*Mini Review*

# Molecular mechanisms of glucose uptake in skeletal muscle at rest and in response to exercise

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**Abstract** — Glucose uptake is an important phenomenon for cell homeostasis and for organism health. Under resting conditions, skeletal muscle is dependent on insulin to promote glucose uptake. Insulin, after binding to its membrane receptor, triggers a cascade of intracellular reactions culminating in activation of the glucose transporter 4, GLUT4, among other outcomes. This transporter migrates to the plasma membrane and assists in glucose internalization. However, under special conditions such as physical exercise, alterations in the levels of intracellular molecules such as ATP and calcium act to regulate GLUT4 translocation and glucose uptake in skeletal muscle, regardless of insulin levels. Regular physical exercise, due to stimulating pathways related to glucose uptake, is an important non-pharmacological intervention for improving glycemic control in obese and diabetic patients. In this mini-review the main mechanisms involved in glucose uptake in skeletal muscle in response to muscle contraction will be investigated.

Keywords: exercise, skeletal muscle, glucose uptake, GLUT4

## Introduction

The knowledge that exercise plays an important role in health is widely disseminated. In ancient Greece, the philosopher Hippocrates had mentioned in his works that both exercise and a healthy diet could promote health and prevent certain diseases. Nonetheless, the author did not imagine that several molecular changes might occur in response to exercise, culminating in many physiological responses, including the glucose uptake increment. With the advent of modern science and especially of molecular biology, the understanding of this process was significantly expanded. One pioneering study that confirmed glucose uptake with muscle contraction measured the arterio-venous difference in glucose derived from the masseter muscle in horses, demonstrating a reduction in the amount of glucose in this tissue after hay-chewing[1].

Some decades later, Lawrence showed that physical exercise could enhance the effects of the hormone insulin, resulting in increased glucose consumption and internalization, suggesting that physical exercise could be relevant for the treatment of diabetic people[2]. Insulin injection prior to exercise resulted in greater reduction in blood glucose when compared to their peers who also exercised but did not receive the hormone. Following this evidence, it was shown that physical exercise increases the rate of glucose utilization in animals and humans[3,5], even suggesting that its capture would occur in a similar manner to situations in which there was insulin stimulation. In a comple-mentary way, it was also observed that the magnitude of the effect depends on the duration and intensity of the exercise[6]. Therefore, insulin and exercise have agonist actions in glucose uptake by skeletal muscle tissue process.

Further investigations have shown that a single exercise session can positively influence glucose uptake by muscle. It was shown that after 16 hours of a single swimming session – as this practice increases the expression of glucose transporter type 4 (GLUT4) by about 50% – there was an increase in glucose

uptake in exercised muscles[7]. The justification lies in the fact that the transport of glucose stimulated by insulin or muscle contraction is increased in proportion to the increase of GLUT-4, as well as their translocation rate to the cell membrane[8]. This effect may remain for up to 48 hours after physical activity, suggesting the need of practice in a chronic manner to obtain its benefits continuously.

The identification of GLUT-4, abundantly expressed in adipose and muscle tissue, favored further studies about the mechanisms by which exercise can influence glucose uptake by skeletal muscle tissue[9]. Studies in animal models[10,11] as well as in humans[12-14] have shown that physical training increases the expression of GLUT-4, promoting glucose uptake in skeletal muscle.

Although the identification of the GLUT-4 has been very important, the following findings allowed to understand that exercise exerts actions on other molecules involved in glucose uptake. Glucose uptake utilizes insulin-dependent and insulin-independent cell signaling pathways. The latter category involves AMP-activated protein kinase (AMPK) and calcium/calmodulin-dependent protein kinase (CaMKK)[15]. The finding and understanding about the mechanisms of action of some proteins that act independently of insulin on glucose uptake allow to definitely recognize the benefits of using exercise as a non-pharmacological tool in the control of glucose homeostasis and in preventing and treating diabetes. Therefore, this mini-review aims to describe the different mechanisms involved in glucose uptake at rest and in response to physical exercise.

## Glucose uptake mediated by insulin hormone

Glucose uptake by insulin stimulation occurs after the hormone binds to its specific membrane receptor called insulin receptor (IR)[16]. The IR is a heterotetrameric protein with intrinsic tyrosine kinase activity. After this binding, the receptor undergoes a conformational alteration, triggering its autophosphorylation on tyrosine residues. Once activated, IR promotes the tyrosine phosphorylation of different substrates, including insulin receptor substrates 1 and 2 (IRS-1 and IRS-2). The tyrosine phosphorylation of IRS-1 and IRS-2 allows them to associate and activate the phosphatidylinositol-3 kinase enzyme (PI3K). PI3K catalyzes the phosphorylation of membrane phosphoinositides at the position 3 of the inositol ring to produce phosphatidylinositol 3-phosphate, phosphatidylinositol 3,4-phosphate and phosphatidylinositol-3,4,5-phosphate. The latter product regulates the activity of phosphoinositide-dependent kinase-1 (PDK-1). Successively, PDK-1 phosphorylates protein kinase B/Akt at serine/threonine kinase residue, which has a key role in insulin signaling pathway. When active, the Akt is responsible for the activation of AS160 (Akt substrate of 160 kDa)[16]. The AS160 can act in two significant ways on GLUT4 translocation to the periphery: 1) by reducing the "tethering" of the vesicle, by proteins called TUG, and thus releasing it to the periphery; 2) by increasing the activity of Rab proteins which will stimulate the translocation of these vesicles containing GLUT4 to

the periphery. Therefore, these activation mechanisms and the release of vesicles containing GLUT4 are essential for glucose homeostasis at baseline. The increase of GLUT4 and its translocation to the sarcoplasm are extremely important for performance and health[16].

Several studies have indicated that compromise at some point in this pathway can decrease glucose uptake, triggering important physiological changes that can lead to the development of various metabolic diseases, such as diabetes[17]. However, it is worth mentioning that there are alternative pathways in addition to insulin that are responsible for regulating glucose uptake, acting jointly in the regulation of glucose homeostasis. For example, studies using MIRKO animals (mice without insulin receptor in skeletal muscles – muscle insulin receptor knockout) show that there is reduced insulin signaling but no effect on the IGF-1 pathway (insulin-like growth factor, a growth factor similar to insulin), indicating that different pathways can act in a compensatory fashion to maintain signaling[18]. As there is homology between the molecules in the absence of insulin, IGF receptor receives the insulin signal and propagates intracellularly, partially compensating its absence. Thus, it is necessary to address some of the main mechanisms to promote glucose uptake, even in conditions of low levels of circulating insulin.

## Glucose uptake through physical exercise

In skeletal muscle, glucose uptake depends on the presence of GLUT-4 on the cell membrane, so that it exerts its function of allowing the entry of glucose by facilitated diffusion. At baseline conditions, the great majority of GLUT4 molecules is stored in vesicles within the cell, keeping it in a quiescent, waiting for the recruitment signal. Therefore, under resting conditions, insulin is essential for glucose uptake in muscle tissue[19]. During exercise, there is an increase in blood flow to the active muscles, creating an incentive for the dilation of blood vessels responsible for irrigation of active muscles, thereby aiming to increase the surface area available for transport of glucose[20]. During muscle contraction, circulating insulin levels suffer no significant change, and in some cases even suffer a decrease. Thus, the muscle contractions and blood flow circulating levels of insulin act in synergism generating signals for translocation of GLUT4 to the membrane of the sarcolemma and t-tubules, thereby increasing glucose uptake by the cell[21].

The amount of GLUT4 present in the sarcolemma and in the t-tubules is influenced by the efficiency of endocytosis and exocytosis processes of vesicles containing the protein in its inactive form. Insulin increases the amount of GLUT4 in muscle membrane primarily by increasing the stimulation of exocytosis[22], while muscle contractions (that activate AMPK protein by depletion of ATP levels) not only increase the exocytosis rate but also decrease endocytosis rate of GLUT-4[23]. This phenomenon may explain the additive effect of exercise on insulin in muscle glucose uptake[24]. The understanding that exercise cooperates in glucose uptake has led many researchers to investigate which mechanisms could be linked to muscle contraction and independently to the hormone insulin.

## Cell signaling in skeletal muscle in response to physical exercise

In general, physical exercise – and muscle contraction specifically – is able to activate a series of proteins that trigger the functional and/or structural protein alterations, ultimately promoting GLUT-4 translocation. Increases of both glucose uptake and metabolism in response to exercise are often associated with the effects of a single session of exercise in the levels of mRNA and in the absolute levels of GLUT-4 [25]. In addition to increased levels of GLUT-4, exercise is able to increase GLUT-4 translocation to myocellular membrane (related to increased exocytosis rate and lower endocytosis rate, as previously discussed), increasing the glucose uptake capacity in muscle cells [19]. The effects of exercise in increasing GLUT4 content and translocation involve some molecules that will be presented below.

## Physical exercise and glucose uptake mediated by AMPK

A few years before the description of AMPK activation by physical exercise and related experimental evidence in humans, animal researches identified a drug compound known to act as a potent activator of this molecule, AICAR (5-aminoimidazole ribonucleotide-4-carboxamide). This compound, that mimics the intracellular increase in AMP, is capable of phosphorylating AMPK promoting glucose uptake and lipid oxidation in skeletal muscle [26]. Animal experiments also suggest that AMPK activation in skeletal muscle is capable of enhancing lipid oxidation and the glycogen resynthesis rate in response to exercise (having a protective effect on muscle glycogen stores) through the muscle contraction stimulus per se and through the increase of calcium release [27].

Continuous and repetitive muscle contraction that is characteristic of exercise acts as a stressor agent. This activity causes depletion (momentary or extended) of ATP levels, which are rapidly resynthesized. However, at any given time, cellular ATP resynthesis becomes insufficient due to decreased levels of the enzyme creatine phosphate (CP) or even glucose, causing an increase in the ADP:ATP ratio and, consequently, in the AMP:ATP ratio. This phenomenon promotes the phosphorylation of LKB1 (liver kinase B1 - B1 liver kinase) which phosphorylates and activates AMPK, which can finally phosphorylate the AS160 protein [28,29]. The AS160 can act in various ways to translate GLUT4-containing vesicles to the periphery. One is through the activation of Rab-GTP protein, which will trigger signal for the translocation of GLUT4 [30]. Another way is by reducing the TUG activity, protein "tethering" which binds to the vesicles, preventing its translocation [31]. Additionally, AMPK phosphorylates HDAC5 (histone deacetylase 5), which is exported from the nucleus promoting the activation of MEF2 (stimulating factor of myocyte 2) and GEF (stimulating factor GLUT4) as well as their combination. Both these transcription factors are related to the GLUT4 expression in skeletal muscle [32]. Thus, in addition to causing the translocation of GLUT4 molecules to the membrane, AMPK (in its active form) is also capable of regulating the expression of new GLUT-4 molecules.

## Muscle contractions and glucose uptake mediated by calcium ions

Calcium ions have gained attention in recent decades due to their contribution to glucose uptake. Early studies found their participation in glucose homeostasis, when Sartorius muscle of frogs were incubated with caffeine [33]. Based on these results, it was observed that caffeine stimulates calcium release from the sarcoplasmic reticulum without plasma membrane depolarization and that this influx per se was sufficient to stimulate glucose uptake. Subsequently, studies with rat muscles incubated with caffeine or a chemical compound, which also stimulates calcium release in sufficient levels to promote muscle contraction [34], also demonstrate the capacity of $Ca^{2+}$ in increasing glucose uptake.

Furthermore, recent studies have brought new evidences of how $Ca^{2+}$ promotes increased glucose uptake. It was observed that caffeine, through stimulation of $Ca^{2+}$ ion pumps from the sarcoplasmic reticulum, increases cellular energy demand and ATP consumption. Since the increase in energy expenditure is responsible for the activation of AMPK, and it triggers processes that signal for the translocation of GLUT4 from intracellular vesicles to the plasma membrane, glucose uptake will be stimulated [15]. This phenomenon shows that $Ca^{2+}$ influences the increase of glucose uptake, but with an indirect action, through AMPK activity.

Another hypothesis currently studied about the participation of $Ca^{2+}$ in AMPK activation was observed in non-muscle tissues. It was observed the participation of calcium kinase-dependent protein/calmodulin (CaMKKs), highlighting the role of the CaMKKβ (which has the ability to phosphorylate AMPK in its active site $Thr^{172}$)[35]. Therefore, it was created the hypothesis that $Ca^{2+}$ directly activates AMPK in skeletal muscle via CaMKK, independent of increasing cellular energy consumption. Due to this, some studies were developed to examine this pathway in muscle tissue of rodents, but they showed controversial results [15,36,37]. Thus, it is not possible to state whether this action of $Ca^{2+}$ in AMPK activation is direct or not. To date, the participation of $Ca^{2+}$ in increasing glucose uptake in muscle is considered to be indirect, by increasing the cellular energy expenditure caused by the action of $Ca^{2+}$ pumps present in the sarcoplasmic reticulum.

## Physical exercise, reactive oxygen species, and regulation of glucose uptake

Some studies suggested the role of ROS in glucose uptake in response to muscle contraction, due to the fact that exercise increases significantly and transiently the production of ROS [38,39]. Some experiments that used donor ROS treatment in isolated skeletal muscle fibers observed that glucose uptake in the

treated muscles was higher[39]. Other studies using non-specific inhibitors of ROS found divergent results, with no effect on glucose uptake in both rodents[40] and humans[41]. Concerning these studies, it is suggested that the involvement of ROS in muscle glucose uptake would be restricted to conditions observed *in vitro* (intense electrical stimulation of isolated muscles fibers), without affecting the effects of exercise on the subjects. In a study with type 1 diabetics, administration of natural antioxidants (e.g., vitamin E) after exercise attenuated glucose uptake, indicating that reactive oxygen species act as regulators of glucose uptake and their inhibition might have a negative effect[42]. These controversial results suggest the need for more studies to further elucidate this issue.

### Physical exercise, nitric oxide synthesis, and glucose uptake

Nitric oxide (NO) is synthesized by three isoforms of nitric oxide synthase: iNOS (inducible nitric oxide synthase), eNOS (endothelial nitric oxide synthase), and nNOS (neuronal nitric oxide synthase). The role of NO is commonly related to vasodilation of the endothelium to facilitate the coming of nutrients distribution to the active muscles as well as the removal and transport of metabolites, maintaining cellular homeostasis[43,44]. Studies using animals showed that 4 and 8 weeks of treadmill running training[47,48] as 4 weeks of swimming training[49] can upregulate nNOS levels in the skeletal muscle of trained mice. On the other hand, studies with humans are not clear in showing if there is an increase in the levels of nNOS and NO in skeletal muscle in response to exercise[45,46].

Infusion of L-NAME (a known pharmacological inhibitor of NOS) in the femoral artery of humans during exercise on a cycle ergometer, performed at 60% of VO$_2$ peak (moderate intensity), substantially attenuates glucose uptake in healthy subjects and in patients with type 2 diabetes, with no changes in blood flow in the lower limbs, blood pressure or insulin and glucose concentrations[51,52], indicating that there must be an effect of NOS on muscle contraction-induced glucose uptake.

These results indicate that the inhibition of molecules that produce NO attenuates glucose uptake in skeletal muscle during muscle contraction, without affecting the flow of glucose into muscle. It is suggested that the release of NO by muscular stimulation occurs only in high-intensity exercises. In humans, the inhibition of NOS in low-intensity exercise did not affect glucose uptake, with significant alterations for moderate intensities[53]. In animal models, the treatment with NO donor enhances glucose uptake in the EDL muscle of C57BL/6J mice and this effect was ceased by the use of the NO inhibitor[54].

On the other hand, excessive concentrations of NO can induce insulin resistance in skeletal muscle by binding of NO to specific cysteine residues present in IRβ proteins, IRS-1 and Akt, reducing the ability to activate these molecules when stimulated by insulin[55]. The modification of protein activity mediated by the binding of NO to cysteine residues is known as S-nitrosation or S-nitrosylation and it is becoming a key mechanism in the insulin resistance process[56].

Specifically, aberrant production of NO in mammals appears to be mediated by the inducible isoform, iNOS, which is highly expressed in certain situations such as oxidative stress and inflammation. In light of these findings, it was demonstrated that iNOS knockout mice are protected from developing insulin resistance induced by obesity[57] and aging[58]. Interestingly, it was observed that acute aerobic exercise can reduce the protein content of iNOS, S-nitrosation of IRβ, IRS-1 and Akt and, thus, decrease insulin resistance in the skeletal muscle of obese rats[59].

In summary, it was shown that at physiological levels NO is required for increasing glucose uptake by skeletal muscle during exercise. This suggests a relationship between NO and AS160 protein; however, the underlying molecular mechanisms remain to be elucidated. Studies have also shown that exaggerated NO production can reduce glucose uptake after insulin stimulation.

### Muscle temperature and glucose uptake

Recently, the increased temperature of skeletal muscle was considered as a potential inductor for increased glucose uptake. An elegant study demonstrated in animal models that the simple increase of skeletal muscle temperature is able to stimulate glucose uptake through the activations of AMPK and Akt, which were partially reversed after the use of pharmacological inhibitors of these proteins, such as compound C and Wortmannin, respectively[60].

Other studies also suggested that the increased glucose uptake, which occurs because of increase in muscle temperature, is due to the increased production of reactive oxygen species (ROS) that regulates the activity of NOS (promoting NO production) favoring the formation of RNS (as a result of the reaction of NO with ROS previously exposed). ROS and RNS provoke a stimulus for the translocation of GLUT4 to the muscle cell membrane in a way not fully understood to date[61,62]. However, it is worth highlighting that the results of increased glucose uptake in response to increased skeletal muscle temperature are transient. Further studies about the mechanisms through which the muscle temperature interferes with this process, the transition of these findings to studies with humans, and the use of this knowledge in practice should be developed. This may bring new insights about the thermal baths (soaking in hot water), preheating at the beginning of physical activity interventions with equipment that causes local heating, etc.

### Actin cytoskeleton and glucose uptake

Several articles have addressed the role of the reorganization of the actin cytoskeleton – which occurs during the shortening/relaxation of the muscle fiber – in glucose uptake. This momentary reorganization would not only be capable of modulating intracellular traffic of GLUT-4, but also the signaling that occurs prior to its translocation into the muscle cell membrane, acting in synergy with other signaling molecules, enhancing GLUT-4

action. This hypothesis was confirmed by experiments using skeletal muscle myotubes and rodents, which demonstrated that insulin signaling (specifically the GLUT-4 translocation signal) depends on transient changes in the actin cytoskeleton[63,64]. Confirming these results, experiments using drugs that are able to break the actin filaments showed that under these conditions, both in cells and in skeletal muscle of rodents, the translocation of GLUT-4 and subsequent glucose uptake by the cell after stimulation with insulin was impaired[65]. More thoroughly, these results are related to the functioning of a specific protein, Rac1. This protein is a GTPase responsible for mediating the stimulatory effect on exocytosis rates of GLUT-4, resulting in release of intracellular vesicles and their subsequent translocation to the membrane myocyte[64]. It was demonstrated, both in rodents and in humans, through the increase in GTP levels, that exercise increases the activation of Rac1. Nevertheless, it was observed that the pharmacological inhibition or genetic ablation of Rac1 impaired the increased glucose uptake obtained as a result of physical exercise[66]. Subsequently, the same group of researchers showed that the signaling of Rac1 would be downregulated in skeletal muscles of rodents that are insulin-resistant[67].

Another protein located in the cytoskeleton is Myo1c, which is also related to motor activity. It was suggested that this protein can act as a contributor to glucose uptake induced by muscle contraction. An elegant study promoted the mutated expression of this protein in skeletal muscles of mice and demonstrated that Myo1c is involved in the translocation of GLUT4, since the animals with mutated Myo1c had glucose uptake attenuated in response to muscle contraction stimulus. These observations indicate that these proteins and possibly others, which are related to cytoskeletal composition, regulate the effect of exercise in increasing glucose uptake in exercised muscles. Accordingly, these proteins can be targeted for future studies on insulin resistance treatment, as well as studies to provide a better understanding of this complex phenomenon[68].

## Conclusion

In this mini-review we addressed the most recent findings with regard to glucose uptake mechanisms in skeletal muscle in response to exercise. Several studies indicate that exercise can promote robust control of glucose homeostasis through various cellular mechanisms. Figure 1 shows a schematic summary of the molecular mechanisms regulated by physical exercise that are related to increased glucose uptake in skeletal muscle. We presented information showing that exercise is capable of regulating insulin-dependent and non-insulin-dependent molecular pathways to increase glucose uptake in exercised skeletal muscle.
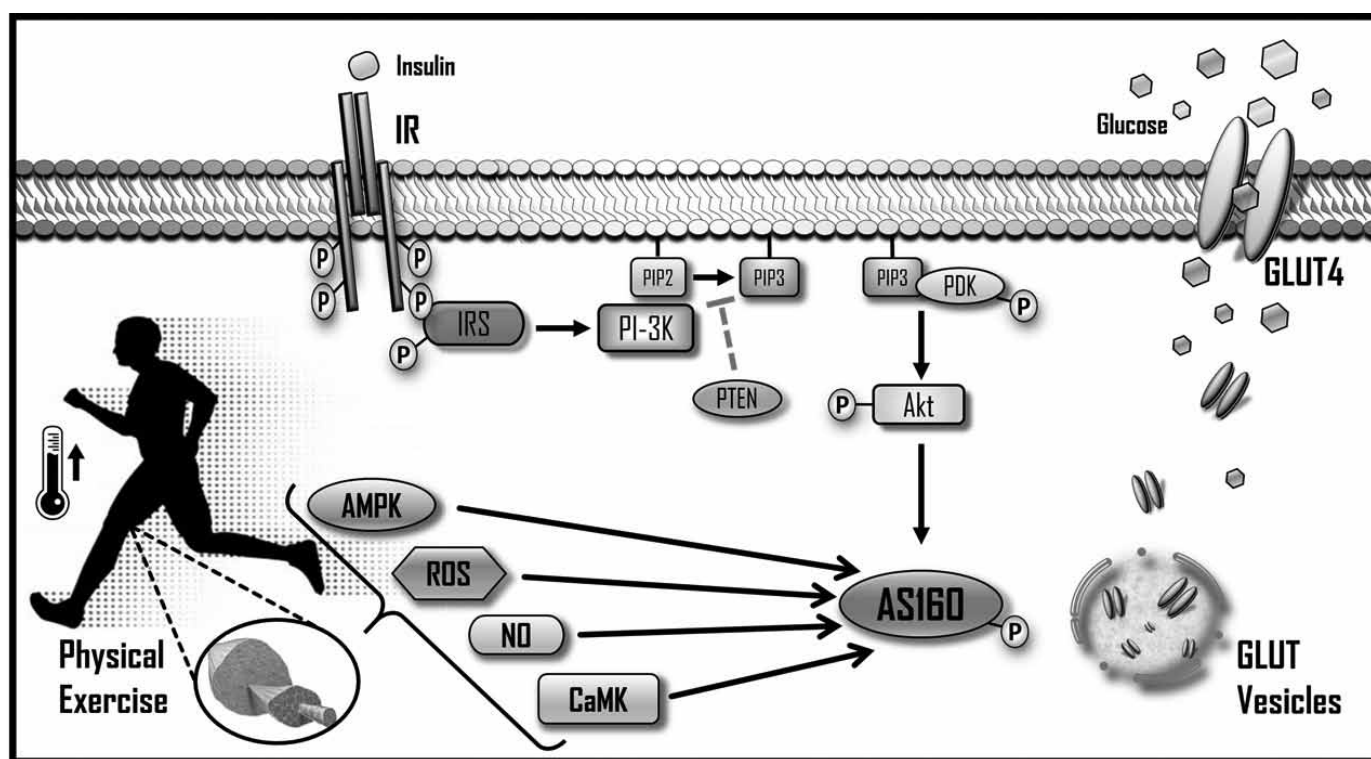


Figure 1. Molecular mechanisms involved in skeletal muscle glucose uptake at rest and in response to exercise. IR, Insulin receptor; IRS, insulin receptor substrate; PI-3K, phosphatidylinositol 3-kinase; PIP2, phosphatidylinositol 4,5-bisphosphate; PIP3, phosphatidylinositol (3,4,5)-trisphosphate; PDK, phosphoinositide-dependent kinase; Akt, protein kinase B; AS160, Akt substrate of 160 kDa; GLUT, glucose transporter; AMPK, AMP-activated protein kinase; ROS, reactive oxygen species; NO, nitric oxide; CaMK, Ca2+/calmodulin-dependent protein kinase.

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