Exercise and type 2 diabetes: molecular mechanisms regulating glucose uptake in skeletal muscle

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Exercise Training Improves Metabolic Health in People With Type 2 Diabetes

While rates of diabetes are on the rise, it has long been recognized that exercise has important health benefits for people with type 2 diabetes. One of the environmental factors considered a risk for the development of insulin resistance and type 2 diabetes is a lack of physical activity, and regular physical exercise can delay or prevent the onset of this disease (18, 58, 78).

Randomized trials have found that lifestyle interventions including ~150 min/wk of physical activity, combined with diet-induced weight loss, reduced the risk of type 2 diabetes by 58% in an at-risk population (58, 78). Interventions of exercise alone have proved to be just as effective in terms of prevention of the progression of type 2 diabetes as programs of diet alone or diet and exercise combined (9). Exercise training in type 2 diabetic patients improves management of blood glucose levels, body weight, lipids, blood pressure, cardiovascular disease, mortality, and overall quality of life (13, 19, 38, 47, 48, 62, 82). The more recent Look AHEAD study has shown that combined weight loss and physical activity in people with type 2 diabetes resulted in modest weight loss of ~6%, improved glycated hemoglobin, improved mobility, and improved kidney function, but no improvement in cardiovascular disease over a 10-yr period (13, 47, 48, 62). However, since the level of fitness was only assessed through year 4 of the study, conclusions on the effects of fitness level on cardiovascular disease cannot be made (13, 47, 48, 62). Another recent study (23) has shown that 6 mo of moderate-intensity exercise training decreased visceral fat mass and decreased hepatic triglyceride content in people with type 2 diabetes and that this program of exercise alone was more effective than programs of diet alone. Another recent study (19) showed that increasing physical activity in adults with type 2 diabetes resulted in partial or complete remission of type 2 diabetes in 11.5% of subjects within the first year of intervention and an additional 7% had partial or complete remission of type 2 diabetes after 4 yr of exercise intervention. Complete remission was defined by glucose normalization without need for drugs, and partial remission was defined as a transition to prediabetic or normal glucose levels without drug treatment (19). Taken together, these data demonstrate the beneficial effects of exercise training to combat type 2 diabetes.

One of the most well-established mechanisms through which type 2 diabetes improve metabolic health with exercise is through adaptations to skeletal muscle, which, in turn, decreases skeletal muscle insulin resistance. Here, we will discuss the effects of exercise on skeletal muscle, because skeletal muscle is responsible for the majority of glucose uptake in the
postprandial state (6, 11). In the following sections, we will discuss specific adaptations of skeletal muscle to both acute and exercise training on skeletal muscle glucose uptake and metabolism.

**Effects of Acute Exercise on Skeletal Muscle Glucose Uptake**

It is well established that insulin is a potent simulator of glucose transport in skeletal muscle. In people with type 2 diabetes, insulin-stimulated glucose uptake in skeletal muscle is impaired. However, exercise-stimulated glucose uptake in people with type 2 diabetes is normal or at near normal levels (51). Because exercise-stimulated glucose uptake is normal in people with type 2 diabetes, defining insulin-independent mechanisms in the control of exercise-stimulated skeletal muscle glucose uptake is of critical importance as a potential means to treat diabetes. During the last several years, researchers have learned much about the signaling mechanisms that regulate exercise-induced glucose transport. There are many lines of evidence that show that exercise activates molecular signals that bypass defects in insulin action in skeletal muscle.

Both insulin and exercise increase skeletal muscle glucose uptake by translocation of glucose transporter 4 (GLUT4), the predominant GLUT in muscle, from an intracellular location to the plasma membrane. Insulin and exercise stimulate GLUT4 translocation through distinct signaling mechanisms. Insulin signaling involves rapid phosphorylation of the insulin receptor, insulin receptor substrate-1/2 on tyrosine residues, and the activation of phosphatidylinositol 3-kinase (14, 17). Exercise, however, has no effect on insulin receptor and insulin receptor substrate-1/2 tyrosine phosphorylation or on phosphatidylinositol 3-kinase activity (17, 75). In fact, mice that lack insulin receptors in skeletal muscle [muscle-specific insulin receptor knockout (KO) mice] have normal exercise-stimulated glucose uptake (85). These data clearly demonstrate that insulin and exercise mediate GLUT4 translocation in skeletal muscle through distinct proximal signaling mechanisms.

Acute exercise activates multiple signaling pathways, but the activated signaling pathways necessary for increased glucose uptake and GLUT4 translocation are not well understood. Muscle contraction involves changes in energy status (i.e., increased AMP/ATP), increases in intracellular Ca²⁺ concentration, increased ROS, and PKC. These changes activate various signaling cascades, some of which likely work to phosphorylate Tre-2/USP6, BUB2, cdc16 domain family member 1 (TBC1D1) and Akt substrate of 160 kDa (AS160) and activate GLUT4 translocation. Here, we will discuss some of the various signaling cascades that have been implicated in exercise-stimulated glucose uptake (Fig. 1) (63, 66).

**AMP-Activated Protein Kinase**

AMP-activated protein kinase (AMPK) and its primary upstream kinase, liver kinase B1 (LKB1), are the most widely studied proteins implicated in skeletal muscle glucose transport in response to exercise. AMPK is a heterotrimeric protein...
composed of a catalytic α-subunit and regulatory β- and γ-subunits. The α- and β-subunits each exist in two isoforms (α1, α2, β1, and β2), and the γ-subunit exists in three isoforms (γ1, γ2, and γ3). AMPK is activated by phosphorylation by one or more upstream kinases, including LKB1 (20, 39). A previous study (53) involving incubation with the AMP-analog 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR) have shown that activation of AMPK is positively correlated with increased skeletal muscle glucose uptake. This increase in glucose uptake by AICAR stimulation is lost in mouse models deficient in AMPK-α2- or γ2-subunits (10, 33). Some studies (1, 28, 30, 33, 57, 69) have shown that mice overexpressing a dominant negative AMPK-α2 construct in muscle or AMPK-α1 and -β KO mice have impaired exercise-stimulated glucose uptake. However, other studies using mouse models with ablated AMPK activity have demonstrated that inhibition of AMPK has little or no effect on exercise-induced glucose uptake (15, 33, 55) or exercise-stimulated glucose uptake in vivo (50). Thus, whether AMPK is necessary for exercise-stimulated glucose uptake is not fully understood. In a mouse muscle-specific LKB1 KO model, AMPK-α2 activation was completely inhibited and exercise-stimulated glucose uptake was severely blunted (41, 67). This impairment could be due to decreased activation of AMPK and one or more of the AMPK-related kinases that are substrates of LKB1, for example, sucrose nonfermenting AMPK-related kinase, which is involved in exercise-induced glucose uptake (42). However, the role of LKB-1 in exercise-stimulated glucose uptake is debatable (31). Whereas previous studies (41, 67) have shown that exercise-stimulated glucose uptake was impaired in LKB-1 KO mice, another recent study (31) showed that glucose uptake during treadmill running was similar, if not higher, in LKB-1 KO mice compared with wild-type control mice. An additional study (23) has also shown that muscle-specific deletion of LKB1 only partially inhibits exercise-stimulated glucose transport. These data suggest that while AMPK and LKB1 are important in the regulation of exercise-stimulated glucose uptake, there are several potential mechanisms involved in the control of exercise-stimulated glucose transport in skeletal muscle.

Ca2+/Calmodulin-Dependent Protein Kinases

A fundamental part of skeletal muscle contraction is the increase in intracellular Ca2+ concentration. More recently, studies have indicated Ca2+/calmodulin signaling and Ca2+/calmodulin-dependent protein kinases (CaMKs) as critical components of Ca2+- and exercise-stimulated skeletal muscle glucose uptake. Incubation of rat skeletal muscle with the Ca2+/calmodulin inhibitor KN-93 decreased skeletal muscle glucose transport in response to contraction (86). Incubation with KN-93 also significantly inhibited exercise-induced CaMKII phosphorylation in the absence of AMPK inhibition. This suggests that CaMKs regulate glucose uptake independently of AMPK signaling (83, 86). These studies also showed that overexpression of constitutively active CaMKKα in mouse skeletal muscle increased AMPK Thr172 phosphorylation and skeletal muscle glucose uptake (83). However, this increase in glucose uptake was also observed in muscle overexpressing dead AMPK-α2 and thus is likely independent of AMPK activation. In contrast, another study (30) using isolated skeletal muscle showed that inhibition of CaMK signaling with KN-93 inhibited contraction-induced skeletal muscle glucose uptake through an AMPK-dependent signaling pathway. These data suggest that CaMKs play important roles in the regulation of contraction-induced skeletal muscle glucose uptake, but the role of AMPK in the regulation of Ca2+/calmodulin-mediated increases in skeletal muscle glucose uptake is unclear. Recently, electroporation of a specific CaMKII inhibitor into mouse tibialis anterior muscle reduced exercise-stimulated glucose uptake by 30% (84). However, a separate study (29) found that increases in Ca2+ concentration in muscle caused very little increase in glucose uptake when the contractile response of the muscle was impaired. These data point to an indirect effect of Ca2+ on muscle glucose uptake.

Downstream Targets of Insulin- and Exercise-Stimulated Contraction (AS160, TBC1D1, and Rac1)

The downstream signaling pathways for insulin and exercise have revealed a converging signal for insulin- and the exercise-stimulated glucose uptake in skeletal muscle. Some of the molecules involved in this point of convergence are AS160 and TBC1D1. The link between AS160 and TBC1D1 as well as GLUT4 translocation involves Rab proteins. Rab proteins are members of the Ras small GTPase superfamily (81). These proteins are involved in many membrane trafficking events, and active Rabs recruit various effectors that are involved in vesicle budding, tethering, and fusion and therefore also in GLUT4 translocation (34, 63, 81). In addition to the well-established roles of Rab proteins, there is evidence that the Rho family GTPase Rac1 is involved in both insulin- and exercise-stimulated GLUT4 translocation (71, 72). Mice deficient in Rac1 (Rac1 KO mice) have decreased insulin-stimulated glucose transport.
GLUT4 translocation (71, 83), and Rac1 inhibition decreased contraction-stimulated glucose uptake in mouse skeletal muscle (72).

AS160. AS160 regulates insulin-stimulated GLUT4 translocation (5, 68, 74). It is phosphorylated on six different phospho-Akt-substrate (PAS) sites in response to both insulin and exercise in skeletal muscle (5, 43, 76). Studies have shown that AS160 PAS phosphorylation is increased after prolonged exercise in both humans (12, 70, 76) and rats (6, 14). Studies have shown that AMPK phosphorylates AS160 (PAS site) in response to AICAR and exercise in skeletal muscle (43) and that mutation of four PAS sites significantly inhibits both insulin and exercise-induced glucose uptake (44). Additional data have demonstrated that mutation of the calmodulin-binding domain on AS160 significantly inhibits exercise-stimulated glucose uptake but not insulin-stimulated glucose uptake (45). Interestingly, whole body KO of AS160 does not result in a significant increase in glucose transport in skeletal muscle (8). These data clearly show that both phosphorylation and calmodulin binding on AS160 are involved in the regulation of exercise-stimulated glucose uptake. These data also suggest that while AS160 may serve as a point of convergence for both insulin- and exercise-dependent signaling in the regulation of glucose uptake, other proteins may be involved in this regulation of glucose uptake.

TBC1D1. Recent studies (5, 36, 37, 43, 64, 73, 77) have identified TBC1D1, the paralog of AS160, as another potential molecular link among signaling pathways converging on GLUT4 translocation in skeletal muscle. TBC1D1 and AS160 are 47% identical overall and have several comparable structural features. TBC1D1 was first identified in adipocytes in culture but is highly expressed in skeletal muscle (64). Insulin increases TBC1D1 PAS phosphorylation in skeletal muscle (2, 73, 80), but, unlike AS160, TBC1D1 can regulate insulin-stimulated glucose transport through a PAS-independent mechanism (2). Studies (2, 80) have shown that distinct mutations of TBC1D1 differentially regulate insulin- and exercise-stimulated glucose transport in skeletal muscle. These data suggest that TBC1D1 regulates both insulin- and exercise-stimulated glucose uptake through distinct phosphorylation sites. Taken together, these data suggest that TBC1D1, along with AS160, may also serve as a point of convergence for the regulation of GLUT4 translocation via both insulin- and exercise-stimulated glucose uptake in skeletal muscle.

Effects of Chronic Exercise Whole Body Metabolic Health

Exercise training has important therapeutic implications for people with type 2 diabetes. Here, we will discuss several adaptations of skeletal muscle to chronic exercise and how these adaptations can improve metabolic health in people with type 2 diabetes, specifically by improving mitochondrial function and increasing GLUT4 protein expression.

Effects of Chronic Exercise on Skeletal Muscle Mitochondria

People with type 2 diabetes have been reported to have smaller, damaged, or dysfunctional mitochondria (59) and decreased expression of peroxisome proliferator-activated receptor-γ coactivator-1α, a marker of mitochondrial biogenesis (47, 55). Skeletal muscle mitochondrial dysfunction has also been linked with insulin resistance and type 2 diabetes (59), although more studies are needed to directly connect mitochondrial deficits with impaired muscle glucose metabolism. It is
also unclear if the mitochondrial defect precedes the development of type 2 diabetes or vice versa.

Endurance exercise training has been shown to increase mitochondrial content and activity (22, 52). A recent study (52) examined the effects of exercise training in people with type 2 diabetes and determined if exercise could restore mitochondrial content and function and insulin sensitivity in a patient population. Eighteen male subjects underwent 12 wk of exercise training. Subjects exercised 3 times/wk, 2 times/wk on a cycle ergometer (55% of their maximal O2 consumption), and 1 time/wk with resistance exercise (at 75% maximal voluntary contraction). After 12 wk of exercise, the defects previously observed in skeletal muscle mitochondrial activity in people with type 2 diabetes were completely negated. Interestingly, this restored mitochondrial function (Fig. 2) was correlated to improved insulin-stimulated glucose disposal (52). These data indicate that the restoration of mitochondrial function in type 2 diabetic patients accompanies the improved skeletal muscle insulin sensitivity.

Effects of Chronic Exercise on Skeletal Muscle GLUT4 Expression

It is well established that endurance exercise training increases skeletal muscle GLUT4 (22, 63). The increase in skeletal muscle GLUT4 causes an increase in skeletal muscle glucose uptake (24, 26, 47, 63). Most studies that have examined the therapeutic effects of exercise in people with type 2 diabetes have focused on low- to moderate-intensity aerobic exercise, which has been established as an effective strategy to improve the metabolic health of people with type 2 diabetes (9). The recommended amount of exercise for people with type 2 diabetes is 150 min of moderate intensity exercise per week (9). However, the amount of exercise required for improvements in whole body and skeletal muscle metabolic health is debatable. Recent studies have examined if the effects of low-volume-high-intensity interval training (HIT), a time-efficient strategy to improve health and fitness, could be applied to people with type 2 diabetes as an intervention to improve glucose regulation and skeletal muscle metabolism (82). This study involved 14 people with type 2 diabetes (45). Eight of these subjects underwent HIT for 2 wk, where they trained on a cycle ergometer a total of six times over the 2-wk period. Each session consisted of a 5-min warmup, 10 × 60 s of cycling at 90% maximal effort with 60 s of recovery, and a 5-min cooldown. After 2 wk of HIT, people with type 2 diabetes had a marked increase in skeletal muscle GLUT4 expression and a significant decrease in blood glucose concentrations. Markers of mitochondrial activity, such as citrate synthase activity and expression of complex I, II, and III, were also significantly increased after 2 wk of HIT (Fig. 3). These data indicate that only 2 wk of HIT, where the weekly training time was 50% lower than the recommended guidelines for exercise in type 2 diabetic patients (9), increased glucose uptake and improved mitochondrial function in people with type 2 diabetes and improved whole body metabolism. This study indicated that HIT is a potential time-efficient exercise strategy for the treatment of type 2 diabetes.

Summary

In summary, exercise is critical in the treatment and prevention of type 2 diabetes. Acute exercise activates alternative molecular signals that can bypass defects in insulin signaling in skeletal muscle, resulting in an insulin-independent increase in glucose uptake. Exercise training results in increased skeletal muscle mitochondria and GLUT4 protein expression, which are associated with improved skeletal muscle insulin sensitivity and whole body metabolic health. Exercise-induced adaptations to skeletal muscle are essential to prevent and combat type 2 diabetes.

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DISCLOSURES

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