Skeletal Muscle Glucose Uptake During Exercise: How is it Regulated?

The increase in skeletal muscle glucose uptake during exercise results from a coordinated increase in rates of glucose delivery (higher capillary perfusion), surface membrane glucose transport, and intracellular substrate flux through glycolysis. The mechanism behind the movement of GLUT4 to surface membranes and the subsequent increase in transport by muscle contractions is largely unresolved, but it is likely to occur through intracellular signaling involving Ca\textsuperscript{2+}-calmodulin-dependent protein kinase, S\textsuperscript{5’}-AMP-activated protein kinase, and possibly protein kinase C.

During dynamic exercise, the turnover of ATP in skeletal muscle increases greatly and is fuelled by the catabolism of carbohydrates (intramuscular glycogen, blood glucose) and fatty acids (intramuscular triglycerides, blood lipids). During exercise in the postabsorptive state, the contribution of blood glucose to ATP resynthesis is initially relatively minor, but as exercise continues and muscle glycogen stores are depleted, the contribution of blood glucose becomes more substantial, reaching ~35% of leg oxidative metabolism and close to 100% of muscle carbohydrate metabolism (1, 2, 28, 104, 139). Probably the most influential factor for the magnitude of increase in muscle glucose uptake during exercise in the postabsorptive state is exercise intensity, with skeletal muscle glucose uptake being greater at higher exercise intensities (FIGURE 1; Refs. 104 and 131). This is probably due to a combination of greater fiber recruitment (45) as well as higher metabolic stress on active muscle fibers (59, 60) at higher exercise intensities. During exercise, the major metabolic fate of blood glucose after entry into skeletal muscle cells is glycolysis (139, 157) and subsequent oxidation (61, 157).

Regular exercise can improve glycemic control (11, 12). This may be due, in part, to the acute effects of exercise on glucose metabolism as well as training-induced adaptations. In individuals with type II diabetes, a single bout of exercise can reduce blood glucose concentrations (83) mainly because the exercise-induced increase in skeletal muscle glucose uptake is intact (79) even when insulin action is impaired. Thus the molecular mechanism resulting in increased muscle glucose transport during exercise is recognized as a clinically relevant alternative pathway to increase glucose disposal in skeletal muscle in states of insulin resistance.

Regulation of Skeletal Muscle Glucose Uptake During Exercise

There are three sites of regulation of skeletal muscle glucose uptake in vivo: glucose delivery to the skeletal muscle cells, surface membrane permeability to glucose (i.e., glucose transport), and flux through intracellular metabolism (FIGURE 2). Whether one or a combination of these factors is rate limiting to tissue uptake has proven difficult to discern. Glucose delivery or supply to a tissue vascular bed is usually expressed as the product of blood flow and blood glucose concentration. Because muscle blood flow can increase up to 20-fold during intense exercise (4), the increase in muscle perfusion is of large quantitative importance for the increase in glucose supply (97). In vitro studies of glucose uptake during electrical stimulation of perfused rat hindlimb muscles indicate that perfusion is important for the rate of glucose uptake during contractions (52), but whether perfusion limits glucose uptake under normal exer-
cise conditions is questionable. If flow were to be limiting during exercise, a decrease in the interstitial glucose concentration would be expected. However, this is not the case, as skeletal muscle interstitial glucose concentration is actually higher when comparing exercising and rested limbs (76). On the other hand, increasing glucose supply to the working muscle by raising glucose concentrations during contractions in vitro (85, 91) or during exercise in vivo (97, 157) increases skeletal muscle glucose uptake during exercise even when insulin levels are prevented from rising (FIGURE 3).

Conversely, during prolonged exercise when blood glucose concentration decreases, leg glucose uptake decreases as well (1). It is noteworthy that within the physiological range of glucose concentrations the relationship between plasma glucose concentration and glucose uptake in muscle during exercise is almost linear, indicating that changes in plasma glucose concentrations during exercise translate almost proportionally to changes in glucose uptake by muscle (FIGURE 3). Thus, whereas the increase in muscle blood flow seems well matched to the metabolic demands of muscle (4) and does not pose an obvious limitation to glucose uptake in healthy individuals, the blood glucose concentration is an important limiting factor for glucose uptake during exercise.

Skeletal muscle expresses multiple isoforms of glucose transporters (93). During exercise the most important of these is GLUT4, because systemic (110) and muscle-specific (158) GLUT4 knockout abolishes contraction-stimulated glucose uptake, at least when studied in vitro. Several studies suggest that glucose transport is rate limiting for glucose uptake in skeletal muscle during moderate-intensity exercise, because intramuscular glucose does not accumulate except perhaps in the first few minutes of exercise (64, 99), which would be expected if phosphorylation of glucose was limiting. However, during intense exercise when glycogenolysis is very rapid and formation of gluco...
cose-6-phosphate (G6P) is pronounced, the ensuing inhibition of hexokinase (HK) may make glucose phosphorylation, rather than transport, limiting (63, 69).

Skeletal muscle glucose transport follows Michaelis-Menten kinetics, and most studies show that exercise increases the $V_{\text{max}}$ of glucose transport without affecting $K_m$ (66, 122, 157), suggesting that the number but not affinity for glucose of individual transporters is higher with exercise. Even so, a study in perfused rat hindlimb demonstrated both a contraction-induced increase in $V_{\text{max}}$ and a decrease in $K_m$ for glucose transport (91) that was subsequently supported by data obtained in isolated plasma membrane vesicles from rat muscle (90). These data indicate that the activity of each glucose transporter may increase with contractions. Similar to the action of insulin, it has been demonstrated that muscle contractions increase the sarcolemmal and transverse-tubular content of GLUT4 (FIGURE 4) using several different methods and models (34, 35, 36, 46, 70, 74, 75, 92, 108, 109, 126), thereby enhancing the facilitated diffusion of glucose into the muscle cells. The increased GLUT4 abundance in the transverse tubules is hypothesized to account for a substantial proportion of the overall increase in skeletal muscle glucose transport, allowing delivery of glucose deep into the myoplasm (33).

Recent studies using genetic manipulation to alter the expression of proteins involved in glucose transport and metabolism have shed some light on the possible limiting steps in glucose uptake during exercise. Wasserman and colleagues have demonstrated that neither GLUT4 overexpression nor partial knockout alter exercise-stimulated increases in skeletal muscle glucose uptake in vivo (40, 42), indicating that the increase in surface membrane GLUT4 is a permissive step in glucose uptake under normal conditions or that only a percentage of the total GLUT4 pool is required for the increase in surface membrane permeability with exercise. Further work by Wasserman and colleagues using mice with skeletal muscle HKII overexpression or partial knockout implicate an important role for HKII in the regulation of glucose uptake by working murine muscle in vivo (40, 41, 42, 48). However, when mice with a partial knockout of skeletal muscle HKII were exercised, there was a limitation to glucose uptake only in the oxidative skeletal muscles during exercise and no discernable effect in muscles of a mixed fiber type (41). Given that human vastus lateralis (the muscle typically sampled for biochemical measures, representative of leg glucose uptake) is of a heterogeneous fiber population (116), it is difficult to extend these findings to humans. Furthermore, in individuals with type II diabetes who have lower maximal HKII activity (88) and hence expression, skeletal muscle glucose uptake is normal (79), perhaps because perfusion (79, 135) and GLUT4 translocation (65) during exercise are not impaired. Lastly, there is very little direct evidence that skeletal muscle HK activity is altered by acute contractile activity or exercise in mammals (133), and there are no studies that have directly examined the relationship between possible acute changes in HK activity and glucose uptake with exercise. However, the apparent lack of fine control of HK activity during exercise may very well be the reason that HK can limit glucose uptake in some circumstances. Overall, the role of HK in regulation of glucose uptake during exercise in humans is ambiguous and probably only limiting to glucose uptake during intense exercise when G6P accumulates and inhibits HK activity.

In summary, glucose supply, transport, and phosphorylation are important regulatory steps of skeletal muscle glucose uptake during exercise (FIGURE 2). Whether one or a combination of these factors is rate limiting to glucose uptake in the intact mammal has proven difficult to discern, because all steps are likely to be closely coupled to the metabolic state of the muscle fiber as well as the percentage of fibers recruited. It may be that oxidative muscles rely more on glucose supply (perfusion) and phosphorylation by HK (41, 77), with glycolytic muscles probably relying more on glucose transport (32, 108). From a hypothetical perspective, it is also likely that even within the same muscle fiber the limiting step for glucose uptake varies depending on a variety of factors, including the intensity and duration of exercise and muscle glycogen and interstitial glucose concentrations.

Given that there is little known about the regulation of HK activity by acute exercise (133) and that there are several recent reviews on the regulation and putative molecular events leading to hyperemia (25) and greater microvascular flow (95) and thus substrate supply in skeletal muscle with exercise, the following section focuses on the regulation of membrane glucose transport by exercise/contraction.

### Regulation of Skeletal Muscle Glucose Transport by Exercise/Contraction

The molecular events involved in stimulating GLUT4 movement within muscle cells are complex (18, 134). There is evidence that there are distinct contraction and insulin-responsive GLUT4 vesicle “pools” in skeletal muscle (26, 35, 74, 92) and that the molecular signals that trigger increased glucose transport and surface membrane GLUT4 are different when comparing insulin and contraction stim-
ulation (58, 73, 75, 151). Indeed, insulin, but not contractions, results in changes in distribution of the rab4 protein (118) and contractions, but not insulin, alter the distribution of the transferrin receptor (74). Even so, there are likely to be similarities between the two stimuli, as both exercise and insulin probably recruit GLUT4 vesicles that contain vesicle-associated membrane protein-2 (70, 94) and insulin-responsive aminopeptidase (26) as well as stimulate higher sarcolemmal content of GTP-binding proteins (36). Although it is not known whether the higher surface membrane GLUT4 with contraction results from a slower endocytosis or faster exocytosis of GLUT4 vesicles in intact mammalian skeletal muscle, insights from work with cultured cardiomyocytes suggest the latter (152).

**Signaling mechanisms involved in contraction-stimulated glucose transport**

Although the signaling mechanisms that are involved in insulin-stimulated glucose transport are still partly unresolved (134), even less is known about the molecular mechanisms responsible for the increase in glucose transport and GLUT4 translocation during muscle contraction (FIGURE 4). It is generally accepted that the underlying signals occur independently of humoral factors and arise from local factors within the contracting skeletal muscle (97). Indeed, it has been demonstrated numerous times that contraction of skeletal muscle ex vivo results in higher glucose transport compared with basal conditions. Furthermore, the use of these reductionist models such as the perfused hindlimb and incubated muscle techniques allow greater insight into the regulation of glucose transport, because glucose supply can be precisely controlled (10, 142).

**Role of Ca²⁺ signaling**

The transient “spikes” in intracellular Ca²⁺ that occur with muscle contractions have for more than 30 years been hypothesized to be involved in contraction-stimulated glucose transport (56). Since the intracellular Ca²⁺ level is related to the activity of the motor nerves, signaling directly influenced by Ca²⁺ can be considered as feedforward regulation. The original studies were performed in frog sartorius muscle incubated with caffeine, which releases Ca²⁺ from the sarcoplasmic reticulum. In rat epitrochlearis muscle, raising intracellular Ca²⁺ by treatment with caffeine in vitro also increases glucose transport (125, 154). In contrast, other in vitro studies have shown no effect of Ca²⁺ ionophores on basal skeletal muscle glucose transport, with an inhibition of insulin-stimulated glucose transport (68, 72). The discrepancies between these findings are difficult to explain but may relate to the magnitude and duration of the increase in cytosolic Ca²⁺ (72). Importantly, at the concentra-
In rats, skeletal muscle PKC activity, as determined by translocation of PKC to a membrane fraction, is higher with exercise/contractions (24, 98), but this finding could not be replicated in humans (106). Furthermore, chronic downregulation (23) as well as chemical inhibition (58, 143) of conventional and novel PKC isoforms results in reduced contraction-stimulated glucose transport. However, the effect of inhibition of DAG-sensitive PKC by the microbial agent calphostin C was much more pronounced in fast-twitch than in slow-twitch muscles (143), a finding that is not surprising given that artificial activation of DAG-sensitive PKCs by phorbol esters results in increased glucose transport in rat fast-twitch but not slow-twitch muscles (150). Thus, although there are some studies that suggest that the conventional and novel PKC isoforms may be involved in contraction-induced glucose uptake, it is clear that more definitive studies are required to resolve the role of these enzymes in this process.

Another ubiquitously expressed Ca²⁺-sensing protein is calmodulin (CaM). Studies using CaM inhibitors show reductions in contraction-stimulated glucose transport, perhaps because CaM inhibition leads to reduced tension development (58), a result likely due to the role of CaM in many signaling processes in skeletal muscle, including excitation-contraction coupling (124, 130). However, specific inhibition by KN62/93 of the multifunctional CaMK isoforms such as CaMKI cannot be ruled out. Indeed, CaMKI is expressed in skeletal muscle (3), whereas CaMKIV is not (3, 105). However, the observation that insulin stimulation of glucose transport is also partially inhibited by KN62 (16, 148) suggests that insulin action is partially dependent on CaMK but also raises the possibility that KN62 inhibits glucose transport nonspecifically. Clearly, further study is required to clarify this issue.

Nitric oxide synthase (NOS) is also activated by Ca²⁺-bound CaM (12). NOS is expressed in skeletal muscle cells, and there is evidence from rodent studies that NOS activity (101) and nitric oxide production (5) are increased during exercise. There is equivocal evidence regarding the involvement of NOS in contraction-stimulated glucose transport in skeletal muscle of rats, with some studies indicating no effect (37, 54, 107) and other studies showing a reduction (6, 102, 121) in muscles treated with NOS inhibitors. Inhibition of NOS leads to reduced glucose uptake without affecting total blood flow across the working limb of humans in vivo (13, 67). This does not rule out an effect of NOS inhibition on glucose uptake, as the inhibitor used in these studies probably affects endothelial NOS, and nitric oxide is believed to be involved in increasing nutritive microvascular flow with exercise/contraction (95). As changes in nutritive flow can occur in the absence of changes in total flow (129), the effect of NOS inhibition on glucose uptake in humans might be explained by regulation of nutritive flow rather than membrane transport, although this remains to be established.

**Signaling related to metabolic status of muscle**

Early studies observed an inverse relationship between the phosphocreatine concentration and glucose uptake by contracting muscle (63, 132), suggesting a role for signals that sense metabolic stress in contraction-stimulated glucose transport. This may be regarded as feedback regulation of glucose transport. In an attempt to address this issue, Ihlemann et al. (59, 60) employed a model in which the force development was manipulated while the stimulation frequency and muscle fiber recruitment were kept constant to alter metabolic stress, presumably without disturbing Ca²⁺ signaling. There was a positive relationship between force development and skeletal muscle glucose uptake in both slow-twitch and fast-twitch muscles (59, 60). Thus an energy-sensing signaling system is likely to be involved in stimulating glucose transport with contractions. Indeed, 5′-AMP-activated protein kinase (AMPK) may be the enzyme that fulfills this role. AMPK is a multifunctional serine/threonine protein kinase that acts as an
important sensor of cellular energy charge, as reflected by the ratios of AMP/ATP and creatine/ phosphocreatine (49). AMPK activity is higher in skeletal muscle during exercise/contraction (21, 31, 43, 62, 83, 128, 145, 146). Furthermore, the magnitude of activation of AMPK is dependent on exercise intensity (22, 145), which may relate to the higher metabolic stress of recruited fibers, particularly in fast-twitch muscles (31, 60). Because activation of AMPK in resting muscle by the drug 5'-aminoimidazole-4-carboxamide-ribonucleoside (AICAR) increases glucose transport/uptake (9, 50, 80), it would seem logical to ascribe a role for AMPK in contraction-stimulated glucose transport. However, experiments with genetically manipulated mice with knockout of catalytic (62) or regulatory (7) AMPK subunits or overexpression of functionally inactive AMPK (44, 82) in skeletal muscles have yielded conflicting results as to whether or not AMPK is involved in contraction-stimulated glucose transport. The studies that have reduced AMPK activity only partially (7, 44, 62) have failed to demonstrate an effect of manipulation of AMPK on contraction-stimulated glucose transport despite the evidence that the knockout completely abolished the effect of AICAR on glucose transport in resting muscle (7, 62). On the other hand, in the study by Mu et al. (82) in which the muscles apparently were without any AMPK enzymatic activity, a 30–40% reduction in contraction-stimulated glucose transport in fast- and slow-twitch muscles was observed. However, later studies on these mice revealed that there were differences in force development during repeated contractions (81), a factor that could explain the lower contraction-stimulated glucose transport (59, 60). In addition, using the perfused rat hindlimb, a dissociation between AMPK activity and glucose uptake was observed in slow-twitch but not in fast-twitch muscle, pointing to a differing role of AMPK in different muscle fiber types (31). It was demonstrated (31) that AMPK activity during contractions of fast-twitch muscle was reciprocally related to muscle glycogen content, a finding that has been replicated in humans during exercise (103, 145). Thus, although AMPK is an attractive candidate for signaling in contraction-stimulated muscle glucose uptake, the available evidence pro and con at this time does not lead to a clear conclusion regarding its role. At the very least, there does not appear to be a simple “dose-response” relationship between AMPK activity and glucose transport during contractions.

Role of insulin-signaling intermediates

Insulin and muscle contractions both increase muscle glucose transport, and an obvious question to propose is whether proteins involved in signaling to insulin-stimulated glucose transport are involved in contraction signaling to glucose transport. It has been shown that exercise or muscle contraction does not increase tyrosine phosphorylation of the insulin receptor or insulin receptor substrate proteins (47, 57). With respect to phosphatidylinositol 3-kinase (PI3K), studies using incubated muscle (73, 75, 153) show that inhibition of PI3K does not influence contraction-stimulated glucose transport, a result not surprising given that skeletal muscle PI3K activity is not higher with exercise/contraction (47, 120, 136, 140, 141, 156), at least for those isoforms examined. However, in the perfused rat hindlimb, the PI3K inhibitor wortmannin was shown to inhibit contraction-induced muscle glucose uptake (140, 142). The discrepancies between ex vivo (73, 75, 153) and perfused hindlimb (140, 142) studies examining the effect of wortmannin on contraction-stimulated glucose transport may possibly be explained by factors not present in the incubated muscle preparation, such as nerve-derived agents. Indeed, stimulation of neuregulin receptors increases skeletal muscle glucose transport in a PI3K-dependent wortmannin-sensitive manner (19, 123), and given that neuregulin receptor tyrosine phosphorylation is higher with nerve-induced contractions (71), this factor may explain differences in wortmannin sensitivity on contraction-stimulated glucose transport between the two models used.

Some (111, 112, 114, 126, 127, 136) but not all (15, 78, 138, 147) studies demonstrate that skeletal muscle protein kinase B (PKB; also known as Akt) activity/phosphorylation is higher during exercise/contractions. It was recently shown that the Ser/Thr phosphorylation of the PKB/Akt substrate of 160 kDa (AS160) that is involved in GLUT4 translocation and perhaps glucose transport with insulin in adipocytes (117, 155) is increased by contractions in rodent skeletal muscle (17). However, both PKB and AS160 are unlikely to be involved in contraction-stimulated glucose uptake, as their activation/phosphorylation with contraction is inhibitable by the drug wortmannin (17, 114), whereas typically contraction-stimulated glucose transport in incubated muscle is not (see above). Indeed, a preliminary report has demonstrated no effect of PKB-β knockout on contraction-stimulated glucose uptake in soleus muscle of mice (115).

A typical PKC (αPKC) isoforms are activated by
phosphorylation and lipid (i.e., phosphatidylinositol triphosphate, phosphatidic acid) binding (36), and studies have consistently shown that aPKC activity is higher in contracting muscle during exercise (8, 20, 86, 89, 100, 106), although the mechanism for activation remains unclear (106). Given that aPKC, like PKB, is linked to insulin-stimulated glucose transport (36, 137) the increase in aPKC activity during exercise may indicate that aPKC also has a role in contraction-induced glucose uptake as well; however, there are currently no studies to directly support this notion.

Mitogen-activated protein kinase isoforms (i.e., ERK, p38, JNK) are activated by insulin and exercise/contraction (113). A link between the MAP kinases ERK and aPKC has been suggested in AICAR-stimulated glucose transport (20), and in a human study the exercise-induced increase in aPKC activity correlated with the increase in ERK activity (86). Contraction-stimulated glucose transport is partially inhibited by the drug SB203580 (119), which inhibits the α- and β-isoforms of p38 MAPK (29). However, as this drug may also directly affect glucose transport by binding and inhibition of GLUT4 activity, as inferred from experiments using measurement of glucose transport during insulin stimulation in adipocytes (96), the effect of this SB203580 on glucose transport may not be entirely attributable to inhibition of p38. Furthermore, Ho et al. (55) have recently demonstrated that p38β, the major p38 isoform in skeletal muscle, is unlikely to be involved in contraction-stimulated glucose transport. Lastly, blockade of the activation of ERK isoforms does not result in inhibition of contraction-stimulated glucose transport (51, 144).

Summary and Perspectives

The increase in skeletal muscle glucose uptake during exercise probably results from a coordinated increase in rates of glucose delivery (higher capillary perfusion), surface membrane transport, and intracellular substrate flux through glycolysis. Despite considerable research, relatively little is known about how exercise/contraction regulates skeletal muscle glucose transport (FIGURE 4). Although there is evidence linking activation of CaMK isoforms and AMPK in this process, the evidence is presently not clear cut. The signaling through CaMK isoforms is probably related to the motor nerve activity and could be regarded as a feedforward level of regulation. On the other hand, AMPK is activated when the energy status of the muscle is decreased, and hence its activation can be regarded as a feedback level of regulation. PKC, NOS, or PKB/Akt isoforms may also be involved in regulation of contraction-induced glucose transport, but this is presently unresolved. Furthermore, there are very few defined substrates that may act downstream of these activated kinases. However, it has been hypothesized that, because insulin and contraction signaling both ultimately result in GLUT4 translocation, there may be convergent signaling intermediates (27). Indeed, aPKC activity is higher with insulin and exercise/contraction stimulation, and this signaling intermediate, as well as other yet-undefined participants, may represent the putative convergent steps toward signaling to glucose transport. Knowledge of these signaling mechanisms is important, as exercise can increase skeletal muscle glucose uptake and GLUT4 translocation normally in individuals with type II diabetes. Thus the exercise-stimulated molecular mechanism resulting in increased muscle glucose transport may be important as an alternative pathway to increase glucose disposal in skeletal muscle in states of insulin resistance. In fact, drugs such as metformin and rosiglitazone that can activate skeletal muscle AMPK (39, 84) are widely used to treat type II diabetes.

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