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AMP-activated protein kinase regulation and action in skeletal muscle during exercise

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Abstract

Physical exercise increases muscle glucose uptake, enhances insulin sensitivity and leads to fatty acid oxidation in muscle. The AMP-activated protein kinase (AMPK) is an energy-sensing enzyme that is strongly activated during muscle contraction due to acute decreases in ATP/AMP and phosphocreatine/creatine ratios. Accumulating evidence suggests that AMPK plays an important role in mediating these metabolic processes. Furthermore, AMPK has been implicated in regulating gene transcription and therefore may play a role in some of the cellular adaptations to training exercise. There is also evidence that changes in AMPK activity result in altered cellular glycogen content, suggesting that this enzyme regulates glycogen metabolism. Recent studies have shown that the magnitude of AMPK activation and associated metabolic responses are affected by factors such as glycogen content, exercise training and fibre type. In summary, AMPK regulates several metabolic pathways during acute exercise and modifies the expression of many genes involved in the adaptive changes to exercise training.

Introduction

Exercise acutely stimulates glucose uptake into contracting skeletal muscles, enhances post-exercise insulin sensitivity and promotes the oxidation of circulating fatty acids. In subjects with Type 2 diabetes, these changes play an important role in the beneficial long term effects of exercise, such as lowering blood glucose concentrations and improving the lipid profiles. Moreover, exercise can prevent or delay the development of this disease.

Despite years of intensive research, the molecular mechanisms by which exercise promotes these effects are still largely unknown. The AMP-activated protein kinase (AMPK) has recently been implicated in the regulation of many of these exercise-induced effects. Furthermore, there is evidence that this enzyme may be involved in the chronic adaptations to exercise training in skeletal muscle through the modification of gene expression of several proteins. In this article we review how AMPK regulates glucose and fat metabolism in muscle and its potential role in exercise-induced gene transcription.

AMPK

AMPK is a heterotrimer formed by an α subunit, which contains the catalytic domain, and by the β and γ subunits, important in maintaining the stability of the heterotrimer and for substrate specificity [1,2]. There are two or more different isoforms for each subunit [1]. AMPK α1 is widely expressed, while the α2 subunit isoform is expressed mostly in liver, heart and skeletal muscle [3]. Decreases in ATP/AMP and in phosphocreatine/creatine ratios causes AMPK activation through Thr172 phosphorylation by one or more upstream kinases (AMPK kinase) and by allosteric modification [1,2]. AMPK activity increases during cellular stress, in conditions such as muscle contraction, hypoxia, ischaemia and hyperosmolarity, and by uncouplers of oxidative phosphorylation [4,5]. AMPK is an energy-sensing enzyme, such that when the cell is under these conditions associated with energy depletion, AMPK switches off ATP-consuming pathways and switches on pathways for ATP regeneration.

Activation of AMPK by exercise

During contraction there are rapid decreases in ATP/AMP and phosphocreatine/creatine ratios, causing robust activation of AMPK. How contraction increases AMPK activity has been reviewed elsewhere [6]. Different experimental systems have consistently shown that contraction increases AMPK activity. For example, exercise in rats [7,8], sciatic nerve-stimulated muscle contractions in situ [9,10] and contraction of isolated muscles in vitro [4,8,11,12] all significantly increase AMPK activity in muscle. Cycle exercise also increases AMPK α2 activity in humans in an intensity- and time-dependent manner [13–15]. AMPK α2 is activated during moderate-intensity exercise whereas, in general, AMPK α1 is more resistant and requires a stronger stimulus to be activated, such as electrically stimulated isolated rat muscle contractions [8] and, in humans,

**AMPK and muscle glucose uptake**

When it was found that, similar to contraction, chemical activation of AMPK with 5-amino-4-imidazolecarboxamide riboside (AICAR) increased insulin-stimulated glucose uptake in perfused rat hindlimb muscles, it became apparent that this enzyme could be playing a role in mediating muscle glucose uptake [17]. AICAR is a compound that, after entering the cell, is metabolized to its monophosphorylated form (5-amino-4-imidazolecarboxamide ribotide, 'ZMP') and increases AMPK activity by mimicking the effects of AMP [18]. Like contraction, AICAR increases muscle glucose uptake in isolated rat muscles in the absence of insulin and through a wortmannin-insensitive mechanism, suggesting that AICAR and contraction may use a similar insulin-independent pathway to acutely stimulate muscle glucose uptake [11,19]. In rat epitrochlearis muscles incubated in vitro, the activation of AMPK in response to stimuli such as AICAR, muscle contraction, hypoxia and hyperosmolarity correlates closely with the increases in muscle glucose uptake [4,8], further supporting a role for this enzyme in regulating muscle glucose uptake.

For the most part, the hypothesis that AMPK plays a role in contraction-stimulated glucose uptake has been based on correlative data and on studies using AICAR, which is not AMPK-specific. In mice carrying a kinase-inactive AMPK mutant in skeletal muscle AICAR- and hypoxia-stimulated glucose uptake is completely abolished [20]. In contrast, contraction-stimulated glucose uptake is only partially reduced in these mice, indicating that while AMPK plays a role in regulating glucose uptake during contraction, it is not indispensable for this effect and other mechanisms are probably involved in this process.

Similar to contraction, AICAR-stimulated muscle glucose uptake involves GLUT4 glucose transporter translocation to the plasma membrane [21]. In H-2Kb skeletal muscle cells, overexpression of constitutively active AMPK stimulates glucose uptake accompanied by GLUT1 and GLUT4 translocation, indicating that AMPK is sufficient to stimulate glucose uptake and, furthermore, that AMPK-mediated glucose uptake involves glucose transporter translocation [22].

Several molecules have been implicated in mediating AMPK-stimulated glucose uptake downstream of this enzyme. It has been proposed that NO is involved in muscle glucose uptake during exercise [23] and it has been shown that AMPK phosphorylates endothelial nitric oxide synthase (NOS) [24,25]. These findings suggest that the effects of AICAR on muscle glucose uptake could be mediated through NOS [26]. However inhibition of NOS does not decrease contraction-stimulated glucose uptake in rat muscles [27,28]; therefore, the role that NOS plays in AMPK-mediated muscle glucose uptake is unclear. The p38 mitogen-activated protein kinase has also been proposed as a mediator of contraction-stimulated glucose uptake [29]. In clone 9 cells, AICAR phosphorylated both p38 and AMPK, while AICAR-stimulated glucose uptake into these cells was p38-dependent [30]. These findings suggest that AMPK stimulates glucose uptake through activation of p38 [30]. It remains to be determined whether the regulation of p38 by AMPK occurs in animal and human tissue in vivo.

Findings from a study done in L6 myotubes and isolated rat muscles suggest that the effects of AMPK on glucose uptake are mediated through the sequential activation of extracellular signal-regulated kinase, proline-rich tyrosine kinase-2, phospholipase D and atypical protein kinase C isoforms [31]. However, inhibition of contraction-induced extracellular signal-regulated kinase phosphorylation by a mitogen-activated protein kinase inhibitor does not affect contraction-stimulated glucose uptake in rat muscles [32,33]. Therefore, further research is needed to clarify the role of these molecules in regulating AMPK-mediated metabolic responses.

Several factors can modify the magnitude of AMPK activation in response to a stimulus. Glycogen supercompensation in rat skeletal muscle significantly blunts the acute increases in AICAR- and contraction-stimulated AMPK activity and glucose uptake [34,35]. In contrast, subjects with McArdle’s disease, who have high muscle glycogen content due to a lack of functional glycogen phosphorylase, have exaggerated muscle AMPK α2 activation and glucose disposal during exercise [36]. This suggests that the inhibitory effect of glycogen on stimulated AMPK activity and glucose disposal is offset when glycogenolysis is impaired.

The nutritional state also modifies the activation of AMPK. In rat epitrochlearis and flexor digitorum brevis (FDB) muscles, there is a tendency for contraction-stimulated AMPK activity to be higher in the fasted compared with the fed state, while AICAR increases AMPK activity in these muscles to a similar degree regardless of the nutritional state [37]. In epitrochlearis, AICAR- and contraction-stimulated glucose uptake are also higher in the fasted state, but in FDB and soleus the increases in glucose uptake by contraction are not affected by the nutritional state.

The activation of AMPK by different stimuli also varies depending on fibre type [37]. In rat muscles, contraction produces a strong activation of AMPK in epitrochlearis (IIB) fibres, FDB (IIs) and soleus (I), while AICAR increases AMPK activity in both epitrochlearis and FDB but has no effect in soleus. Similarly, AICAR stimulates glucose uptake in epitrochlearis and FDB but has no effect in soleus [37].

Endurance training also modifies the activation of AMPK by acute exercise [38]. In red quadriceps muscle from rats, exercise strongly activated AMPK α2 but not α1, while 7 weeks of exercise training blunted exercise-stimulated AMPK α2 activity. In white gastrocnemius, acute exercise did not increase AMPK activity, but interestingly training led to increased basal AMPK α2 activity and AMPK Thr172 phosphorylation. Exercise caused only a small increase in AMPK α1 and α2 activities in soleus, and training did not alter this response.
Because AMPK is an interesting pharmacological target [39,40], the activation of AMPK by contraction has been investigated in animal models of Type 2 diabetes and human subjects with this disease. In lean Zucker rats, contraction of isolated epitrochlearis muscles increased both AMPK α1 and α2 activities, but in obese Zucker rats only α2 activity increased [41]. This differential activation of the α subunit isoforms did not affect contraction-stimulated muscle glucose uptake [41]. We studied subjects with Type 2 diabetes, and found that moderate-intensity exercise increased AMPK α2 activity to a similar degree in skeletal muscle from these subjects compared with non-diabetic controls, while the α1 isoform did not change in either group [42]. The findings from these studies suggest that AMPK α1 is not indispensable for contraction to increase glucose uptake in muscle [41] and that AMPK α2 is the predominant isoform involved in AMPK-regulated responses to exercise [13]. Moreover, subjects with Type 2 diabetes have a normally functioning AMPK amenable for pharmacological activation.

**Insulin sensitivity**
Besides directly stimulating muscle glucose uptake, AMPK may also play a role in enhancing insulin sensitivity. A 5 day AICAR treatment to Wistar rats increases insulin-stimulated GLUT4 translocation and glucose uptake in isolated muscles [43]. Preincubation of isolated rat muscles in vitro with AICAR for 1 h also results in enhanced insulin-stimulated glucose uptake [44]. In insulin-resistant high-fat-fed rats, a single subcutaneous AICAR dose increases whole-body, liver and muscle insulin sensitivity measured 1 day after the AICAR dose using a hyperinsulinaemic euglycaemic clamp study [45]. This insulin-sensitizing effect of AICAR is analogous to the effects of exercise; however, it remains to be determined how activation of AMPK subsequently enhances insulin sensitivity.

**Regulation of fatty acid oxidation**
The first evidence that the activity of AMPK increases during exercise came from studies investigating the role of this enzyme in exercise-induced fatty acid oxidation in muscle. Activation of AMPK during exercise causes phosphorylation of acetyl-CoA carboxylase (ACC) by AMPK, decreasing its activity [9,10,46]. Lower ACC activity leads to a fall in malonyl-CoA content in muscle, relieving the inhibitory effect of malonyl-CoA on carnitine palmitoyltransferase 1, causing entry of fatty acids to the mitochondria for its oxidation. In rat skeletal muscle, stimulation of AMPK by electrically stimulated contractions [9,10,46,47] and exercise [46,47] leads to phosphorylation and inactivation of ACC. In human muscle, exercise also phosphorylates [16,48] and inactivates [49] ACC, accompanied by higher fat oxidation [49].

It has been reported that, in rats, malonyl-CoA decarboxylase (MCD) activity increases during in situ muscle contractions [50], treadmill exercise [51] and AICAR treatment [50], suggesting that AMPK may activate MCD during exercise. However, another group did not observe increases in MCD activity with contraction of isolated rat muscles or after exposure to AICAR [52]; therefore, further research is needed to clarify the regulation of MCD by AMPK.

**Glycogen metabolism**
The hypothesis that AMPK plays a role in glycogen metabolism comes from the following findings: (i) in vitro, AMPK phosphorylates glycogen synthase and phosphorylase kinase [53]; (ii) mutations of AMPK γ2 and γ3 are associated with increased glycogen content in human heart [54] and skeletal muscle of Hampshire pigs respectively [55], and (iii) similar to exercise training, chronic AICAR treatment in vivo increases muscle glycogen content in rats [43,56,57]. Two recent studies further investigated the potential regulation of glycogen metabolism by AMPK. In perfused rat hindlimb muscles, AICAR caused phosphorylation and inactivation of glycogen synthase [34]. In contrast, administration of AICAR to rats in vivo increased glycogen synthase activity in red and decreased it in white gastrocnemius muscle; however, isolated rat muscle incubations with AICAR had no effect on either glycogen synthase or glycogen phosphorylase, indicating that AMPK does not directly regulate their activity [58]. While chronic AMPK activation may lead to increased cellular glycogen content, based on these conflicting findings [34,58], the mechanism responsible for this effect remains unclear.

**Gene expression**
Similar to the effects of exercise training, chronic AICAR treatment of Sprague–Dawley rats increases GLUT4 protein content and hexokinase activity [56]. AICAR treatment for 28 days also caused an increase in the content of GLUT4 and the mitochondrial enzymes cytochrome c and δ-aminolevulinic acid synthase and in the activity of citrate synthase, succinate dehydrogenase and malate dehydrogenase [57]. Incubation of isolated rat muscles in vitro for 18 h with AICAR also increased GLUT4 expression and hexokinase activity [59]. Consistent with these findings, in vivo AICAR treatment in rats caused a significant increase in the rate of uncoupling protein-3 and hexokinase II gene transcription in muscle [60]. To exclude the possibility of a systemic effect, single-leg arterial infusions with AICAR were performed. Gene transcription of uncoupling protein-3 and hexokinase II increased in muscle from the leg infused with AICAR, but not from the saline-infused leg.

These results suggest that the activation of AMPK is involved in some of the biochemical adaptations that take place during exercise training. Furthermore, these findings indicate that AMPK may regulate gene transcription. In line with this, AICAR treatment rapidly induced GLUT4 gene transcription in muscle within a few hours of administration [61]. Treatment of rats for 9 weeks with β-guanadinopropionic acid, a compound that by depleting
cellular phosphorelay and ATP increases AMPK activity, caused an increase in cytochrome c content, mitochondrial density and nuclear respiratory factor-1 DNA-binding activity [62]. This transcription factor plays a role in mitochondrial replication and regulates gene transcription of respiratory proteins. In Sprague–Dawley rats, swimming exercise also stimulated peroxisome proliferator-activated receptor γ co-activator-1 (PGC-1) gene expression in muscle, and this effect was reproduced by incubating isolated muscles with AICAR for 18 h [63]. This suggests that AMPK may induce exercise-stimulated mitochondrial biogenesis through PGC-1, and gives further support to the hypothesis that AMPK is involved in the cellular adaptations to exercise training by regulating gene transcription.

Summary

AMPK regulates several exercise-induced metabolic responses, such as increasing glucose uptake and fatty acid oxidation in muscle. Activation of AMPK during exercise may play a role in some of the chronic adaptations to physical training, including gene expression of GLUT4, PGC-1 and mitochondrial enzymes. There is evidence suggesting that AMPK may regulate cellular glycogen content. However, the precise role that this enzyme plays in glycogen metabolism remains to be determined in future investigations.

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