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AMPK signaling in skeletal muscle during exercise: Role of reactive oxygen and nitrogen species

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ABSTRACT

Reactive oxygen and nitrogen species (RONS) are generated during exercise depending on intensity, duration and training status. A greater amount of RONS is released during repeated high-intensity sprint exercise and when the exercise is performed in hypoxia. By activating adenosine monophosphate-activated kinase (AMPK), RONS play a critical role in the regulation of muscle metabolism but also in the adaptive responses to exercise training. RONS may activate AMPK by direct an indirect mechanisms. Directly, RONS may activate or deactivate AMPK by modifying RONS-sensitive residues of the AMPK-α subunit. Indirectly, RONS may activate AMPK by reducing mitochondrial ATP synthesis, leading to an increased AMP:ATP ratio and subsequent Thr^{172}-AMPK phosphorylation by the two main AMPK kinases: LKB1 and CaMKKβ. In presence of RONS the rate of Thr^{172}-AMPK dephosphorylation is reduced. RONS may activate LKB1 through Sestrin2 and SIRT1 ($NAD^+/NADH.H^+$-dependent deacetylase). RONS may also activate CaMKKβ by direct modification of RONS sensitive motifs and, indirectly, by activating the ryanodine receptor (Ryr) to release Ca^{2+}. Both too high (hypoxia) and too low (ingestion of antioxidants) RONS levels may lead to Ser^{485}-AMPKα1/Ser^{491}-AMPKα2 phosphorylation causing inhibition of Thr^{172}-AMPKα phosphorylation. Exercise training increases muscle antioxidant capacity. When the same high-intensity training is applied to arm and leg muscles, arm muscles show signs of increased oxidative stress and reduced mitochondrial biogenesis, which may be explained by differences in RONS-sensing mechanisms and basal antioxidant capacities between arm and leg muscles. Efficient adaptation to exercise training requires optimal exposure to pulses of RONS. Inappropriate training stimulus may lead to excessive RONS formation, oxidative inactivation of AMPK and reduced adaptation or even maladaptation. Theoretically, exercise programs should be designed taking into account the intrinsic properties of different skeletal muscles, the specific RONS induction and the subsequent signaling responses.

1. Introduction

Adenosine monophosphate-activated kinase (AMPK) is considered a metabolic master switch, which turns off several anabolic processes at the same time that turns on catabolic processes [123]. AMPK is usually activated, in response to hypoxia, exhausting exercise, and caloric restriction [114,73]. This is accompanied by activation of several metabolic pathways in an attempt to acutely increase the energy level of the cell [78]. Concomitantly, additional signaling pathways are activated to elicit chronic adaptations, which produce a switch to a more oxidative phenotype [124] ultimately increasing the chances of survival [15]. AMPK has become a therapeutic target since AMPK activation promotes Glut4 translocation and mitochondrial biogenesis in skeletal muscle [101]. However, the regulation of AMPK activation is modulated depending on the tissue and the micro-environment [73]. Just in skeletal muscle, the response to one bout of high-intensity exercise may elicit phosphorylation in 1004 phosphosites on 562 proteins, several of which are related with AMPK but with a previous unknown implication on exercise signaling [52].

2. AMPK activation/inhibition mechanisms

2.1. Canonical AMPK activation

AMPK is an enzyme with a heterotrimeric structure composed by one catalytic subunit (α) and two regulatory (β and γ). The γ

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subunit has a cystathionine β-synthase (CBS) motif capable of binding adenosyl nucleotides (ATP, ADP and AMP) [18]. The binding of AMP to AMPK causes an increase of AMPK activity by three mechanisms: (1) direct allosteric activation; (2) facilitation of phosphorylation of the 172 threonine residue; and (3) inhibition of Thr^{172} dephosphorylation [45]. While AMP activates, ATP inhibits AMPK activation such that only an increase of AMP:ATP ratio produces AMPK activation. When AMP:ATP ratio increases, AMP binds to γ subunit and increases AMPK activity (allosteric activation) even in the absence of LKB1 [41]. In addition, binding of AMP to γ subunit induces a conformational change exposing the 172 threonine residue of the AMPK α subunit [46]. This allows the phosphorylation of 172 threonine residue by a heterotrimeric complex containing the tumor suppressor kinase LKB1, the main AMPK upstream kinase [47], which increases further AMPK activity. Although LKB1 appears to be constitutively active [105], LKB1 activity is also regulated by phosphorylation, deacetylation and compartmentalization [113,6].

The γ subunit of AMPK has three specific binding sites for AMP, ADP or ATP [45]. Binding of AMP to site 1 elicits conformational changes in AMPK, which facilitate Thr^{172} phosphorylation by LKB1. When AMP or ADP binds to the site 3 of the γ subunit protects Thr^{172}-AMPKα phosphorylation from the action of phosphatases 2A and 2C, which dephosphorylate and deactivate AMPK [117,15,96]. Wright et al. [125] have shown that tyrosine and Ser/Thr phosphatases may be inhibited by exposure to RONS in skeletal muscle. RONS play a more important role in the non-canonical regulation of AMPK activity [15].

As illustrated in Fig. 1, LKB1 compartmentalization is regulated by STe20 Related ADaptor (STRAD). STRAD binds to and phosphorylates LKB1 facilitating the migration from the nucleus to the cytoplasm [6]. The phosphorylation produced by STRAD is not necessary to produce an increase on LKB1 activity [8] since Ser^{431}-LKB1 (orthologous to Ser^{428} in humans) is distal to the kinase domain [32]. The formation of the STRAD-LKB complex requires MO25. MO25 binds to COOH-terminal Trp-Glu-Phe residues of STRADα and to an additional binding site created by the union of STRAD-LKB1 [1], which produces a more stable complex LKB1-STRAD-MO25 [79]. The complex LKB1-STRAD-MO25 remains in the cytoplasm where AMPK can be phosphorylated.

Sestrin2 has been reported to increase directly AMPK activity [10] (Fig. 1). Sestrins 1 to 3 constitute a family of proteins that are induced in mammalian cells in response to environmental stressors [100]. Sestrin2 expression is upregulated by human myotubes exposed to H_2O_2 [88] and accumulates in cardiomyocytes during ischemia [85]. Sestrin2 stabilizes the LKB1/AMPK complex, which is involved in AMPK activation in response to ischemia [85]. Sestrin2 expression is modulated by p53 [10] and upregulates

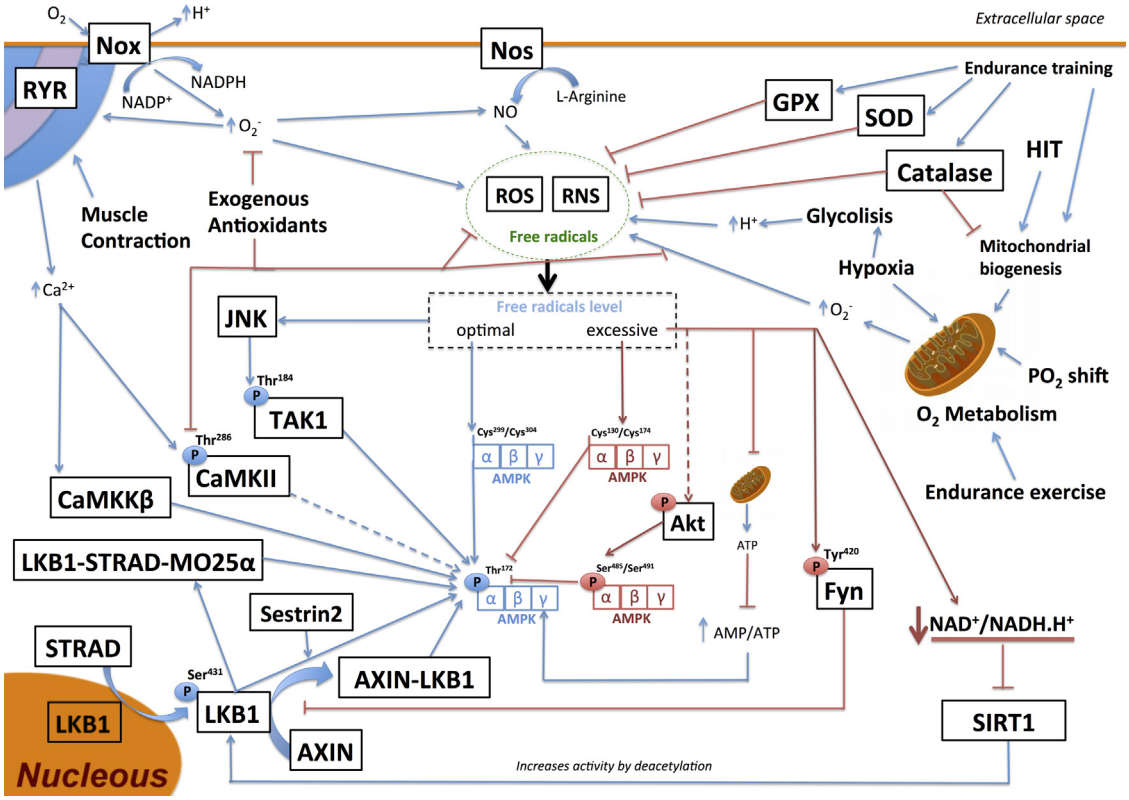

Fig. 1. The main sources of reactive oxygen and nitrogen species (RONS) during muscle contraction are mainly NADPH oxidase (NOX) and Mitochondria. Optimal levels of RONS elicit AMPK activation by Thr^{172}-AMPKα phosphorylation. Acute effects of RONS: JNK phosphorylates Thr^{184}-Tak1, an upstream AMPK kinase. Ca^{2+} release through ryanodine receptors (Ryr) increases Thr^{286}-CaMKII phosphorylation and CaMKKβ total protein amount. Moderate levels of RONS lead to PKC activation, which phosphorylates LKB1 at Ser^{431} activating LKB1. LKB1 activates AMPK by Thr^{172} phosphorylation. AMPK increases the protein expression of SIRT1. SIRT1 deacetylates and activates LKB1. Sestrin2 stabilizes the LKB1–AMPK complex and promotes Thr^{172}-AMPKα phosphorylation. Excessive levels of RONS cause JNK phosphorylation (activation), which phosphorylates SIRT1 at Ser^{47} committing SIRT1 to proteasome degradation. A lower amount of SIRT1 reduces LKB1 deacetylation and hence, Thr^{172}-AMPKα phosphorylation. High RONS levels increase Fyn autophosphorylation at Tyr^{420}, which leads to AMPKα1/2 phosphorylation at $Ser^{485/491}$ inhibiting AMPKα phosphorylation at Thr^{172}. Tyr^{420}-Fyn phosphorylates tyrosine residues (Tyr^{261} and Tyr^{365}) in LKB1 in the nucleus preventing LKB1 cytoplasmic translocation, which is necessary for AMPK activation. AMP increases the binding between AXIN and LKB1, resulting in Thr^{172}-AMPKα phosphorylation. Exogenous antioxidants decrease RONS levels blunting Thr^{286}-CaMKII phosphorylation and increase the inhibitory Ser^{485}-AMPKα1/Ser^{491}-AMPKα2 phosphorylation. Moderate RONS levels inhibit mitochondrial respiration causing an increase of the AMP:ATP ratio and Thr^{172}-AMPKα phosphorylation. Moderate RONS may also activate directly AMPK. Excessive RONS may cause oxidative inactivation of AMPK. Chronic effects of RONS: repeated exposure to RONS pulses by endurance or high-intensity exercise (HIT) enhances the endogenous antioxidant defense mechanisms and nitric oxide synthase (NOS) levels. In the trained state, the endogenous antioxidant system blunts the RONS-mediated signaling resulting in lower or absent Thr^{172}-AMPKα phosphorylation in response to exercise, unless unaccustomed exercise bouts are performed. Dashed arrows indicate unknown mechanism.