Advances in the Energy-Sensing Network AMPK/SIRT1/PGC-1α Regulation of Skeletal Muscle Fiber Type Transformation: Postprint

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Abstract

Adenosine monophosphate-activated protein kinase (AMPK), peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α), and silent information regulator 1 (SIRT1) constitute a signaling network that regulates cellular energy output, i.e., the energy sensing network, which collectively regulates organismal energy metabolism, mitochondrial function, and skeletal muscle fiber type transformation. This paper aims to synthesize the latest domestic and international research reports, summarizing the important role played by the AMPK/SIRT1/PGC-lα energy sensing network in the process of skeletal muscle fiber type transformation, to provide a reference for livestock production and human nutrition research.

Full Text

Preamble

Advances in Research on Energy Sensing Network AMPK/SIRT1/PGC-1α Regulating Skeletal Muscle Fiber Type Transformation[1]

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Abstract: AMP-activated protein kinase (AMPK), peroxisome proliferator-activated receptor γ co-activator 1α (PGC-1α), and silent information regulator 1 (SIRT1) constitute a signaling network that regulates cellular energy output, known as the energy sensing network, which collectively modulates whole-body energy metabolism, mitochondrial function, and muscle fiber type transformation. This review synthesizes the latest research findings both domestically and internationally to summarize the critical roles of the AMPK/SIRT1/PGC-1α energy sensing network in muscle fiber type transformation, providing references for livestock production and human nutrition research.

Keywords: muscle fiber type transformation; AMPK/SIRT1/PGC-1α; mitochondrial function; energy metabolism

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Skeletal muscle comprises 40%~50% of mammalian body composition and regulates whole-body energy metabolic homeostasis [1]. Skeletal muscle is primarily composed of mature muscle cells known as muscle fibers, which exhibit strong plasticity by altering their type composition to adapt to various physiological states—a process termed "muscle fiber type transformation."

Muscle fiber proliferation is completed during the embryonic period, with the number of fibers essentially fixed at birth, whereas muscle fiber type transformation occurs throughout the animal' s entire growth and development period. Investigating skeletal muscle fiber types provides theoretical references for studying muscle development, plasticity, and nutritional intervention approaches.

1.1 Muscle Fiber Structure and Classification

Based on the differential expression of myosin heavy chain (MyHC), skeletal muscle fibers are classified into four types [2]: MyHC Type I (slow oxidative, marker gene *Myh7*), MyHC Type IIa (fast oxidative, marker gene *Myh2*), MyHC Type IIb (fast glycolytic, marker gene *Myh4*), and MyHC Type IIx (intermediate, marker gene *Myh1*).

Type I muscle fibers are considered oxidative metabolism fibers, also known as slow oxidative fibers, with smaller diameters that primarily rely on aerobic metabolic pathways for energy supply, depending on fatty acid oxidation to generate ATP. Type II muscle fibers, also known as fast glycolytic fibers, mainly depend on glycolytic pathways for energy supply, using glucose as the energy substrate. Types IIx and IIb fibers are considered glycolytic metabolism fibers, while Type IIa fibers represent oxidative-glycolytic mixed metabolism fibers.

The contractile and metabolic characteristics of Type IIa fibers are intermediate between Types I and IIx, with Types IIb and IIx being relatively similar. The content of triglycerides in different muscle fiber types follows the order: I > IIa > IIx = IIb [3], whereas mitochondrial content ranking varies among species [4]. Mitochondria constitute 1%~45% of skeletal muscle cell volume [5], with significant differences in both mitochondrial volume and function among different fiber types. Oxidative fibers obtain the vast majority of their energy from mitochondria, while glycolytic fibers also depend on mitochondria to maintain basal metabolism and repair metabolism after high-intensity exercise [6].

1.2 Muscle Fiber Growth and Development

Skeletal muscle satellite cells are highly active and possess the capacity to regulate muscle fiber type composition and remodel skeletal muscle. Satellite cells are stem cells residing in muscle tissue that normally remain quiescent but become activated and enter the cell cycle of proliferation, differentiation, and fusion upon muscle trauma or other special physiological conditions [7]. The expression levels of marker genes in satellite cells vary depending on their stage. From birth to adulthood, the number of satellite cells shows no significant change, while muscle fiber diameter increases markedly; however, the diameter of Type II fibers significantly decreases with age, accompanied by a reduction in satellite cell number [8]. The Type I fiber-specific protein Myh7 is also expressed in proliferating myoblasts. Once Myh7 expression is initiated, myoblasts enter the differentiation stage, but multiple possibilities exist for differentiation into slow or fast muscle tubes [9], which contradicts the traditional view that Myh7 is only expressed in differentiated muscle cells. Nucleoside analog reverse transcriptase inhibitor (zidovudine)-induced mitochondrial myopathy shows a significant increase in Type II fibers, upregulated expression of myogenic differentiation antigen (MyoD), and downregulated expression of myogenin and estrogen-related receptor γ (ERRγ); mitochondrial ultrastructure abnormalities, DNA depletion, decreased cytochrome C oxidase activity, and loss of reconstructive capacity for fiber type transformation [10], demonstrating that mitochondrial function is intimately linked with muscle fiber type.

2 Energy Sensing Network and Muscle Fiber Transformation

Muscle fiber type composition is closely related to mitochondria, and energy metabolism represents the primary function of mitochondria; in other words, cellular energy metabolism regulates muscle fiber type. AMP-activated protein kinase (AMPK), peroxisome proliferator-activated receptor γ co-activator 1α (PGC-1α), and silent information regulator 1 (SIRT1) are all known as "energy sensors" with interconnected functions and regulatory roles, forming an energy sensing network that controls cellular energy output [11-12] and thereby regulates muscle fiber type composition.

Abbreviations: CaMK, calcium/calmodulin-dependent protein kinase; CaMKK, calcium/calmodulin-dependent protein kinase kinase; AMPK, AMP-activated protein kinase; SIRT1, silent information regulator 1; PGC-1α, peroxisome proliferator-activated receptor γ co-activator 1α.

[Figure 1: see original paper] Figure 1. Energy metabolism network of AMPK/SIRT1/PGC-1α involved in mitochondrial biogenesis and oxidative metabolism in type I muscle fiber [12].

AMPK exists in cells as a heterotrimeric complex composed of a catalytic α subunit and regulatory β and γ subunits. AMPK expression differs among muscle fiber types: $ $3 is expressed only in skeletal muscle, with the highest expression in Type IIb fibers, followed by Type IIa fibers, and is barely detectable in Type I fibers [13]. Over the past decade, research using transgenic and knockout mice as well as chemical activators or inhibitors has deeply investigated muscle fiber type transformation, identifying a series of important regulators of skeletal muscle fiber type, among which the most critical is the energy sensor AMPK, which regulates mitochondrial biogenesis, Type I fiber formation, and endurance adaptation during long-term training [14-15].

Increased intracellular calcium ion (Ca^{2+}) concentration and AMP/ATP ratio can activate AMPK, which maintains energy homeostasis by regulating mitochondrial biogenesis, nicotinamide adenine dinucleotide (NAD^+) levels, ATP production, and autophagy, while simultaneously shutting down the ATP-consuming mTOR-mediated cell growth and protein synthesis pathways, shifting metabolism toward ATP-generating catabolic pathways. AMPK regulates the expression of PGC-1α and PGC-1β through synergistic signaling with the Ca^{2+} second messenger, modulating metabolism and differentiation related to muscle cells, thereby promoting mitochondrial biogenesis, aerobic metabolism, and slow-twitch fiber formation while limiting muscle growth [16-17]. Recent studies show that the R615C mutation in the ryanodine receptor 1 (RyR1) in porcine skeletal muscle increases muscle fiber diameter by approximately 35%, while the R200Q mutation in AMPK$ $3 simultaneously increases mitochondrial protein content by approximately 50% and oxidative capacity [18], indicating that muscle fiber diameter and oxidative capacity can be improved concurrently.

The sirtuin (SIRT) family comprises seven members (SIRT1-7) that participate in regulating cellular energy status based on the NAD^+/NADH ratio [19]. To date, the regulatory mechanisms of SIRT1 have been most thoroughly investigated. AMPK enhances the activity of another energy sensor—SIRT1—by increasing intracellular NAD^+ levels, leading to deacetylation and activity modulation of SIRT1 downstream targets, among which the most important is PGC-1α. In muscle tissue, SIRT1 promotes PGC-1α transcription through an autoregulatory loop [20]. AMPK-induced, SIRT1-mediated target deacetylation explains many convergent biological effects of AMPK and SIRT1 on energy metabolism [21].

The SIRT1/PGC-1α energy axis plays a crucial role in skeletal muscle energy metabolism, demonstrating that cells can regulate energy metabolism through transcriptional mechanisms, though the interaction mechanism between SIRT1 and PGC-1α under energy stress remains unclear. Mice with skeletal muscle-specific SIRT1 overexpression show a shift from fast-twitch to slow-twitch fibers, with upregulated expression of PGC-1α—a marker of oxidative metabolism and mitochondrial biogenesis—whereas mice with SIRT1 knockout show no significant change in muscle fiber type composition [22], indicating that alternative pathways exist to regulate normal muscle fiber type composition and maintain metabolic homeostasis; however, under normal conditions, SIRT1 plays a special role. Recent reports indicate that PGC-1α can selectively regulate SIRT3 or SIRT5, with SIRT5 exerting positive effects on oxidative phosphorylation, while AMPK and PGC-1α have opposite effects on SIRT5 regulation [23]. We speculate that SIRT family members are differentially involved in energy metabolism and mitochondrial biogenesis.

2.3 PGC-1α

Mitochondrial biogenesis in skeletal muscle cells requires a series of transcription factors, including PGC-1α and its downstream proteins mitochondrial transcription factor A, nuclear respiratory factor 1 and 2 [24]. PGC-1α resides in mitochondria and requires post-translational modifications—including acetylation and phosphorylation—for activation [25]. Increased mitochondrial biogenesis can promote activation of calcium/calmodulin-dependent protein kinase (CaMK), p38-mitogen-activated protein kinase (MAPK), and AMPK. AMPK activation induces phosphorylation of PGC-1α at threonine 177 (Thr177) and serine 538 (Ser538), initiating and activating PGC-1α [26]. In addition to these phosphorylation sites, PGC-1α has multiple different acetylation sites, and both in vivo and in vitro studies have demonstrated that SIRT1 can mediate PGC-1α deacetylation to activate it [27-28]. Therefore, both the deacetylase SIRT1 and the kinase AMPK can increase PGC-1α activity. PGC-1α is a master regulator of mitochondrial oxidative metabolism and cellular energy homeostasis, exerting its effects through downstream effector proteins including peroxisome proliferator-activated receptor α (PPARα) and estrogen-related receptor α (ERRα).

PGC-1α plays a critical role in maintaining normal muscle fiber type composition and integrity. PGC-1α is highly expressed in slow-twitch Type I fibers and promotes slow-twitch fiber formation [29]. Skeletal muscle-specific PGC-1α knockout mice exhibit a shift from Types I and IIa to Types IIx and IIb fibers, decreased endurance, and significantly elevated inflammatory factor levels after exercise [30]. Other studies report that muscle-specific PGC-1α overexpression in mice enhances regulation of mitochondrial oxidative metabolism [32] and increases mitochondrial content and capillary density in both cardiac and skeletal muscle [31]. Conversely, muscle-specific PGC-1α knockout reduces skeletal muscle mitochondrial content and decreases expression of mitochondria-related

genes [30]. Although this study did not examine muscle fiber type composition, we speculate that composition changes with alterations in mitochondrial content. PGC-1α also regulates lipid droplet formation in muscle cells [32], further reflecting its key role in muscle cell energy metabolism. In rodent and human skeletal muscle, PGC-1α expression is higher in Type IIa fibers than in Type I fibers, which seemingly indicates that PGC-1α is not the sole determinant in muscle fiber type specification and may need to work in concert with other factors such as calcineurin, nuclear factor of activated T cells, CaMK, p38-MAPK, and/or PPARδ [4].

3.1 Improvement of Pork Quality

Pork quality is primarily determined by the biological characteristics of porcine muscle, with Type I fibers being a crucial factor. Increasing the proportion of Type I fibers in muscle contributes to improved meat color, water-holding capacity, and tenderness, while reducing the rate and extent of pH decline. Studies have found that the proportion of MyHC Type I fibers in Meishan pigs is significantly negatively correlated with drip loss and significantly positively correlated with pH_{45} and intramuscular fat content [33]. Comparative studies of meat quality between Bama Xiang pigs and Landrace pigs demonstrate that AMPK and PGC-1α gene expression in the longissimus dorsi muscle is extremely significantly positively correlated with oxidative fiber Types I and IIa [34]. Compared with Large White pigs, Erhualian pigs contain higher proportions of MyHC Type I and IIa fibers in the longissimus dorsi muscle [32]. Rongchang pigs show lower proportions of MyHC Type IIb fibers but significantly elevated PGC-1α gene expression levels compared with three-way crossbred pigs [35], all of which may be related to the superior meat quality of local pig breeds.

3.2 Improvement of Human Health

While genetic factors determine the fundamental muscle fiber type composition, physiological factors such as exercise can influence fiber type composition, mitochondrial biogenesis, and energy metabolic pathways after birth. Whole-body insulin sensitivity and insulin-stimulated glucose transport are positively correlated with the proportion of Type I fibers [36]. Long-term exercise training increases the proportion of oxidative fibers and mitochondrial biogenesis, which in turn enhances exercise capacity and fatty acid and glucose oxidation [37-38], accompanied by AMPK- and SIRT1-mediated PGC-1α deacetylation [39]. Conversely, reduced physical activity, particularly in obesity or chronic disease states, decreases the proportion of Types I and IIa fibers and significantly reduces skeletal muscle glucose utilization capacity and insulin sensitivity [40]. Therefore, exercise training can improve whole-body health by altering skeletal muscle fiber type composition.

Muscle fiber type composition determines the fundamental biological characteristics of skeletal muscle tissue. Energy sensing network AMPK/SIRT1/PGC-

1α-mediated muscle fiber type transformation accompanies animal growth and development, though the precise mechanisms require further investigation. Myoblasts during the embryonic period prior to muscle cell differentiation exhibit molecular differences [41], making it necessary to understand the differences in muscle fiber types during embryogenesis and the molecular mechanisms of the myogenic lineage that produce these differences. Targeted nutritional interventions based on these differences can improve livestock production while providing references for human nutrition research.

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