Molecular Mechanisms of Muscle Glucose Uptake in Response to Resistance Exercise: A Review

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ABSTRACT

Santos JL, Araujo SS, Estevam CS, Lima CA, Carvalho CRO, Lima FB, Marcal AC. Molecular Mechanisms of Muscle Glucose Uptake in Response to Resistance Exercise: A Review. JEPonline 2017;20(4):200-211. Evidence shows that physical exercise carried out systematically promotes numerous health benefits, including improvements in glucose homeostasis. Of all types of physical activity, resistance training is effective in promoting increased strength, speed, power, hypertrophy, and metabolic effects such as increased insulin sensitivity and control in glycogen metabolism. Since there are still gaps requiring attention in regards to the molecular mechanisms leading to effective insulin signaling, this paper attempts to review the effects and implications of resistance exercise on the major intracellular protein activities. In this regard, we set out to understand the role of resistance training on IRS1, IRS2, PI3K, Akt/PKB, and GLUT4 activity, which can lead to the development of possible future interventions to improve physical performance and mitigate the effects of altered metabolic conditions such as obesity, insulin resistance, and type 2 diabetes mellitus.

Key Words: Insulin Resistance, Insulin Signaling, Intracellular proteins, Resistance Exercise
INTRODUCTION

Physical exercise is an activity carried out through systematic repetitions of oriented movements that are associated with increased oxygen consumption due to muscular demand, therefore, generating work. Aerobic training has been associated with several benefits related to health and improvement in cardiovascular performance, inducing significant acute endocrine and immune physiological responses (36,40). On the other hand, several authors (5,17,20,48) have reported the importance of the practicing resistance exercise due to its important effects on muscle hypertrophy, increased strength, and muscle quality in healthy individuals and diabetic patients.

In particular, progressive resistance training is associated with changes in individuals with advanced diabetes with reduced lean body mass, decreased enzyme glycogen synthase (GS) activity, and the conversion of type 2 to type 1 muscle fibers prior to insulin resistance (17). While there is a consensus that both aerobic and resistance exercises increase insulin sensitivity (6,33), the exact mechanism by which this happens in association with each of these exercise modalities has not been fully elucidated.

In this context, Richter and Ruderman (42) and Sarvas et al. (44) have reported on the importance of the cellular energy state during strength exercise in inducing the activation of AMP kinase (AMPK). A phylogenetically conserved enzyme, AMPK occurs in unicellular and multicellular organisms (mainly mammals). It is very sensitive to cellular energy stress that is regulated allosterically by increasing the concentration of AMP and prior phosphorylation by AMPK. Ha and colleagues (20) indicate that AMPK activation triggers the translocation of vesicles of glucose transporters (GLUT4) and catabolic processes with the release of energy for synthesis and, concomitantly, the inhibition of anabolic processes consuming ATP. As a result, the cell increases the uptake of glucose independent of insulin and glucose oxidation of fatty acids in the mitochondrial matrix, respectively (Figure 1).

![Figure 1. Process of AMPK Activation Induced by Resistance Exercise and Its Metabolic Consequences, Increased Cellular Uptake of GLUT4, and Fatty Acid Oxidation.](image)

Details of this process are described in the text.
In contrast, physical inactivity is a risk factor for the development of type 2 diabetes and obesity (2,30). The close association of intracellular changes observed with physical inactivity and obesity seems to be a function of insulin resistance (14). Thus, given the above, this review examines the molecular pathways of muscle glucose uptake in response to resistance exercise. We also set out to understand the molecular mechanisms involving the activity of the insulin receptor (IR), the IR substrates 1 and 2 (IRS1/2), the phosphoinositol-3-kinase protein (PI3K), and protein kinase B (Akt/PKB), and the regulation of glucose transporter isoform 4 (GLUT4) in response to acute and chronic resistance exercise.

METHODS

The data for this study comes from the following databases: (a) SciElo, the Scientific Electronic Library Online; (b) PubMed/MEDLINE, the National Library of Medicine; (c) Scopus; and (d) ScienceDirect. A combination of terms was used in Portuguese and English, including resistance exercise, insulin resistance, insulin resistance, and insulin signaling as well as the descriptors, PI3K, Akt/PKB, and GLUT4. The search included studies published until 2017. Using the above keywords, 100 articles were retrieved of which 50 suited the purpose of this review, which comprised the adjustments promoted by resistance exercise in the intracellular insulin signaling pathway and the proteins downstream in this pathway (IR, IRS, PI3K, Akt/PKB, GLUT4).

RESULTS

Activation of the Insulin Receptor and Insulin Receptor Substrates

The hormone insulin binds to its receptor (IR) located in the cell membrane, which triggers a signaling cascade completed in the effectors downstream of the pathway (15). The IR is a glycoprotein ubiquitously expressed in all cell tissues that consists of two α subunits and two β subunits. The binding of insulin to the alpha subunit of the IR (Figure 1) promotes transphosphorylation of tyrosine residues (Tyr) in the beta subunit of the receptor (located in the intracellular region), which mediates the phosphorylation of IRSs (13,32). These substrates play an essential role in the transmission of intracellular signaling through their interaction with the Grb2 adapter protein via a specific recognition of the SH2 and SH3 domains, characterizing a pleiotropic effect of the insulin (49).

In the skeletal muscle, IRS-1 occupies an essential role in glucose uptake. When tyrosine residues in IRS-1 are phosphorylated, IRS-1 becomes the main substrate following activation of the IR in the skeletal muscle, promoting the translocation of vesicles containing GLUT4 to the membrane (9,35,41). Several serine residues in the IRS-1 protein are a target of phosphorylation following IR activation, including Ser612, Ser632, and Thr446, resulting in intracellular signal transduction (11). However, in conditions with metabolic imbalance such as obesity and insulin resistance, the activation of the inhibitor of nuclear factor kappa-B kinase (IKKβ) induces phosphorylation in residue Ser307, resulting in reduced glucose uptake (21).

In general, phosphorylation of tyrosine residues in the IRS-1 isoform are followed by activation of the protein PI3K and, subsequently, Akt/PKB – important molecules in glucose uptake (11). Therefore, the levels of IRS-1 phosphorylation determine the effectiveness of insulin in the process of glucose uptake in the skeletal muscle. Araki and colleagues (4)
indicated that mice with deletion of the IRS-1 gene have retardation of intrauterine and postnatal growth associated with moderate insulin resistance. In addition to these consequences, Najeeb and Zou (37) highlighted developmental deficits and effects on glucose homeostasis.

Despite the studies cited above, there is still some controversy regarding the changes in IRS expression induced by exercise. Expression of IRS-1 has been reported to be increased one day after exhaustive aerobic training and decreased 5 days later (31); whereas, Aguirre et al. (1) found no change in the expression of IRS-1 and IRS-2 seven days after exercise. In line with these studies, Aoi et al. (3) also demonstrated that mice submitted to eccentric and exhaustive aerobic exercise had impaired IRS-1 tyrosine residue phosphorylation and PI3K/Akt signaling in the skeletal muscle when compared with a control group not submitted to exercise, resulting in decreased GLUT4 translocation to the membrane. This evidence suggests that the effects promoted by aerobic exercise on the expression of IRS-1 are dependent on different conditions, such as duration and intensity of the exercise. A study by Jorge et al. (28) has shown increased glucose uptake in humans undergoing different training modalities. The authors submitted overweight and obese patients to different types of training (resistance, aerobic, and combined), and verified increased IRS-1 expression in the groups involved with resistance and combined training. Therefore, the increased expression of IRS-1 and the consequent phosphorylation of its tyrosine residues explain a higher uptake of glucose that persisted for several hours after exercise.

**PI3K Activity**

PI3K enzymes are members of a family of intracellular heterodimeric kinases, composed of regulatory and catalytic subunits that recruit lipids as second messengers. When activated, these molecules modulate several important intracellular functions such as the inhibition of apoptosis, growth, differentiation, metabolism, chemotaxis, and transit of cellular vesicles (10,39).

The various functions of PI3K are associated with its isoforms, comprising of three distinct classes categorized according to their structure and activation mechanisms. Class I isoforms are the most studied isoforms, due to their participation in tyrosine kinase receptor signaling. Through adapter proteins, such as the IRS, converts class I isoforms phosphorylate membrane phosphatidylinositol 4,5-bisphosphate (PIP2) into phosphatidylinositol 3,4,5-trisphosphate (PIP3) (28). The class I subfamily consists of four catalytic subunits: three IA subunits (p110α, p110β, and p110δ) and one class IB subunit (p110γ). The class I regulatory subunits that are essential for the interaction with receptor tyrosine kinases are p85α, p85β, p55α, p55γ, and p50α. Class II comprises three isoforms (PI3K-C2α, PI3K-C2β, and PI3K-C2γ) activated by G protein-coupled receptors. Finally, the class III isoform is also called Vps34 (3,7).

Subunit p85 of PI3K plays an important role in mediating interactions resulting from the activation of insulin and insulin-like growth factor (IGF) receptors via IRS-1 and IRS-2 (30,31) and activation of the protein pyruvate dehydrogenase kinase (PDK), resulting in Akt/PKB phosphorylation. This cascade is essential for phosphorylation of the mammalian target protein kinase (mTOR), which enhances protein synthesis by activating p70S6K and 4E-BP1, and consequently regulating skeletal muscle hypertrophy (9,32,54). On the other hand, Hamilton et al. (22) have found no changes associated with resistance exercise in the
PI3K/Akt cascade with subsequent activation of mTOR, despite blocking phosphatase and tensin homolog (PTEN), a negative regulator of the interaction between insulin and PI3K. Reports (26,29,40) have pointed out to a direct association between regular exercise on the availability of glucose in the skeletal muscle, as well as increased insulin sensitivity with concomitant insulin-mediated PI3K activity.

As demonstrated by Melo et al. (36), the activation of the cascade downstream of PI3K potentiates concentric hypertrophy of cardiac myocytes in rats submitted to resistance training. This is consistent with the study by Luo et al. (34), in which a deletion of PI3K markedly damaged glucose uptake and contributed to the development of muscle atrophy. In patients with type 2 diabetes undergoing a resistance exercise program, there are increases in PI3K activity, Akt content, GLUT4, and GS activity.

**Akt/PKB Activation during Exercise**

Akt/PKB is a serine/threonine (Ser/Thr) kinase activated by PI3K. Also termed RAC-PK ("related to the A and C kinases"), the Akt isoforms comprise a family of three members – Akt1 (PKB-α), Akt2 (PKB-β), and Akt3 (PKBγ) – and their activities are very susceptible to metabolic alterations such as obesity, insulin resistance, and type 2 diabetes (18). The Akt1 and Akt2 isoforms are expressed predominantly in the skeletal muscle, brain, heart, and lungs; whereas, Akt3 is primarily expressed in the brain. Akt/PKB phosphorylates regulatory target proteins that are important for glucose uptake and inhibits apoptosis (27,35,36).

In addition to the effects above, Akt/PKB may be activated by mechanisms independent of PI3K signaling, treatment with growth hormone, and increase in the intracellular concentration of calcium ions; these effects are due in part to the increase in the observed cAMP concentration during muscle contraction (12,46). In addition, there are reports of increased activity and phosphorylation of Akt/PKB Ser/Thr related to muscle hypertrophy through the activation of mTOR and inhibition of glycogen synthase kinase 3β (GSK-3β), leading to important changes in the degree of insulin sensitivity and circulating concentrations of this hormone (43). A study published by Hers et al. (24) has demonstrated that the regulation of Akt/GSK-3β and Akt/mTOR in human skeletal muscle is fundamental in muscle hypertrophy; a pathway that parallels the decrease in FOXO1, a transcription factor associated with the stimulation of muscle atrophy (50).

Corroborating the above-mentioned activities of Akt, Choi and Bum Kim (12) found that Akt1 knockout mice present impaired body growth pattern. Meanwhile, Ogasaswara and colleagues (38) emphasize the effector role of Akt in muscle hypertrophy through a pathway involving rapamycin-sensitive mTOR. Although, as already confirmed in previous study (43), this signaling pathway is dependent contractile stimulus. Interestingly, diverging with this line of evidence, Zanchi and Lancha (53) emphasized that both aerobic and resistance exercise may not cause Akt phosphorylation with subsequent activation. This can occur for several reasons not yet clarified, mainly by the existence of multiple isoforms of Akt in skeletal muscle, especially when Akt1 does not prevail.

**Akt/PKB-GLUT4 Pathway**

Glucose transporters of different isoforms allow the transport of glucose into the intracellular environment. In the muscle cells, GLUT4-containing vesicles translocate to the plasma membrane following stimulation by insulin to promote the uptake of glucose into the cell. The
interaction of insulin with its receptor on the membrane is the main extracellular stimulus determining GLUT4 translocation and promoting glucose uptake into skeletal muscle cells, although recent studies have shown availability of signaling molecules in GLUT4 through an insulin-independent pathway and other ligands (Figure 2) (45,52).

Increased insulin sensitivity culminates in effects that are due in part to the increase in the degree of phosphorylation of Akt/PKB (23,47) by PI3K, which allows the phosphorylation of its substrate AS160 (Rab GTPase-activating protein [GAP]) Osorio-Fuentealba et al. (40). In muscle fibers, contractile stimulation promotes molecular changes triggering the activation of two independent pathways, including mTOR and GSK-3β, leading to skeletal muscle hypertrophy (43).

**Figure 2. Intracellular Insulin and Insulin-Independent Signaling Pathways Triggered by Exercise.** Binding of Insulin to the Insulin Receptor α Subunit Causes the Receptor to Autophosphorylate, Leading to Phosphorylation of Intracellular Substrates such as the IRS. Phosphorylation is followed by Activation of Intracellular Signaling Pathways such as the PI3K Pathway, Akt/PKB, and the AMPK Cascade.

This evidence suggests that exercise can activate molecular pathways mobilizing GLUT4 vesicles independent of insulin. However, studies have demonstrated that the benefits of resistance exercise on GLUT4 expression and translocation result from the recruitment of molecules related to cellular stress promoted by the contractile process, such as those in the AMPK signaling dependent pathway (Figure 2). This enzyme may be activated by Ca++/calmodulin-dependent protein kinase (CaMK), liver kinase B1, protein kinase C (PKC), heat shock protein 90 (HSP90), and nitric oxide (NO) (3,5,8,16,25).
In another line of observation (5), increased AMPK activity in response to improved glucose tolerance has also been associated with physical training. Andersen et al. (2) have observed in the skeletal muscle increased transcription of GLUT4 mRNA and several other proteins involved in insulin signal transduction and glucose metabolism, such as PI3K, Akt, AMPK, and mTOR after the practice of resistance exercise.

Once activated, AMPK influences several molecular targets that trigger different long-elucidated cellular processes including lipid and carbohydrate metabolism, cell signaling, IRS phosphorylation, and ion transport (17, 51). The AMPK signaling sequence involves a direct phosphorylation of the AS160 substrate, which controls the recycling of GLUT4 vesicles and their membrane translocation (37), resulting in the uptake of glucose independent of insulin.

CONCLUSIONS

In the present review, we presented important evidence about the effects of resistance exercise on the modulation of intracellular signaling pathways leading to glucose uptake. In this sense, the interaction of the IR and its substrates (IR / IRS-1 / IRS-2), PI3K, and Akt leads to protein synthesis and translocation of GLUT4 to the membrane. The signaling pathways triggered by insulin are potentiated by regular practice of resistance exercise. Glucose uptake is also evident by the activation of AMPK by molecular markers resulting from cellular stress in response to resistance exercise. While these mechanisms contribute directly to the elucidation of pathways that have not been described, they may be involved in response to resistance physical exercise. This review offers perspectives for the development of possible interventions for the improvement of physical performance and attenuation of the effects of metabolic disorders such as obesity, insulin resistance, and type 2 diabetes.

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REFERENCES


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