HIGHLIGHTED TOPIC | Role of Exercise in Reducing the Risk of Diabetes and Obesity

Contraction signaling to glucose transport in skeletal muscle

Niels Jessen1,2 and Laurie J. Goodyear1

1Research Division, Joslin Diabetes Center, and Department of Medicine, Brigham and Women’s Hospital and Harvard Medical School, Boston, Massachusetts; and 2Medical Research Laboratory and Department of Clinical Pharmacology, University of Aarhus, Aarhus, Denmark

Jessen, Niels, and Laurie J. Goodyear. Contraction signaling to glucose transport in skeletal muscle. J Appl Physiol 99: 330–337, 2005; doi:10.1152/japplphysiol.00175.2005.—Contracting skeletal muscles acutely increases glucose transport in both healthy individuals and in people with Type 2 diabetes, and regular physical exercise is a cornerstone in the treatment of the disease. Glucose transport in skeletal muscle is dependent on the translocation of GLUT4 glucose transporters to the cell surface. It has long been believed that there are two major signaling mechanisms leading to GLUT4 translocation. One mechanism is insulin-activated signaling through insulin receptor substrate-1 and phosphatidylinositol 3-kinase. The other is an insulin-independent signaling mechanism that is activated by contractions, but the mediators of this signal are still unknown. Accumulating evidence suggests that the energy-sensing enzyme AMP-activated protein kinase plays an important role in contraction-stimulated glucose transport. However, more recent studies in transgenic and knockout animals show that AMP-activated protein kinase is not the sole mediator of the signal to GLUT4 translocation and suggest that there may be redundant signaling pathways leading to contraction-stimulated glucose transport. The search for other possible signal intermediates is ongoing, and calcium, nitric oxide, bradykinin, and the Akt substrate AS160 have been suggested as possible candidates. Further research is needed because full elucidation of an insulin-independent signal leading to glucose transport would be a promising pharmacological target for the treatment of Type 2 diabetes.

insulin receptor substrate-1; phosphatidylinositol 3-kinase; Type 2 diabetes

THE PREVALENCE OF DIABETES is increasing dramatically in the United States and worldwide, which has resulted in some health organizations and researchers to consider it as a possible “epidemic.” Although the rate of diabetes is on the increase, it has long been recognized that exercise has important health benefits for people with Type 2 diabetes. Physical exercise can positively moderate glucose homeostasis in people with Type 2 diabetes, due to enhanced glucose transport and insulin action in the working skeletal muscles, which are the major tissues responsible for total body glucose disposal (22). The effects of exercise to increase skeletal muscle glucose transport and insulin sensitivity may also be a key mechanism to explain the strong epidemiological evidence that regular exercise prevents or delays the onset of Type 2 diabetes (55, 101). Despite the physiological importance of exercise in regulating glucose transport in skeletal muscle, the molecular mechanisms that mediate this important phenomenon are still not fully understood.

GLUCOSE TRANSPORT SYSTEM IN SKELETAL MUSCLE

Under most physiological conditions, glucose transport across the cell membrane is the rate-limiting step in glucose utilization in skeletal muscle (17, 57). Insulin stimulation and physical exercise are the most physiologically relevant stimulators of glucose transport in skeletal muscle (29, 37), and interestingly in patients with Type 2 diabetes, insulin- but not contraction-stimulated glucose transport is impaired (54, 123). GLUT4 is the major glucose transporter isoform expressed in skeletal muscle, and the translocation of GLUT4 from an intracellular location to the plasma membrane and T tubules is a major mechanism through which both insulin and exercise increase skeletal muscle glucose transport (Fig. 1) (29, 37). Contractile activity can stimulate GLUT4 translocation in the absence of insulin (29, 37), and some studies suggest there are different intracellular “pools” of GLUT4, one stimulated by insulin and one stimulated by exercise (18, 24, 84). These findings have provided the basis for our understanding of the glucose transport system with exercise in skeletal muscle, and a detailed discussion of exercise regulation of the glucose transport system has been published previously (29, 37).

SIGNSIGNALING MECHANISMS IN CONTRACTING SKELETAL MUSCLE

In recent years, considerable research has focused on elucidating the signaling mechanisms leading to exercise-stimulated GLUT4 translocation. Early data have demonstrated that there are distinct mechanisms for the stimulation of glucose transport by exercise and insulin. Insulin signaling involves the rapid phosphorylation of the insulin receptor, insulin receptor substrate-1 and -2 on tyrosine residues and the activation of phosphatidylinositol 3-kinase (PI3-kinase) (26, 28). In skeletal...
AMPK (AMP-activated protein kinase) is a heterotrimer complex that consists of \( \alpha \), \( \beta \), and \( \gamma \) subunits, each of which contains two or more different isoforms. In skeletal muscle, the \( \alpha_2 \) (31, 71), \( \beta_2 \) (11, 98), and \( \gamma_1 \) (13) or \( \gamma_2 \) (64) isoforms are the major isoforms expressed and form the majority of AMPK heterotrimer complexes (109). The \( \alpha \)-subunit of AMPK exhibits catalytic activity (67), whereas the \( \beta \)- and \( \gamma \)-subunits appear to be important in substrate specificity and maintenance of heterotrimer stability (32, 53). The \( \beta \)-subunit acts as a scaffold for the binding of the \( \alpha \)- and \( \gamma \)-subunits (114) and is also speculated to be involved in the regulation of glycogen metabolism due to having a putative glycogen-binding domain (45, 76). The \( \gamma \)-subunit has also been proposed to be involved in binding of AMP, and mutations of this subunit can also lead to aberrations in glycogen metabolism (1, 13, 21, 66).

AMPK is activated by an increase in the AMP-to-ADP and ADP-to-ATP ratios via a complex mechanism that involves allosteric modification and phosphorylation (32, 53). It has been proposed that AMPK acts as a “fuel gauge” in mammalian cells (32). When the cell senses low fuel (decreased ATP), AMPK acts to switch off ATP-consuming pathways and switch on alternative pathways for ATP regeneration. Recently, the serine/threonine kinase LKB1 in complex with the two accessory subunits, STRAD and MO25, has been identified as an upstream kinase for AMPK (33, 44, 89). LKB1 is also a kinase for 11 of the 12 proteins in the AMPK family (61, 90). Regulation of AMPK activity seems to depend on the presence of AMP for making the AMPK heterotrimer a better substrate for the LKB1-STRAD-MO25 complex, possibly by binding to the \( \gamma \)-subunit. Phosphorylation of the Thr172 site on the \( \alpha_1 \) and \( \alpha_2 \) catalytic subunits is essential for AMPK activity (20, 93), but it is still not known whether LKB1 is the only kinase responsible for Thr172 phosphorylation of AMPK in skeletal muscle. Immunoprecipitation with LKB1 antibody has been shown to reduce AMPK-kinase activity by 80% in cultured cells (33) and 85% in rat liver (113), suggesting that LKB1 accounts for at least the major part of AMPK-kinase activity in these tissues.

Contractile activity alters the fuel status of skeletal muscle, and depending on the intensity of the contractions, there can be significant decreases in both phosphocreatine and ATP concentrations. Thus it is not surprising that AMPK is activated in the rat in response to exercise in vivo (77, 78), sciatic nerve-stimulated muscle contractions in situ (46, 105), and contraction of isolated muscles in vitro in the absence of systemic factors (35, 36, 48). The greater the force production generated by contraction (48) or the greater the intensity of treadmill exercise (78), the greater the activation of AMPK. In humans, moderate-intensity aerobic cycle exercise (27, 95, 111), as well as high-intensity “sprint” exercise (12), will increase skeletal muscle AMPK activity. LKB1 appears to be constitutively active in skeletal muscle, and, in fact, neither muscle contraction nor 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR) treatment increases LKB1 activity (86). Interestingly, increased LKB1 expression seen after long-term endurance training also has no effect on activity (96).

AMPK AS A MEDIATOR OF SKELETAL MUSCLE GLUCOSE TRANSPORT

Initial evidence in support of a role for AMPK in contraction-stimulated glucose transport came from studies using AICAR. AICAR is a compound that is taken up into skeletal muscle and metabolized by adenosine kinase to form 5-aminoimidazole-4-carboxamide-1-beta-D-ribofuranoside, the monophosphorylated derivative that mimics the effects of AMP on AMPK (36, 65). AICAR infusion enhances insulin-stimulated glucose transport in perfused rat hindlimb skeletal muscles (65). In the isolated intact rat epitrochlearis muscle, AICAR can stimulate glucose transport in the absence of insulin, similar to the effects of contraction (5, 36). Insulin, muscle contraction, and AICAR all robustly increase glucose transport in isolated rat epitrochlearis muscles incubated in vitro, and,
similar to contraction-stimulated transport, AICAR-stimulated transport is not inhibited by wortmannin. Furthermore, the increase in glucose transport with the combination of maximal AICAR plus maximal insulin treatments is partially additive, whereas there is no additive effect on glucose transport with the combination of AICAR plus contraction (36). Short-term infusion of rats with AICAR (and glucose to maintain euglycemia) also increases glucose transport in multiple muscle types (5). Hindlimb perfusion studies reveal that AICAR-induced increases in skeletal muscle glucose transport are mediated by the translocation of GLUT4 to the plasma membrane (58), comparable to the effects of exercise and muscle contraction (37).

Although experiments using AICAR as an AMPK activator have generated important information for the function of AMPK, there are limitations to this approach because AICAR does not have strict specificity to AMPK (30, 106, 120). Specific activation and inhibition of AMPK by pharmacological agents would be a valuable approach to more clearly define the role of AMPK in glucose transport and other metabolic effects, but unfortunately such compounds are not currently available. Two putative AMPK inhibitors, iodotubercidin and araA, are not specific to AMPK, and although these compounds inhibit AICAR-induced activation of AMPK, they fail to inhibit contraction-induced AMPK activation in skeletal muscle (72). Compound C, which can inhibit AMPK activity in hepatocytes (122), is ineffective in attenuating AMPK activity in skeletal muscle (Fujii N, Hirshman MF, and Goodyear LJ, unpublished observations).

The use of transgenic and knockout mouse models is beginning to make an impact on understanding the role of AMPK in contraction-mediated glucose uptake. Transgenic mice that express a muscle-specific mutant subunit of AMPK with reportedly dominant inhibitory effects on the α-subunit have been used to study glucose transport (69). Consistent with the hypothesis that AMPK is a positive regulator of glucose transport in skeletal muscle, this same group found that AICAR-stimulated glucose transport was fully inhibited in mutant AMPK transgenic mice and that contraction-stimulated glucose transport was reduced by 30–40% (69). In contrast, studies of either α1 or α2 whole body AMPK knockout mice showed that contraction-induced glucose transport in isolated muscles was not altered despite inhibition of AICAR-stimulated glucose transport in α2 knockout mice (51). Although the lack of inhibition of contraction-stimulated glucose transport in the knockout studies could be due to compensatory regulation by the other catalytic isoform, these studies raise important questions regarding the role of AMPK in contraction-stimulated glucose transport. One theory is that there are redundant signaling pathways involved in the stimulatory effect of contraction on glucose transport. If AMPK activity is ablated, then an alternative pathway or pathways compensate for the lack of AMPK function. The large family of AMPK-related proteins could function as redundant proteins in signaling to glucose transport. Although one study has shown that, under normal conditions, contraction does not increase the activity of any of these proteins in rat skeletal muscle (86), it is possible that under conditions of ablated AMPK activity there is a compensatory upregulation or activation of one or more of the AMPK-related proteins. The identification of LKB1 as a kinase of all the proteins in the AMPK family opens up possibilities to further study the involvement of this protein kinase family in exercise regulation of glucose transport.

**CALCIUM-REGULATED SIGNALING TO GLUCOSE TRANSPORT**

Contraction of skeletal muscle fibers is initiated by depolarization of the plasma membrane and T tubules, triggering the release of calcium from the sarcoplasmic reticulum. The spike in intracellular calcium leads to the interaction of actin and myosin filaments and the development of tension in the fibers. The increase in myocardial calcium concentrations has been proposed to be a signal in the initiation of contraction-stimulated glucose transport and GLUT4 translocation (41, 42). Caffeine, an agent that causes contraction by increasing calcium release from the sarcoplasmic reticulum in the absence of membrane depolarization, was first shown to increase glucose transport in isolated amphibian muscle (43). Several studies have shown that glucose transport is increased in mammalian muscle when cytoplasmic calcium concentrations are raised using various agents (38, 50, 118), and dantrolene, which prevents the release of calcium from sarcoplasmic reticulum, blocks glucose transport in skeletal muscle (74, 118). More recent work using isolated rat epitheclearis muscles has shown that low doses of caffeine stimulate an increase in intracellular calcium levels (97) and that this leads to an increase in glucose uptake without inducing contractions or changes in high-energy phosphate (118).

The mechanism by which calcium might regulate exercise-stimulated glucose transport is not known. However, it is unlikely that calcium ions directly activate the glucose transport system because cytoplasmic calcium concentrations are elevated for only a fraction of a second after each muscle contraction, whereas the increase in muscle glucose transport can remain elevated for a considerable period of time after the contractile activity ceases. Instead, one or more of the calcium-regulated intracellular proteins may lead to GLUT4 translocation. Potential candidates include calmodulin, the family of calmodulin-dependent protein kinases (CaMK), and the protein kinase C (PKC) family, all of which are important intermediaries in cellular signal transduction.

CaMK are an evolutionarily conserved protein family that phosphorylates substrates important in transcription, secretion, ion channel regulation, and morphogenesis (99). Inhibition of the CaMK pathway with different pharmacological inhibitors leads to a reduction in contraction-stimulated glucose uptake (47, 116). However, both direct inhibition of calmodulin using various compounds (47) and inhibition of CaMK with the specific inhibitor KN62 (7, 115) (Witczak CA, Jessen N and Goodyear LJ, unpublished observations) have also been shown to inhibit insulin-stimulated glucose uptake. This can mean either that calmodulin and CaMK represents a point of convergence of the insulin- and contraction-stimulated glucose transport pathways or that the inhibitors have unspecific effects on glucose transport.

Another potential candidate for calcium-induced glucose transport is PKC. In mammalian cells, 12 different PKC isoforms have been identified that have been classified into three subfamilies based on amino acid similarity and mode of activation: conventional PKCs, novel PKCs, and atypical PKCs (aPKC). Muscle contraction has been shown to increase
PKC activity (16, 75, 80, 83), although early studies that measured PKC activity in muscle used the nonspecific kinase substrate histone H3, and it is now known that contraction can increase the activity of numerous kinases in skeletal muscle (2, 70). Downregulation of PKC by long-term phenol ester treatment (16) and inhibition of PKC using polymyxin B (39, 119) have both been associated with decreases in contraction-stimulated glucose transport. However, polymyxin B is not a specific inhibitor of PKC, and both of these treatments can result in decreased contractility of the muscle fibers. Another PKC inhibitor, calphostin C, can reduce contraction, but not insulin-stimulated transport (47), without affecting contractility or inhibiting other effects of contractions such as mitogen-activated protein kinase signaling (34). Calphostin C inhibits PKC by covalent modification of the lipid-binding regulatory domain and competition at the binding site for diacylglycerol (8), suggesting that conventional and novel PKC isoforms may play a role in contraction-stimulated glucose transport. Again, data with inhibitors needs to be interpreted with caution due to nonspecificity of most of these compounds.

Other intriguing evidence that PKCs may be involved in contraction-stimulated glucose transport comes from a study suggesting that aPKC-ζ and -λ are downstream of AMPK. This study, performed in L6 myotubes and isolated rat muscles, suggests that the effects of AMPK on glucose transport are mediated through the sequential activation of extracellular signal-regulated kinase, proline-rich tyrosine kinase-2, phospholipase D, and aPKCs (10). This hypothesis must also be interpreted with caution since inhibition of contraction-induced extracellular signal-regulated kinase phosphorylation by a mitogen-activated protein kinase kinase inhibitor does not affect contraction-stimulated glucose transport in rat muscles (34, 110). Clearly, further research is needed to clarify the role of aPKCs and these other molecules in regulating contraction-stimulated glucose transport in skeletal muscle.

Some observations raise the question of whether changes in calcium concentrations can be the sole mediator of contraction-induced glucose transport. It would be expected that the increases in glucose transport would depend on the stimulation frequency (i.e., high increases in calcium concentrations) rather than the force production (i.e., high metabolic stress). But, in incubated rat soleus muscles, contraction-stimulated glucose uptake is more dependent on the force production than on the stimulation frequency (48, 49). The role for calcium as the only mediator of contraction-stimulated glucose uptake seems therefore less likely, but further studies of the calcium-stimulated glucose uptake, preferably using other approaches than the inhibitors, will be important.

AKT AND AS160

Akt is a Ser/Thr kinase with three isoforms (Akt1, Akt2, and Akt3) that are all expressed in skeletal muscle (107). Akt is activated by a wide variety of growth factors in a PI3-kinase-dependent manner, through translocation to the membrane and phosphorylation on two regulatory sites (104). Various lines of evidence suggest that Akt2 is critical in insulin-stimulated glucose transport (31). Akt1-deficient animals have a marked impairment in embryonic and postnatal growth patterns but show no abnormalities with respect to insulin sensitivity or glucose and lipid metabolism (15). On the other hand, Akt2-deficient mice have no apparent growth defects but do exhibit several diabetes-like characteristics such as pancreatic β-cell hypertrophy, aberrant hepatic glucose production, as well as impaired resting glucose homeostasis and insulin-stimulated whole-body glucose disposal (14). Furthermore, insulin-stimulated glucose transport is markedly reduced in isolated extensor digitorum longus muscles from Akt2 knockout mice (14).

Some, but not all, findings have shown that muscle contractions increase Akt activity and/or phosphorylation (6, 63, 73, 85, 102). In contrast to insulin-stimulated Akt regulation, the role of Akt activation in contraction-mediated glucose uptake is unknown. The time course of contraction-stimulated glucose transport and Akt activation are similar, raising the possibility that Akt may function in signaling to glucose transport in the working muscle. On the other hand, the PI3-kinase inhibitors wortmannin and LY294002 fully inhibit contraction-stimulated Akt phosphorylation and activity (87) but do not decrease contraction-stimulated glucose transport (36, 59, 62, 117). Although this dissociation between Akt activity and glucose transport suggests that Akt does not function to increase transport with contraction, additional studies using knockout mice will be important to fully resolve this question.

The use of the phosho-Akt-substrate antibody has identified a novel Akt substrate of a molecular weight of 160 (AS160). AS160 contains a GTPase-activating domain for Rabs, which are small G proteins required for membrane trafficking (88). AS160 has been proposed to connect insulin signaling to membrane trafficking (52) and to be necessary for insulin-stimulated glucose uptake in adipocytes (88). Contraction and AICAR both increase AS160 phosphorylation in rat skeletal muscle (9), suggesting that AS160 could be a site of signal integration for insulin- and contraction-stimulated signaling to GLUT4 translocation. However, similar to contraction-induced Akt activity, contraction-induced AS160 phosphorylation is inhibited by wortmannin (9). The role of AS160 in contraction-induced glucose transport will need to be investigated in future work.

NITRIC OXIDE SYNTHASE AND SKELETAL MUSCLE GLUCOSE TRANSPORT

Nitric oxide (NO) is produced in a variety of tissues through the activation of different isoforms of NO synthase (NOS) (68), and skeletal muscles express both neuronal NOS and endothelial NOS (56). NO is released from isolated extensor digitorum longus muscles incubated at rest, with further increases following electrical stimulation that generates contractile activity (3). Treadmill running exercise can activate NOS in gastrocnemius muscles (81), providing additional evidence that NO production in skeletal muscle increases during exercise. Exogenously administered NO, which is generated from the NO donor sodium nitroprusside, stimulates glucose transport in isolated skeletal muscles (4, 25, 40, 121) by increasing GLUT4 concentrations at the cell surface (25). One group has proposed that NO mediates exercise-stimulated glucose transport in skeletal muscle (4, 82). In studies where rats were first exercised on a treadmill (82) or had hindlimb muscles contracted via nerve stimulation (4) followed by isolation of muscles and measurement of glucose transport, NOS inhibition blocked exercise/contraction-
stimulated glucose transport. In contrast, we (40) and others (25, 94) have found that when muscles are contracted or AICAR is treated in the presence of a NOS inhibitor, there is normal activation of glucose transport. Addition of the NOS inhibitor NG-nitro-L-arginine methyl ester (1 mg/ml) to the drinking water of rats for 2 days failed to affect the increase in muscle 2-deoxyglucose uptake in response to treadmill running exercise. These data suggest that NO stimulates glucose transport by a mechanism that is distinct from the insulin and contraction signaling pathways.

BRADYKININ

Bradykinin is a nonapeptide hormone that is locally released from contracting skeletal muscles (92, 108) and mediates physiological effects, such as pain, inflammation, vascular permeability, hypotension, edema formation, and smooth muscle contraction (79). Infusions of bradykinin to healthy volunteers have been shown to increase glucose uptake in the forearm independent of insulin (23). This finding has interesting clinical potential since treatment with ACE inhibitors, commonly used as antihypertensive drugs in patients with Type 2 diabetes, can increase the circulating level of bradykinin (103). However, infusion of bradykinin in the forearm has potent effects on blood flow and is known to cause edema, and the technique does not allow a direct estimate of glucose transport into the muscle. Later studies using in vitro incubated muscles, where glucose uptake is independent of blood flow, have not seen any effect of bradykinin on skeletal muscle glucose uptake (19), and a role for bradykinin in contraction-stimulated glucose uptake seems less likely.

SUMMARY AND FUTURE DIRECTIONS

Over the past several years, there has been considerable research focused on elucidating the mechanisms through which exercise regulates glucose transport in skeletal muscle. It has been clearly demonstrated that exercise functions via a different signaling mechanism from insulin for regulation of glucose transport. Distinct signaling pathways provide the molecular basis for the ability of exercise to normally (or near normally) increase glucose uptake in patients who are otherwise resistant to the actions of insulin. Numerous studies have identified AMPK as a part of a distinct, insulin-independent signaling mechanism for the stimulation of glucose transport in skeletal muscle. Studies of exercise signaling have been led to the identification of the AMPK pathway as a target for pharmacological development. In fact, metformin, the single most highly prescribed oral antidiabetic agent in the United States, increases AMPK activity in rat muscle (122) and patients with Type 2 diabetes (71), indicating that stimulation of this pathway might already have been used in the treatment for almost 50 years. Although there are numerous studies suggesting that AMPK functions in contraction-stimulated glucose transport, there is now convincing evidence that AMPK is not the only mechanism involved in exercise regulation of glucose transport. It seems likely that there may be a number of signaling proteins involved in this exercise signaling mechanism. The role of potential mediators like the calcium-activated proteins are under investigation, and determining the functions of these proteins, as well as the identification of new proteins, is the immediate goal for understanding the mechanism of glucose transport regulation by exercise in skeletal muscle.

GRANTS

Work in the author’s laboratory associated with these studies was supported by National Institute of Arthritis and Musculoskeletal and Skin Diseases Grants AR-45670 and AR-82238 (to L. J. Goodyear).

REFERENCES

CONTRACTION REGULATION OF GLUCOSE TRANSPORT


CONTRACTION REGULATION OF GLUCOSE TRANSPORT


72. Neubauer SC, Jennings IG, Campbell DJ, Witters LA, and Kemp BE. Progression of Type 1 diabetes by 10.220.33.5 on April 8, 2017 http://jap.physiology.org/ Downloaded from
CONTRACTION REGULATION OF GLUCOSE TRANSPORT


