Review article

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Regulation of exercise-stimulated glucose uptake in skeletal muscle

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AMP-activated protein kinase (AMPK) is a Ser/Thr kinase that has been thought to be an important mediator for exercise-stimulated glucose uptake in skeletal muscle. Liver kinase B1 (LKB1) is an upstream kinase for AMPK and AMPK-related protein kinases, of which the function in skeletal muscle has not been well documented. Our group and others have generated mice lacking AMPK activity in skeletal muscle, as well as muscle-specific LKB1 knockout mice. In this review, we discuss the potential role of AMPK and LKB1 in regulating exercise-stimulated glucose uptake in skeletal muscle. We also discuss our recent study, demonstrating the molecular mechanism of obesity-induced development of skeletal muscle insulin resistance.

Keywords: Skeletal muscle, Glucose uptake, Exercise, Obesity, Insulin

Introduction

The incidence of type 2 diabetes is increasing at epidemic rates worldwide and skeletal muscle is the tissue responsible for the majority of glucose disposal upon insulin stimulation and exercise, which have been known to increase glucose uptake into the tissue[1]. Although the ability of insulin to promote glucose uptake into skeletal muscle is impaired, the exercise-stimulated glucose uptake is nearly normal in the patients with type 2 diabetes[2,3]. Given the incidence of type 2 diabetes and the physiological importance of exercise in regulation of glucose uptake in skeletal muscle, it is important to understand the molecular mechanisms that mediate this phenomenon, which is still not fully understood.

AMP-activated protein kinase and glucose uptake during exercise

AMP-activated protein kinase (AMPK) is a Ser/Thr kinase that functions in the regulation of energy metabolism[4]. Active AMPK consists of heterotrimeric complexes containing a catalytic α subunit and regulatory β and γ subunits. The phosphorylation of the Thr^{172} site on the α catalytic subunit by upstream kinase(s) is essential for AMPK activation[5-7]. Liver kinase B1 (LKB1)[5,8,9] and Ca^{2+}/calmodulin kinase kinase (CaMKK)[10-12] have been shown to phosphorylate and activate AMPK. Recent studies have demonstrated that knockout of LKB1 in muscle results in a decreased AMPKα2 activity, suggesting that LKB1 appears to be a major AMPK kinase in skeletal muscle[13,14].

AMPK is activated upon increase in the AMP/ATP ratio, such as exercise[15,16] and hypoxia[17], and regulates multiple signaling pathways whose overall effects are to increase ATP production, including fatty acid oxidation and glucose uptake[4,18]. Consistently, incubation of isolated muscle with AICAR (5-aminoimidazole-4-carboxamide-1-β-D-ribofuranotide), an AMPK activator, stimulates glucose uptake in the absence of insulin, comparable to the effects of exercise and muscle contraction[19-21]. Furthermore, lack of AMPK in skeletal muscle abolished the effect of AICAR to stimulate glucose uptake in skeletal muscle[22,23]. Collectively, these studies suggest that AMPK plays an important role in glucose uptake during exercise.

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Despite considerable effort has been made to understand the role of AMPK on exercise-stimulated glucose uptake using transgenic and knockout mouse models, the results are not consistent and are somewhat controversial. Transgenic mouse models depleting AMPK activity in a muscle-specific manner show different phenotypes; a partial impairment[22,24,25] or no reduction[23,26] in exercise-stimulated glucose uptake. Furthermore, knockout of AMPKα1, AMPKα2, AMPKβ2, and AMPKγ3 do not alter glucose uptake during *in vitro* contraction[27-29]. These data suggest that AICAR-stimulated increases in skeletal muscle glucose uptake are mediated by AMPKα2, but AMPK cannot be the sole mediator of contraction-stimulated glucose uptake. Although the role of AMPK in exercise-stimulated glucose uptake cannot be excluded, there may be multiple, potentially redundant, signaling mechanisms mediating contraction-mediated glucose uptake in skeletal muscle.

Role of LKB1 and AMPK-related protein kinases in glucose uptake

In order to determine the potential role of the AMPK upstream kinase, LKB1, our group has generated a muscle-specific LKB1 knockout mouse (MLKB1KO)[13]. Furthermore, Sakamoto et al.[14] have studied a hypomorphic LKB1 mouse where whole body LKB1 protein is decreased by 70%–80% and skeletal muscle LKB1 is ablated. Exercise-stimulated glucose uptake was significantly inhibited in these two LKB1 knockout mouse models[13,14]. The mechanism(s) by which LKB1 regulates exercise-stimulated glucose uptake in skeletal muscle has not been elucidated. We speculated that decreased glucose uptake cannot be explained by inactivation of AMPKα2 alone and could be due to decreased activity of one or more other LKB1 substrates. In addition to AMPK, LKB1 has been known to phosphorylate at least 12 AMPK-related protein kinases that are similar in structure and/or function to AMPK[30,31]. The role of AMPK-related protein kinases in regulating skeletal muscle glucose uptake has not yet been understood but one report suggests that only some of the AMPK-related kinases (QSK, QIK, MARK2/3, and MARK4) are expressed in rat skeletal muscle, and that none of these proteins are activated by *in situ* muscle contraction[32]. Another study demonstrated that phosphorylation of the AMPK-related protein kinase 5 (ARK5) is increased by both muscle contraction and AICAR in rat skeletal muscle. However, the increased ARK5 phosphorylation was not associated with elevated enzyme activity in this study[33]. Taken together, it is likely that exercise-stimulated glucose uptake is regulated by one or more alternative downstream substrates of LKB1.

Role of SNARK in exercise-stimulated glucose uptake

Studies using transgenic and knockout mouse models lacking AMPK activity in skeletal muscle and muscle-specific LKB1 knockout mouse model raise the possibility that one or multiple AMPK-related kinases play an important role in exercise-stimulated glucose uptake in skeletal muscle. The role of the proteins in this process has not been well understood and therefore remains to be elucidated. However, our group has recently reported that Sucrose Nonfermenting AMPK-related Kinase (SNARK) plays an important role in exercise-stimulated glucose uptake in skeletal muscle[34]. The enzyme activity is activated by muscle contraction and exercise in mice and humans. Overexpression of dominant mutant SNARK by a direct DNA injection and heterozygotic SNARK knockout mice decrease SNARK activity in skeletal muscle, which is associated with impaired exercise-stimulated glucose uptake. More recently, SNARK has also been reported to regulate muscle mass[35]. The expression of SNARK in skeletal muscle is increased by high fat diet and aging. Transgenic mice overexpressing a dominant negative form of SNARK in skeletal muscle display a loss of muscle mass and an increased adiposity, showing a sarcopenic obesity (aging-related muscle mass loss and obesity). These findings indicate that SNARK plays important roles in muscle metabolism as well as muscle mass maintenance. Further studies are needed to determine the role of other AMPK-related protein kinases in skeletal muscle metabolism. Thus, LKB1 and its downstream pathway, including AMPK and SNARK, play an important role in exercise-stimulated glucose uptake and muscle metabolism.

Skeletal muscle and insulin resistance

The importance of skeletal muscle in the regulation of whole body glucose metabolism has been clearly established. In addition to exercise, insulin is a major mediator for glucose uptake in skeletal muscle. Most patients with type 2 diabetes display insulin resistance in skeletal muscle. Although the insulin signaling molecules have been well understood, the development of insulin resistance remains unclear. Our group recently studied the role of Tribbles 3 (TRB3) in skeletal muscle. TRB3 is a pseudokinase[36] that is expressed in various tissues, including liver, adipose tissue, heart and skeletal muscle[13,37-39], and its expression is regulated through multiple mechanisms. In liver and adipose tissue, TRB3 expression is induced by fasting via activation of PGC-1α and PPAR-α[37,38,40]. In 3T3-L1 adipocytes and L6 myotubes, glucose deprivation, dexamethasone, and the unfolded protein response regulate TRB3 expression[41]. Endoplasmic reticulum (ER) stress is known to increase TRB3 expression in various cell lines[42-44]. TRB3 is induced by various forms of ER stress via enhanced promoter activity[44]. These indicate that TRB3 is regulated by metabolic stress in multiple tissues. Recent studies demonstrate that TRB3 binds and inhibits Akt activity, leading to impaired insulin signaling in liver[37,40]. Our study found[45] that high-fat feeding in mice, and obesity and type 2 diabetes in humans increase TRB3 in skeletal muscle. Overexpression of TRB3 impairs insulin signaling and insulin-stimulated glucose uptake in skeletal

muscle. Consistently, TRB3 knockout mice are protected from high-fat diet-induced insulin resistance in skeletal muscle. Taken together, these data strongly suggest that TRB3 mediates development of insulin resistance in skeletal muscle. Furthermore, the results demonstrate that TRB3 may be a new therapeutic target for effectively managing insulin resistance.

Conclusions

It has been clearly shown that AMPK activates glucose uptake in skeletal muscle, which is comparable to insulin. Skeletal muscle LKB1, an upstream kinase of AMPK and AMPK-related protein kinases, has been shown to regulate exercise-stimulated glucose uptake. The underlying mechanisms appear to be AMPK-independent and still need to be elucidated. SNARK, an AMPK-related protein kinase, plays an important role in exercise-stimulated glucose uptake in skeletal muscle. Further investigations need to be done to determine if the LKB1/SNARK pathway is a useful therapeutical target for the treatment of type 2 diabetes, as the role of the pathway in other tissues has not been studied. Our recent study also identified a novel protein, TRB3, as an important signaling molecule in developing insulin resistance in skeletal muscle. Inhibition of TRB3 function may assist in preventing obesity and type 2 diabetes.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

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