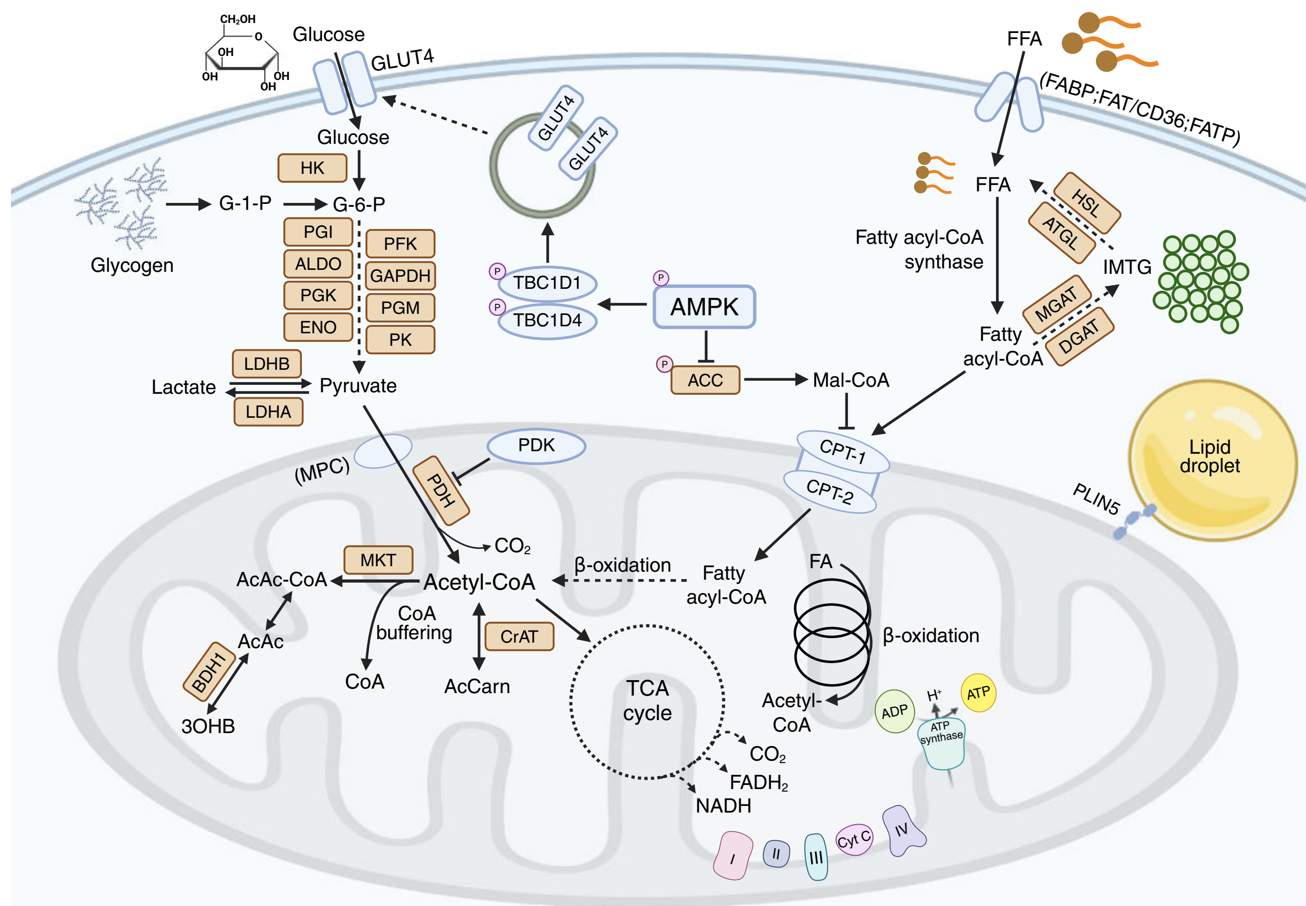

Figure 3. Mitochondrial regulation of substrate utilization in skeletal muscle during exercise. This figure illustrates the integrated pathways generating ATP from carbohydrates and lipids in skeletal muscle. Glucose enters muscle cell via TBC1D1/4-regulated GLUT4 and is metabolized to pyruvate via glycolysis. Pyruvate is converted to acetyl-CoA by the PDH complex, a step negatively regulated by PDK. Concurrently, fatty acids derived from lipolysis or intramuscular triglycerides (IMTGs) undergo β-oxidation to yield acetyl-CoA. The figure highlights several key regulations discussed in the text. AMPK phosphorylates ACC to relieve inhibition on the rate-limiting enzyme CPT-1. Carnitine- and CoA-buffering enzymes such as CrAT, MKT, and BDH1 help preserve the mitochondrial free CoA pool. LDs are positioned near mitochondria, with PLIN5 acting as a molecular interface. Ultimately, these convergent pathways fuel TCA cycle and oxidative phosphorylation to meet the energy demands of exercise. Figure was created using BioRender (www.biorender.com). GLUT4: glucose transporter 4; HK: hexokinase; PGI: phosphoglucose isomerase; PFK: phosphofructokinase; ALDO: aldolase; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; PGK: phosphoglycerate kinase; PGM: phosphoglycerate mutase; ENO: enolase; PK: pyruvate kinase; LDHA/LDHB: lactate dehydrogenase A/B; TBC1D1/4: TBC1 domain family member 1/4; FABPpm: plasma membrane-associated fatty acid-binding protein; FAT/CD36: fatty acid translocase; FATP: fatty acid transport protein; HSL: hormone-sensitive lipase; ATGL: adipose triglyceride lipase; MGAT: monoacylglycerol acyltransferase; DGAT: diacylglycerol acyltransferase; CPT-1/2: carnitine palmitoyltransferase 1/2; AMPK: AMP-activated protein kinase; ACC: acetyl-CoA carboxylase; MPC: mitochondrial pyruvate carrier; PDH: pyruvate dehydrogenase; PDK: pyruvate dehydrogenase kinase; CrAT: carnitine acetyltransferase; BDH1: beta-hydroxybutyrate dehydrogenase 1; MKT: medium-chain ketothiolase; PLIN5: perilipin 5; acetyl carnitine (AcCarn).

translocation of glucose transporter 4 (GLUT4) to the sarcolemma and T-tubules, independent of insulin signaling [3,58]. This process is mediated by contraction-induced signals, including AMPK activation, increased intracellular calcium, and mechanical stress [3,59–61]. Two important proteins, TBC1 domain family member 1 (TBC1D1) and TBC1D4 (AS160), have been shown to regulate GLUT4 translocation by modulating Rab-GTPase activity, and their phosphorylation is influenced by AMPK signaling [3]. Additionally, the small Rho family GTPase Rac1 has emerged as another regulator of GLUT4 mobilization [62], integrating cytoskeletal remodeling with metabolic signaling to optimize glucose uptake. Exercise training enhances GLUT4 expression in skeletal muscle, likely via AMPK–PGC-1α activation [29], thereby increasing its availability for translocation during subsequent exercise bouts. This adaptation facilitates insulin-independent glucose uptake and promotes glycogen storage. Beyond glucose uptake, exercise also alters lactate handling and mitochondrial oxidation, which is summarized in Box 2.

Lipid uptake and lipid oxidation in exercising muscle

Preferential utilization of IMTGs during exercise is highly dependent on training status. In endurance-trained individuals, acute exercise induces substantial IMTG breakdown. By contrast, sedentary, obese, and type 2 diabetic individuals exhibit little to no IMTG utilization during comparable exercise bouts [69]. In trained muscle, lipid droplets (LDs) are strategically positioned near mitochondria [70]. Consequently, at exercise onset, endurance-trained skeletal muscle preferentially oxidizes IMTGs may due to their close proximity to mitochondria, minimizing reliance on plasma-derived fatty acids [69]. Lipolysis of IMTGs is mediated by hormone-sensitive lipase (HSL) and adipose triglyceride lipase (ATGL) [69] (Figure 3). As exercise duration increases, lipolysis, driven by catecholamines and reduced insulin levels, releases FFAs from adipose tissue into circulation, and plasma NEFAs become a progressively more important fuel source [71].

Lipid transport into skeletal muscle is a highly coordinated process involving membrane-bound transporters, intracellular lipid carriers, and enzymatic regulators [3,69]. Long-chain fatty acids (LCFAs) must be transported across both the plasma membrane and mitochondrial membranes before undergoing β-oxidation. CD36, a critical lipid transporter, facilitates the uptake of LCFAs from circulation into skeletal muscle cells. The contraction-induced exocytosis of CD36 to the sarcolemma enhances LCFA uptake. AMPK has been implicated in regulating LD–mitochondrial proximity through signaling pathways involving RAB8A and the LD protein perilipin 5 (PLIN5),

Box 2. Lactate as mitochondrial fuel in exercising skeletal muscle

Once considered a metabolic byproduct of anaerobic glycolysis, lactate is now recognized as an important vehicle for carbohydrate carbon distribution under fully aerobic conditions [63,64]. Glycolysis proceeds to lactate may not be due to a lack of oxygen, but to facilitate high-flux energy metabolism. Seminal work has established the lactate shuttle hypothesis, demonstrating that lactate is continuously produced under fully aerobic conditions and dynamically exchanged between producer and consumer tissues [63,64]. The lactate shuttle theory highlights that lactate links glycolytic and oxidative metabolism across three distinct layers: systemic, intercellular, and intracellular. Through these shuttles, lactate functions as a fuel, gluconeogenic precursor, and signaling molecule [64]. Lactate flux is driven by concentration and pH gradients via monocarboxylate transporters (MCTs). Biochemical and immunogold electron microscopy have identified lactate dehydrogenase (LDH) within mitochondrial or mitochondrial-associated compartments [64–66], supporting the concept that lactate can be directly oxidized in close proximity to the electron transport chain. Recent work using genetically encoded lactate biosensors further suggests that mitochondrial lactate concentrations can exceed cytosolic levels and are dynamically regulated by mitochondrial membrane potential [67], consistent with mitochondria acting as active hubs for lactate utilization. Although earlier studies using isolated mitochondria reported minimal mitochondrial lactate, these discrepancies likely reflect methodological limitations. In skeletal muscle, exercise induces a coordinated transcriptional program that remodels lactate handling. The NR–PGC-1α axis regulates lactate metabolism by modulating LDH isoform expression, shifting the balance from LDHA-driven lactate production toward LDHB-mediated lactate-to-pyruvate conversion for mitochondrial oxidation. Such an isoenzyme shift enhances mitochondrial ATP generation, improves exercise capacity, and reduces postexercise blood lactate in mice [68]. Moreover, the monocarboxylate transporter 1 (MCT1)-mediated lactate shuttle further integrates lactate utilization with mitochondrial biogenesis and TCA cycle flux [65].

which are proposed to facilitate coordination between lipid mobilization and mitochondrial FAO under energy stress [72]. LCFAs bind to cytoplasmic fatty acid-binding proteins (FABPs), which shuttle them toward mitochondria. LCFAs are activated by thioesterification into long-chain fatty acyl-CoA by acyl-CoA synthetase long-chain family member 1 (ACSL1) [73]. Since the inner mitochondrial membrane is impermeable to LCFAs, these molecules require the carnitine shuttle system, where carnitine palmitoyltransferase 1 (CPT1) catalyzes their conversion into acylcarnitines, allowing mitochondrial entry. CPT1, located on the outer mitochondrial membrane, regulates the entry of activated LCFAs into mitochondria by converting them into acylcarnitines [74]. Exercise-induced AMPK activation enhances FAO in part through inhibitory phosphorylation of acetyl-CoA carboxylase (ACC), thereby reducing CPT1 restraint and promoting mitochondrial fatty acid import. Once inside mitochondria, LCFAs undergo β-oxidation, generating acetyl-CoA, NADH, and $FADH_2$, which fuel the TCA cycle and the ETC for ATP synthesis. Trained individuals exhibit a higher capacity for LCFA oxidation, supported by increased mitochondrial content and elevated expression of mitochondrial enzymes involved in fatty acid metabolism. During exercise, incomplete β-oxidation is often accompanied by increased formation of acylcarnitines, which are used as biomarkers of mitochondrial metabolic congestion [75–78]. In rodent models, excessive acylcarnitine accumulation has been linked to impaired metabolic flexibility and reduced exercise tolerance [75]. In humans, however, exercise robustly increases skeletal muscle and plasma acylcarnitines across training states, and current evidence indicates that these changes are more closely tethered to absolute ATP turnover rather than a direct cause of muscle fatigue [77,78].

The mitochondrial fuel substrate preference shifts during exercise

Exercise induces dynamic shifts in mitochondrial fuel substrate preference to optimize energy production and sustain performance. The ability to efficiently transition between carbohydrate and fat oxidation is crucial for optimizing muscle performance and preventing early fatigue. The Randle cycle describes the reciprocal inhibition between glucose and FAO, ensuring efficient fuel utilization [79]. During high-intensity exercise, glucose oxidation is prioritized due to its rapid ATP production, whereas during prolonged or low-to-moderate intensity exercise, a gradual decrease in the respiratory exchange ratio (RER) occurs concurrently with decreased glucose availability, and hence, lipid oxidation predominates to conserve glucose and sustain exercise endurance.

Evidence is emerging that mitochondria play a crucial role in determining muscle fuel flexibility, orchestrating the selection between carbohydrates and lipids as fuel sources during exercise. Defects in mitochondrial quality-control mechanisms impair mitochondrial energy, leading to mitochondrial fuel inflexibility and reduced exercise performance. For instance, FUN14 domain-containing 1 (FUNDC1), a mediator of mitophagy, plays a critical role in controlling muscle mitochondrial quality [80]. Skeletal-muscle-specific ablation of FUNDC1 in mice resulted in an LC3-mediated mitophagy defect, leading to impaired mitochondrial energetics [80]. Consequently, FUNDC1 deficiencies result in excessive reliance on glucose metabolism, increased postexercise blood lactate levels, and impaired endurance capacity. Metabolic assessments of FUNDC1 mKO mice reveal elevated RER and increased lactate accumulation, indicating a shift toward glycolysis and an inability to efficiently oxidize fats as an energy source [80]. Similarly, the loss of lon peptidase 1 (LONP1), a mitochondrial protease that controls mitochondrial protein turnover, impairs mitochondrial integrity and respiratory function [81]. LONP1 mKO mice also show diminished oxygen consumption, higher RER and levels of blood lactate, greater dependence on carbohydrates, and reduced exercise tolerance [81], reinforcing the role of mitochondrial quality-control mechanisms in regulating muscle fuel substrate utilization during exercise.

Conversely, enhancement of mitochondrial biogenesis and energetic function can improve **mitochondrial fuel flexibility**, facilitating a shift from carbohydrate reliance to lipid oxidation

during endurance exercise. As discussed above, mitochondrial function and muscle fuel utilization can be enhanced through activation of the key NR–PGC-1α pathways. PPARδ and PGC-1α activation promote muscle mitochondrial biogenesis, oxidative metabolism, and FAO, leading to improved endurance [20,82]. PPARδ overexpression in skeletal muscle enhances glucose sparing, shifting reliance toward lipid oxidation and delaying glycogen depletion [19,20]. Similar effects—extended running time and improved glucose sparing—were observed in mice treated with PPARδ agonists [14], underscoring the importance of mitochondrial regulation in substrate selection. More critical regulators, ERRs, enhance mitochondrial respiration function and FAO. Mice with constitutively active ERRγ and ERRα exhibit superior endurance, fatigue resistance, and stable blood lactate levels during exercise [15,17], whereas genetic models demonstrate that ERRα-deficient mice exhibit impaired FAO and reduced endurance capacity [18,83].

Mice overexpressing miR-499 in skeletal muscle display a lower RER during exercise [34], reflecting a metabolic shift toward enhanced fat oxidation over carbohydrate utilization. These mice also show improved endurance and reduced postexercise blood lactate levels [34], closely resembling the metabolic advantages observed in PPARδ and ERRγ transgenic (Tg) models. Furthermore, FNIP1 mKO mice also display a remarkable increase in running time and maximal speed, along with significantly greater oxygen consumption (VO_2 max) and a heightened reliance on fat oxidation for sustained energy production, and reduced postexercise lactate accumulation [31]. Together, all these findings highlight mitochondria's pivotal role in orchestrating muscle fuel flexibility to optimize exercise capacity.

At the molecular level, mitochondria act as the central hub orchestrating metabolic crosstalk between glucose and fat oxidation pathways (Figure 3). When exercise intensity surpasses 50–65% of VO_2 max, mitochondrial preference shifts from fat to glucose oxidation, regulated by increased glycolytic flux and depletion of the muscle free-carnitine pool in humans [55,84]. This impairs the carnitine shuttle, limiting mitochondrial import of long-chain fatty acids and reducing fat oxidation [55,84]. The regulation of carbohydrate-derived acetyl-CoA availability to the TCA cycle from glycolysis-produced pyruvate is mediated by pyruvate dehydrogenase (PDH), an enzyme complex linking glycolysis with oxidation. When FAO is elevated, it suppresses glucose metabolism by increasing acetyl-CoA, NADH, and ATP levels, which allosterically inhibit PDH, limiting glucose oxidation [85]. Pyruvate dehydrogenase kinase (PDK) is an upstream kinase of PDH and inhibits the activity of PDH. This same set of allosteric effectors activates a family of PDH kinases that phosphorylate the complex, further inhibiting its catalytic activity [86,87]. Additionally, carnitine acetyltransferase (CrAT), which is highly enriched in muscle [88] and localized to the mitochondrial matrix, facilitates acetylcarnitine (AcCarn) cycling, preventing metabolic inertia and maintaining glucose homeostasis by alleviating PDH inhibition [89]. Acyl-carnitine (AC) accumulation signals incomplete FAO, often due to a bottleneck in long-chain FAO (LCFA) that limits free CoA and carnitine availability, restricting mitochondrial efficiency [90]. Furthermore, exercise-induced adaptations, such as upregulation of medium-chain ketothiolase (MKT/Acaa2) reverse flux, restore CoA levels, ensuring sustained FAO and preventing metabolic congestion [76,90]. Muscle creatine kinase (MCK)-PGC1α Tg mice, which exhibit higher MKT expression, also demonstrate superior mitochondrial fuel flexibility and endurance [90]. Additionally, beta-hydroxybutyrate dehydrogenase 1 (BDH1) buffers the acetyl-CoA pool, supporting MKT flux and preventing CoA trapping in mice [76]. These tightly regulated mitochondrial regulatory mechanisms together enable efficient substrate switching between carbohydrate and lipid oxidation.

Mitochondrial fuel flexibility and the athlete paradox: intramuscular lipids and metabolic flexibility in endurance athletes

Chronic nutrient overload and excess substrate availability could disrupt mitochondrial fuel partitioning, leading to simultaneous oxidation of competing substrates and impaired

mitochondrial function. Mitochondrial fuel inflexibility is a hallmark of insulin resistance and metabolic disorders such as type 2 diabetes, obesity, and cardiovascular disease [85,91]. Recent studies in heart failure with preserved ejection fraction (HFpEF) demonstrate that, while preclinical models in mice suggest a compensatory reliance on FAO, human metabolomic data indicate a broader perturbation in substrate handling. Notably, both contexts exhibit a failure to appropriately increase glucose oxidation during hemodynamic stress, leading to inefficient substrate utilization and impaired exercise capacity [91]. Skeletal muscle lipid accumulation has long been associated with insulin resistance and metabolic disorders [92–94]. Ectopic lipid accumulation in muscles can cause insulin resistance by activating cytosolic kinase cascades that disrupt molecular insulin signaling, a process referred to as lipotoxicity [94,95]. However, endurance athletes exhibit a paradoxical metabolic phenotype in which elevated IMTG stores coexist with heightened insulin sensitivity [93,96]. This phenomenon, termed the '**athlete's paradox**', challenges conventional models linking intramyocellular lipids (IMCLs) with insulin resistance [97]. This paradox suggests that lipid storage *per se* is not pathogenic but is instead dictated by the ability of mitochondria to efficiently oxidize these substrates. Instead, endurance training enhances mitochondrial oxidative capacity, LD dynamics, and substrate flexibility, enabling efficient lipid oxidation without impairing glucose homeostasis [98]. In endurance-trained muscle, mitochondria facilitate rapid lipid oxidation, efficient fatty acid flux, and dynamic LD remodeling, allowing IMTG to serve as an energy reservoir rather than a source of lipotoxic intermediates.

Studies have shown that IMTG utilization is highly dynamic, with LDs positioned in close proximity to mitochondria, facilitating rapid substrate mobilization during exercise [70,72]. Current evidence supports a model in which LD–mitochondrial interactions contribute to metabolic flexibility and lipid handling in trained muscle, while their precise contribution to exercise fuel oxidation and metabolic health remains an active area of investigation. FNIP1 mKO and PGC-1α Tg mice exhibit enhanced mitochondrial function and improved endurance capacity**,** mirroring the metabolic adaptations seen in elite endurance athletes [31,99]. Electron microscopy of FNIP1-deficient muscles reveals a substantial increase in both mitochondrial density and LD content [31], signifying enhanced lipid oxidation as the primary energy source during exercise. At the molecular level, PGC-1α has been shown to regulate IMTG storage and turnover, optimizing mitochondrial lipid utilization [99]. PGC-1α-regulated mitochondrial remodeling enhances FAO, increases LD-associated gene expression, and promotes efficient lipid trafficking [99]**.** These findings further highlight the interplay between IMTG utilization and mitochondrial function in promoting exercise endurance. Moreover, translational studies comparing trained versus untrained individuals reveal a strong correlation between intramuscular lipid gene expression and improved insulin sensitivity [92,99], indicating that the ability to utilize stored lipids efficiently, rather than the absolute IMTG content, determines metabolic health**.**

Concluding remarks and future perspectives

Skeletal muscle mitochondria represent a highly specialized and plastic system capable of continuous remodeling to meet the dynamic metabolic demands imposed by exercise. This review highlights the critical role of mitochondrial biogenesis and quality control, regulated by the integrated action of PGC-1α, AMPK, and FNIP1, in orchestrating skeletal muscle mitochondrial adaptation, substrate flexibility, and endurance capacity. Mitochondria serve as central regulators of metabolic fuel selection, enabling a flexible shift between carbohydrate and lipid oxidation in response to exercise intensity and duration. Importantly, the efficiency of mitochondrial lipid utilization, rather than total intramuscular lipid content, emerges as a key determinant of metabolic health, providing a mechanistic resolution to the 'athlete's paradox'.

Understanding how mitochondria fuel utilization supports skeletal muscle function during exercise remains a critical frontier in muscle biology. While much progress has been made, several

Outstanding questions

How do mitochondria form spatially organized contact sites with contractile structures and membrane systems to meet the localized adenosine triphosphate demand during exercise? Are there specialized mitochondrial contact sites with excitation–contraction structures or lipid droplets that mediate excitation–fuel metabolism coupling? Are there exercise-responsive mechanisms that govern the formation, maintenance, or remodeling of mitochondria–organelle contact sites?

How is mitochondrial protein homeostasis regulated in skeletal muscle during exercise adaptation? How do emerging AMP-activated protein kinase effectors contribute to mitochondrial proteostasis? To what extent do pathways such as folliculin-interacting protein 1-dependent regulation integrate with canonical quality-control networks, and are these mechanisms conserved between rodents and humans?

How do sex differences shape the mitochondrial responses of skeletal muscle to exercise training? Do sex hormones differentially regulate mitochondrial remodeling and fuel utilization in skeletal muscle during exercise adaptation? Can sex-specific mitochondrial programs explain differential metabolic advantages in male versus female muscle during distinct exercise modalities?

What are the molecular signatures of excessive exercise training in skeletal muscle mitochondria? How does excessive exercise training disrupt the balance between mitochondrial biogenesis and quality control? Do sex-specific factors modulate skeletal muscle susceptibility to mitochondrial dysfunction under excessive training stress?

How do mitochondrial metabolites function as active signaling mediators in skeletal muscle adaptation to exercise? How do specific mitochondrial-derived metabolites regulate gene transcriptional programs in skeletal muscle during and after exercise? Which exercise-responsive metabolites mediate interorgan communication to regulate systemic metabolic homeostasis? Can selective targeting of mitochondrial metabolite

signaling enhance exercise adaptation or treat metabolic disease?

key questions remain unanswered (see Outstanding questions). Emerging technologies such as super-resolution microscopy, multiomics profiling [100], metabolite biosensors [67], and AI-based analytical platforms now offer unique opportunities to resolve these knowledge gaps at unprecedented spatial and molecular resolution. These tools will help delineate how mitochondria form specialized contact sites with contractile structures, membrane systems, or LDs to meet spatiotemporal ATP demand during contraction, as well as how mitochondrial quality control and metabolic rewiring are orchestrated during acute and chronic exercise. Recent studies have revealed significant sex-specific metabolic remodeling in skeletal muscle [101]. Specifically, estrogen estradiol (E2) in women was positively correlated with the expression of ribosomal genes, a key indicator of protein synthesis capacity, with inhibitory effects on metabolic gene transcription, whereas higher E2 in men was negatively correlated with ribosomal genes and instead positively associated with inflammatory pathways [101]. This sex divergence likely reflects evolutionary adaptations to differing physiological demands and hormonal landscapes. Similarly, excessive or unbalanced exercise can perturb mitochondrial homeostasis, leading to maladaptive responses such as overtraining syndrome [102,103]. Future research should investigate how sex and training load shape mitochondrial quality control and metabolic reprogramming. Traditionally, mitochondrial metabolites were viewed as passive byproducts or energy substrates generated to meet cellular demands. However, accumulating evidence reveals that these metabolites also act as potent signaling molecules that influence epigenetic states, gene expression, and interorgan communication [104,105]. These discoveries suggest that mitochondrial metabolites may orchestrate complex systemic responses to exercise beyond simple bioenergetics. Yet, the mechanisms by which specific exercise-responsive mitochondrial metabolites are sensed, transported, and decoded in skeletal muscle remain largely unknown. Future studies are needed to uncover how mitochondrial metabolism generates selective signaling outputs, how these signals are integrated with transcriptional programs and interorgan communications, and whether they can be therapeutically harnessed to enhance exercise adaptation or treat metabolic disease. Such efforts will not only deepen our understanding of exercise biology but also provide a foundation for novel therapeutic strategies, leveraging mitochondrial plasticity and fuel flexibility to optimize human performance and combat metabolic diseases.

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Declaration of interests

The authors declare no competing interests.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used ChatGPT in order to assist with English language editing. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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