I. INTRODUCTION

More than 120 years ago, contraction-induced skeletal muscle glucose uptake was observed from measurements of arteriovenous glucose differences and venous outflow in equine masseter muscle during chewing (39). The importance of glucose as a fuel for endurance exercise in humans, and the links between hypoglycemia and fatigue, were identified in applied physiology studies in the 1920s and 1930s (44, 188). In the 1950s, studies on rats and dogs confirmed that contractions increased muscle glucose uptake (92, 131). However, it was during the 1960s and 1970s that quantitative studies on muscle glucose uptake during exercise were undertaken in humans utilizing radiolabeled glucose tracers or arteriovenous glucose difference and blood flow measurements across active forearm and leg muscles (4, 5, 113, 151, 241, 272, 310, 320). A direct comparison of both methods indicated that the exercise-induced rise in glucose disposal was similar in magnitude when measured either isotopically or by catheterization (163). Largely on the basis of these studies, notably those from John Wahren and colleagues (4, 5, 151, 310), it was recognized that exercise intensity and duration were the primary determinants of muscle glucose uptake during exercise and that blood glucose could account for up to 40% of oxidative metabolism during exercise, when exercise is prolonged and muscle glycogen is depleted (4, 52, 310). Finally, the identification of the insulin- and contraction-regulated glucose transporter isoform GLUT4 (25, 38, 137) paved the way for enhanced understanding of the molecular bases of sarcolemmal glucose transport and muscle glucose uptake during exercise.

II. CONTROL OF SKELETAL MUSCLE GLUCOSE UPTAKE DURING EXERCISE

Glucose uptake by contracting skeletal muscle occurs by facilitated diffusion, dependent on the presence of GLUT4 in the surface membrane and an inward diffusion gradient for glucose. There are three main sites/processes that can be regulated: 1) glucose delivery, 2) glucose transport, and 3) glucose metabolism. Under resting conditions, it is generally believed that glucose transport is the rate-limiting step for muscle glucose uptake, since GLUT1 expression is relatively low and the vast majority of muscle GLUT4 resides within intracellular storage sites, excluded...
from the sarcolemma and T-tubules. With exercise, skeletal muscle hyperemia, capillary recruitment, and GLUT4 translocation to the sarcolemma and T-tubules effectively remove delivery and transport as major barriers to glucose uptake, with glucose phosphorylation becoming potentially limiting, especially at high exercise intensities (156, 314). Studies from Wasserman and colleagues, using isotopic glucose analogs and the principles of countertransport (104), as well as transgenic manipulation of muscle GLUT4 and hexokinase II (HKII) expression in mice (76, 77, 79), have provided support for this hypothesis.

A. Glucose Delivery

Skeletal muscle blood flow can increase up to 20-fold from rest to intense, dynamic exercise (7). Since glucose uptake is the product of blood flow and the arteriovenous glucose difference, this increase in blood flow is quantitatively the larger contributor to the exercise-induced increase in muscle glucose uptake since the arteriovenous glucose difference only increases two- to fourfold during exercise (261). In addition to the large increase in bulk flow to contracting skeletal muscle during exercise, there is also recruitment of capillaries which increases the available surface area for glucose delivery and exchange. Ultrasound imaging techniques have been used to characterize exercise-induced increases in microvascular blood volume, an index of muscle capillary recruitment, in rats and humans (56, 133, 281, 307). Although the extraction of glucose across a working muscle in vivo under most conditions is relatively low (2–8%), an increase in tissue glucose uptake has the potential to decrease interstitial glucose concentrations; however, the increase in glucose delivery and rapid transfer of glucose from the capillaries to the interstitium through the endothelial pores ensures that interstitial glucose levels are well maintained during exercise of increasing intensity (193).

Studies in the perfused rat hindlimb have demonstrated the importance of increases in perfusion for the contraction-induced increases in muscle glucose uptake (118, 277, 278). The increase in both glucose and insulin delivery, secondary to increased perfusion, contributes to enhanced muscle glucose uptake. Indeed, it has been estimated that this accounts for ~30% of the total exercise-induced increase in limb glucose uptake in dogs (344). Although plasma insulin levels decline during exercise, the increase in skeletal muscle blood flow may increase, or at least maintain, insulin delivery to contracting skeletal muscle. Muscle contractions and insulin activate muscle glucose transport by different molecular mechanisms (93, 97, 184, 190, 233, 312), and contractions, flow, and insulin have synergistic effects on glucose uptake in perfused, contracting rat muscle (118) and exercising humans (57, 315). In the former at least, the interaction between insulin and contractions appears to be critically dependent on adenosine receptors (305).

The arterial glucose level is the other important determinant of muscle glucose uptake during exercise. Because glucose uptake across an exercising limb follows saturation kinetics with a $K_m$ found to be around 5 mM in dog muscle (343).
and 10 mM during knee-extension exercise in humans (247), changes in plasma glucose concentration within the physiological range translate almost directly into proportional changes in leg glucose uptake. With prolonged exercise, as the liver becomes depleted of glycogen and glucose-neogenesis is unable to fully compensate, liver glucose output is reduced and hypoglycemia can limit muscle glucose uptake (4, 68). In contrast, increasing arterial glucose availability, by ingestion of carbohydrate-containing beverages, results in increased muscle glucose uptake and oxidation during prolonged exercise (3, 149, 196). The increase in glucose diffusion gradient, as well as a potential glucose-induced GLUT4 translocation (86), drives this increase in muscle glucose uptake; however, since metabolic clearance rate (MCR = glucose Rd/glucose) is also higher during exercise following carbohydrate ingestion, relatively higher plasma insulin (196) and lower plasma nonesterified fatty acids (110) could also contribute to the higher muscle glucose uptake.

B. Glucose Transport

Although it has been known for many years that muscle glucose transport was carrier mediated, it is only relatively recently that the specific transport protein responsible for insulin- and contraction-stimulated glucose transport in skeletal muscle was identified (25, 38, 138). GLUT4 (Gene: SLC2A4) is one of 13 facilitative glucose transport proteins encoded in the genome and is expressed most abundantly in adipose tissue and cardiac and skeletal muscle. It comprises 12 transmembrane domains, and characteristic sequences in both its COOH- and NH2-terminal domains are important determinants of its intracellular localization and trafficking (129). The increase in muscle glucose transport during exercise is primarily due to translocation of GLUT4 from intracellular sites to the sarcolemma and T-tubules, although it is possible that changes in intrinsic activity may also occur. The mechanisms responsible for increased glucose transport during exercise will be discussed in more detail in the next section. The fundamental importance of GLUT4 for muscle glucose uptake during electrical stimulation has been provided in GLUT4 knockout (KO) mice in which muscle contractions have negligible effect on glucose uptake (266, 345). Furthermore, during in vivo exercise, muscle glucose uptake is markedly reduced along with exercise tolerance in mice with muscle-specific GLUT4 deletion (78), although there does seem to be reserve capacity in GLUT4, since partial (~50%) knockout of GLUT4 did not affect skeletal muscle glucose uptake during exercise in mice (77).

In rodent skeletal muscle, there is a direct relationship between muscle GLUT4 content and glucose transport during intense electrical stimulation of selected limb skeletal muscles (116). Somewhat paradoxically, an inverse relationship was observed between skeletal muscle GLUT4 expression and tracer-determined glucose disposal during submaximal exercise in humans (197). Since higher GLUT4 levels are generally associated with a higher muscle oxidative capacity, this may reflect the possibility that those subjects with the lower rates of glucose disposal (and higher GLUT4 levels) were relatively fitter than the other subjects. It is known that endurance training reduces muscle glucose uptake during exercise (49, 250), an adaptation that is associated with reduced sarcolemmal glucose transport and GLUT4 translocation (250), at least during exercise at the same absolute power output. When exercise is performed at the same relative intensity, differences between untrained and trained subjects/limbs are smaller or nonexistent (22, 50, 72, 175). In fact, during dynamic knee extension exercise at peak power output, glucose uptake was higher in the trained, compared with the untrained, limb as were GLUT4 expression and oxidative capacity (175). Thus it appears that the skeletal muscle GLUT4 level after all does correlate with the capacity for glucose uptake during very intense exercise, a finding consistent with the relationship between muscle GLUT4 content and glucose transport during intense electrical stimulation of rat skeletal muscles (116) and the relationship between GLUT4 expression and insulin action in skeletal muscle. In this regard, increased skeletal muscle GLUT4 expression would also facilitate postexercise glucose uptake and glycogen storage (100, 198).

C. Glucose Metabolism

Once glucose has been transported across the sarcolemma, it is phosphorylated to glucose 6-phosphate (G-6-P) in a reaction catalyzed by HKII. This is the first step in the metabolism of glucose via either the glycolytic and oxidative pathways responsible for energy generation during exercise or conversion to glycogen in the postexercise period. Glucose phosphorylation is another site of regulation and a potential barrier to glucose uptake and utilization. During maximal dynamic exercise, increases in intramuscular glucose concentration suggest hexokinase inhibition and a limitation to glucose phosphorylation and utilization, in association with elevated intramuscular G-6-P concentration, secondary to increased rates of muscle glycogenolysis (156). Similarly, during the early stages of exercise, G-6-P-mediated inhibition of hexokinase appears to limit glucose uptake and utilization (156). As exercise continues, there is an increase in glucose uptake and a decrease in intramuscular glucose concentration as the hexokinase inhibition is relieved by a lower G-6-P concentration (156). Such a mechanism contributes to the explanation for the temporal relationship between the decrease in muscle glycogen and the progressive increase in glucose uptake during moderate intensity exercise (112). That said, the progressive increase in sarcolemmal GLUT4 is also likely to contribute to this increase in glucose uptake during exercise (177). Increasing preexercise muscle glycogen levels, resulting in greater glycogenolysis during subsequent contractions, is associated
with reduced rat muscle glucose uptake (117, 249), most likely via effects on glucose utilization mediated by increased G-6-P concentration. However, since GLUT4 translocation during contractions is also affected by muscle glycogen availability (60, 157), the changes in muscle glucose uptake may also be mediated by reduced sarcolemmal glucose transport. It has been more difficult to demonstrate a direct relationship between muscle glycogen and glucose uptake in human skeletal muscle, since alterations in substrate (glucose and NEFA) and hormone levels, secondary to the exercise and dietary regimens used to manipulate muscle glycogen availability, may confound the results obtained (111, 287, 328). However, when substrate and hormone levels are constant, decreased muscle glycogen prior to exercise is associated with increased glucose uptake during exercise (287).

Epinephrine infusion has been shown to reduce muscle glucose uptake during exercise (139, 318). A widely held view is that this is due to inhibition of glucose phosphorylation by elevated G-6-P concentration secondary to greater flux through glycogenolysis (318). However, epinephrine infusion during exercise that commenced with relatively lower muscle glycogen levels resulted in a similar reduction in glucose uptake and no change in muscle G-6-P concentration, suggesting that the effects of epinephrine on muscle glucose uptake may also be partly mediated via effects on sarcolemmal glucose transport (317). It is also possible that epinephrine has a negative effect on the intrinsic activity of GLUT4 (28).

Using radioisotopically labeled glucose analogs and transgenic approaches (GLUT4 and/or HKII overexpression or deletion), Wasserman and colleagues have suggested that glucose phosphorylation is the rate-limiting step for skeletal muscle glucose uptake during exercise (76, 77, 79, 104). The results of some of the transgenic studies are summarized in Figure 3. GLUT4 overexpression, in the absence of HKII overexpression, had little effect on muscle glucose uptake during exercise. Equally, the full effect of HKII overexpression, had little effect on muscle glucose uptake independently of HKII expression. During exercise, HKII overexpression leads to increased glucose uptake at normal and increased levels of GLUT4 expression. Furthermore, GLUT4 overexpression does not in itself lead to increased glucose uptake during exercise. The figure shows that at rest, overexpression of GLUT4 leads to increased glucose uptake independently of HKII expression. During exercise, HKII overexpression leads to increased glucose uptake at normal and increased levels of GLUT4 expression. Furthermore, GLUT4 overexpression does not in itself lead to increased glucose uptake during exercise. On the abscissa, 1 arbitrary unit denotes the average WT level (n = 8–11 per data point). [From Wasserman (316).]

III. EXERCISE-INDUCED GLUT4 TRANSLOCATION

An increase in sarcolemmal and T-tubular glucose transport is fundamental for the contraction-induced increase in skeletal muscle glucose uptake during exercise. This is due to an increase in sarcolemmal and T-tubular GLUT4 (translocation) and perhaps an activation of GLUT4 (increased GLUT4 intrinsic activity). There has been ongoing debate on whether GLUT4 intrinsic activity can be increased by stimuli such as insulin and exercise, and some studies have suggested that GLUT4 intrinsic activity can indeed be altered (8, 340). The technical challenge is that there is no direct assay of GLUT4 intrinsic activity, and any stimuli-induced changes must be inferred from accurate measurements of cell surface GLUT4 and glucose transport/uptake in the same system. Notwithstanding the possibility that GLUT4 intrinsic activity may be increased by exercise, the consensus view at present is that the increase in sarcolem-
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A. GLUT4 Vesicular Trafficking

In a resting muscle, GLUT4 is mainly retained in intracellular vesicle structures by a recycling pathway that largely keeps GLUT4 in intracellular compartments and not inserted in the surface membranes (71, 295). Ploug et al. (235) described that ~23% of intracellular GLUT4 in rat muscle is associated with large structures including multivesicular endosomes located in the trans-Golgi network region, and 77% within small tubulovesicular structures, and much of GLUT4 resides just beneath the sarcolemma (235). Studies with different labeling techniques and intravital imaging of tagged GLUT4 in living mice have shown that insulin as well as muscle contractions translocate GLUT4 to the sarcolemma as well as to the T-tubular system (59, 60, 155, 181–183, 195, 313). The content of GLUT4 in sarclemma and T-tubules is regulated by the relative efficiency of the two processes, endocytosis and exocytosis of GLUT4 containing vesicles. Insulin increases the muscle membrane GLUT4 content primarily via increased exocytosis (71, 155), although recent data also show that the endocytotic pathway is reduced by insulin in L6 myocytes (67). With regard to contraction, there are no studies in differentiated skeletal muscle, but in cardiomyocytes, contraction increases the rate of exocytosis while activation of AMPK due to metabolic stress or treatment with the AMPK activating compound AICAR decreases the rate of endocytosis both in human and rat muscle in vitro, L6 myocytes, and cardiomyocytes (9, 67, 155, 338). Since muscle contractions lead to activation of AMPK, it is likely that contractions/exercise lead to both increased exocytosis and decreased endocytosis of GLUT4. It appears that there may be two intracellular pools of GLUT4 and that one is recruited primarily by insulin and the other by contractions (47, 62, 187, 235). The contraction pool is differentiated from the insulin responsive pool by consisting of mainly transferrin receptor positive structures (187, 235). The existence of two pools of GLUT4 is perhaps one of the reasons for the finding that insulin and contraction have additive effects on glucose transport in rat muscle (51, 219, 234).

In humans, translocation of GLUT4 in skeletal muscle is rather difficult to demonstrate due to the technical and ethical limitations. However, insulin has been shown to increase GLUT4 abundance in an enriched muscle plasma membrane fraction (94, 103), and increased GLUT4 surface membrane content evaluated by surface labeling (191) has been demonstrated after insulin stimulation. Exercise has been shown to increase the sarclemma content of GLUT4 (159, 176, 177), and in addition, increased GLUT4 abundance in the sarclemma during exercise was accompanied by increased sarclemma VAMP2 abundance (176). During prolonged submaximal exercise, the increase in GLUT4 abundance in sarclemma vesicles was shown to be progressive over time (177) as is glucose uptake (5, 110, 310). This suggests that translocation of GLUT4 is critical for increasing glucose uptake in muscle during exercise in humans.

The actual docking and fusion of the GLUT4 vesicles to the surface membrane is only fragmentarily understood but seems to require complex interactions between several proteins. Much of the knowledge about mechanisms regulating docking and fusion of GLUT4 vesicles to the surface membrane comes from studies of insulin-induced GLUT4 translocation in cell culture and adipocytes, and it is tacitly assumed that the basic mechanisms during contraction-induced GLUT4 translocation in mature muscle are the same although this is likely an over simplification. The membrane events that occur during insulin-stimulated GLUT4 translocation are thought to be controlled by proteins known as SNARE proteins (soluble N-ethylmaleimide-sensitive factor-attachment protein receptors) and proteins that regulate SNAREs. Vesicle (v-) SNARES are SNARE proteins located in the GLUT4 vesicles, and target (t-) SNARES are membrane proteins that are located at the cell membrane. When v-SNARES interact with the relevant t-SNARE, a SNAREpin complex is formed in which four SNARE motifs assemble into a twisted parallel four-helical bundle (for review, see Ref. 125). It appears that this helical structure then catalyzes the fusion of vesicles with their target membrane. The specific SNARE proteins that so far have been involved in insulin-induced docking and fusion of GLUT4 vesicles are VAMP2, syntaxin 4, and SNAP23. These proteins interact with and are regulated by proteins that include munc18C, synap, and perhaps synaptotagmin (for review, see Refs. 71, 164). In addition to the SNARE proteins, the actin cytoskeleton has been shown to play an important role in GLUT4 translocation stimulated by insulin (43, 295, 303). Recent data also implicate the actin cytoskeleton in contraction-stimulated glucose uptake via the activation of the actin cytoskeleton-regulating GTPase Rac1 (292).

There is little firm evidence for the mechanisms that regulate docking and fusion of GLUT4 vesicles to the surface membrane during muscle contractions. In muscle, the following v-SNARE isoforms have been described: VAMP2 (synaptobrevin 2) (176, 237, 258, 308), VAMP3 (cellubrevin) (258, 308), VAMP5 (myobrevin) (258, 341), and VAMP7 (tetrat-997Physiol Rev  VOL 93  JULY 2013  www.prv.org

nus toxin-insensitive VAMP, TI-VAMP) (239, 258), but not VAMP1 (synaptobrevin 1) (308). It was recently described that contraction in rat skeletal muscle induced a translocation of skeletal muscle v-SNARE isoforms VAMP2, VAMP5, and VAMP7, but not VAMP3, from intracellular compartments to cell surface membranes together with GLUT4, transferrin receptor, and insulin-regulated aminopeptidase (IRAP) (258). Importantly, it was also shown that all of these v-SNARE isoforms communo-precipitate with GLUT4 from low-density membranes of muscle.
skeletal muscle. This indicates that these VAMPs associate
with intracellular GLUT4 vesicles and may participate in
the contraction-induced docking and fusion of GLUT4 to
the surface membrane. Whereas such findings do not pro-
vide conclusive evidence for the molecular mechanism in-
volved in GLUT4 translocation with muscle contraction,
they at least suggest that the VAMPs are involved in this
process.

**B. Signals to GLUT4 Trafficking**

Transport of glucose across the sarcolemma and T-tubule
membranes occurs by facilitated diffusion by the glucose
transporters GLUT1 and GLUT4. Whereas GLUT1 is ex-
pressed at a low level in mature muscle and does not trans-
locate, GLUT4 is expressed in higher amounts and translo-
cates from intracellular membrane compartments and ves-
icle structures to the plasma membrane and T-tubules as
described above.

The molecular signaling mechanisms that lead to GLUT4
translocation during muscle contraction are not well un-
derstood. It is generally believed that contractions stimulate
GLUT4 translocation via a molecular mechanisms distinct
from that of insulin (93, 97, 184, 190, 233, 312). However,
these two pathways at least partially converge in their
distal parts, and there are now a number of signaling
molecules involved in GLUT4 translocation that are ac-
tivated both by insulin and muscle contractions, e.g.,
TBC1D1 and TBC1D4 (32, 73, 84, 170, 171, 231, 306)
and Rac1 (292). Perhaps this convergence explains the
observation that the phosphatidylinositol 3-kinase (PI3K)
inhibitor wortmannin at the lowest concentration
(1 \( \mu \)M) that blocks insulin-induced PI3K activation in
perfused rat skeletal muscle, also inhibits contraction
induced glucose uptake (330).

Conceptually, the signals underlying contraction-induced
in glucose uptake have been divided into feed-forward signal-
acting directly by depolarization-induced \( \text{Ca}^{2+} \) re-
lease from the sarcoplasmic reticulum and feedback signal-
ing arising as a consequence of \( \text{Ca}^{2+} \)-activated contraction
and ion pumping and the consequent energy stress to the
muscle cell. However, this may be a simplistic view, and the
relative role of the various signaling mechanisms is unclear
at present. Our current understanding of the molecular
mechanisms that regulate exercise-induced muscle GLUT4
translocation is summarized in **FIGURE 4**.

1. \( \text{Ca}^{2+} \) activation of muscle glucose transport

The original studies were performed in frog sartorius mus-
cle incubated with caffeine. Caffeine causes release of \( \text{Ca}^{2+} \)

![FIGURE 4](https://physrev.physiology.org/)
from the sarcoplasmic reticulum, and it also causes an increase in glucose transport. These early studies showed that the increase in muscle glucose uptake during contractions does not require membrane depolarization but only release of Ca\(^{2+}\) (121, 122). Later studies in incubated rat muscle showed increased glucose uptake when incubated with concentrations of caffeine (2.5–3.0 mM) that were too low to cause muscle contractions and alterations in adenine nucleotide status (334, 336, 339). Also, incubations with the Ca\(^{2+}\)-releasing compound N-((6-aminoethyl)-5-chloro-1-naphthalenesulfonamide (W-7) at concentrations that did not cause muscle contraction increased transport of the nonmetabolizable glucose analog 3-O-methylglucose (3-O-MG) 6–8 times (339). These findings suggested that Ca\(^{2+}\) per se is sufficient to stimulate substantial increases in muscle glucose uptake. However, it was subsequently demonstrated by several groups that incubation with caffeine increased AMPK activation and nucleotide turnover in muscles from mice and rats even though no muscle contraction was apparent (64, 145, 240) presumably due to the considerable energy demand posed by sarcoplasmic reticulum Ca\(^{2+}\)-ATPase (SERCA)-dependent Ca\(^{2+}\) reuptake (223). These findings therefore raise the possibility that the effect of increase in cytosolic Ca\(^{2+}\) concentration in muscle in fact is due to the increased energy demand of ion pumping even if the muscle is not contracting. This assumption was directly experimentally confirmed by Jensen et al. (145) who showed that the ability of caffeine to increase glucose uptake in mouse soleus muscle was markedly impaired when caffeine was administered to muscles that overexpressed a dominant negative AMPK construct and therefore had very little endogenous AMPK activity. From this study it can be concluded that increase in Ca\(^{2+}\) per se is unlikely to increase muscle glucose uptake but that the effects of caffeine are due to the subsequent energy stress of the muscle which via activation of AMPK causes increased glucose uptake.

Still, in nonmuscle tissues, calcium/calmodulin-dependent protein kinase kinases (CaMKKs), particularly CaMKK\(\beta\), have been found to be able to phosphorylate AMPK on the activating Thr-172 site (114, 130), and it could therefore be hypothesized that Ca\(^{2+}\) via activation of CaMKK in muscle might be able to increase glucose uptake due to direct AMPK activation independently of energy turnover. In line with these observations, Witczak et al. (322) found that overexpressing a constitutively active CaMKK\(\alpha\) in mouse skeletal muscle increased AMPK Thr-172 phosphorylation and muscle glucose uptake, but the effect on glucose uptake was also found in muscle overexpressing a dead \(\alpha\)2AMPK and therefore likely independent of AMPK activation. Massive overexpression of a protein may lead to effects that are unphysiological; nevertheless, this experiment does not support that CaMKK affects glucose uptake via activation of AMPK. To further study the role of CaMKK in contraction-induced glucose uptake, Jensen et al. subjected muscles from CaMKK\(\alpha\) or CaMKK\(\beta\) KO mice to electrical stimulation in vitro. The data revealed no impairment of muscle AMPK Thr-172 phosphorylation or glucose uptake in either KO mouse during electrical stimulation (Jensen and Richter, unpublished observations). Taken together, the evidence indicates that in skeletal muscle CaMKK is likely not an important AMPK kinase, and it is doubtful if activation of CaMKK during muscle contractions is of any physiological importance for muscle glucose uptake.

Calcium has been thought to increase glucose uptake via activation of other calcium-sensitive downstream signaling molecules. One possibility is the family of calcium/calmodulin-dependent protein kinases (CaMK). In human skeletal muscle CaMKII and CaMKIII (also termed eukaryotic elongation factor 2 kinase (\(\varepsilon\)EF2K)) are highly expressed, as is the upstream kinase CaMKK (257, 259), whereas CaMKIV and CaMKI are not (259). In mice, CaMKI as well as the other CaMKs and CaMKII have been detected (1, 2, 146, 322). CaMKII has been implicated in contraction-induced glucose uptake because the unselective CaMKII inhibitor KN62 and KN93 have been shown to decrease contraction-induced glucose uptake in muscle (146, 333). Recently, electroporation of a specific CaMKII inhibitor into mouse tibialis anterior muscle reduced contraction-induced glucose uptake by 30% (324). However, in a preliminary report it was found that increases in Ca\(^{2+}\) concentration in muscle caused very little increase in glucose uptake when preventing energy expenditure during Ca\(^{2+}\) release in muscle by blocking the contractile response as well as the SERCA pump (144). This suggests that the increase in glucose uptake during contraction is mainly due to the energy expenditure which in turn activates energy-sensing pathways to increase glucose uptake. This again points to an indirect effect of Ca\(^{2+}\) on muscle glucose uptake.

The conventional protein kinase C isoforms are activated by Ca\(^{2+}\) and diacylglycerol, and since DAG increases in muscle during contractions (46), it is expected that the conventional PKC isoforms are activated during contractions. In rats, skeletal muscle PKC activity, as determined by translocation of PKC to a membrane fraction, was increased with muscle contractions/exercise (46, 248), although this was not found in humans (260). The reason for implicating PKC in contraction-induced glucose uptake is that chronic downregulation (45) as well as chemical inhibition (132, 143, 331) of conventional and novel PKC isoforms results in reduced contraction-stimulated glucose transport. In addition, phorbol ester activation of DAG-sensitive PKCs increased glucose transport in rat fast-twitch but not slow-twitch muscles (335). However, recently it was demonstrated that KO of the predominant conventional PKC isoform, PKC\(\alpha\), did not impair contraction-induced glucose uptake in mouse muscle (143). Taken together, the present data do not convincingly implicate
conventional PKC isoforms in regulation of contraction-induced glucose uptake.

The atypical isoforms of PKC have been shown to increase activity in human muscle during exercise (251), and since they have been implicated in insulin-stimulated glucose uptake (66), it could be envisioned that they are also involved in activating glucose transport during exercise. However, muscle specific KO of the predominant PKC lambda isoform in mouse muscle did not impair running-induced muscle glucose uptake (267), suggesting that aPKC activity is not important for exercise-induced muscle glucose uptake.

Taking all of these experimental results together, it appears that the evidence for an independent role of Ca$^{2+}$ on muscle glucose uptake is much less strong than thought only a few years ago. Rather, it appears that Ca$^{2+}$, by causing muscle contraction and activation of the SERCA pump, causes metabolic stress to the muscle cell and that this stress by activation of AMPK causes an increase in muscle glucose uptake.

2. Mitogen-activated protein kinases

The mitogen-activated protein (MAP) kinases ERK1 and ERK2, p38, and JNK are activated during muscle contractions and exercise (11, 251, 265, 268). Regarding the effect of ERK activation on glucose uptake during contractions, two studies have shown that blockade of ERK activation by inhibiting the upstream kinase MEK does not inhibit contraction-induced glucose uptake in rat muscle (115, 327). As regards JNK, this kinase has been involved in causing inflammation and insulin resistance (222, 304), and it has therefore been hypothesized that its activation during exercise/contraction might in fact inhibit glucose uptake (323). However, even though JNK1 KO mice display decreased fasting plasma glucose and insulin, ablation of JNK1 does not lead to changes in contraction-induced glucose uptake in mouse muscle (323).

p38 MAP kinase has been implicated in contraction-induced glucose uptake because the drug SB203580, which inhibits the α- and β-isoforms of p38 MAPK, decreases contraction-induced glucose uptake in rat muscle (285). However, it was subsequently shown that SB203580 directly binds to GLUT4 in adipocytes and may interfere with its activity (10, 246), and therefore, the effect of SB203580 may not be attributable to inhibition of p38 MAPK. In muscle, the main but not sole isoform of p38 MAPK is the γ-isoform, and overexpression of this isoform in mature mouse muscle via electroporation led to a trend towards decreased contraction-induced glucose uptake (120). However, the data were complicated by the fact that GLUT4 expression was decreased by overexpressing p38 MAPK. These results then if anything implicate p38 MAPK as a negative regulator of contraction-induced glucose uptake and contrast the above-mentioned data obtained with the SB203580 blocker. Interestingly, activation of p38 MAPK in resting muscle by the drug anisomysin increases glucose uptake in rat muscle (89). Taken together, however, the data at hand do not establish p38 MAPK as an important regulator of contraction-induced glucose uptake in skeletal muscle.

3. The actin cytoskeleton

The actin cytoskeleton has been implicated in intracellular traffic and the control of signal transduction and particularly signaling to GLUT4 translocation induced by insulin in various cells. In rat epitrochlearis muscle, actin has been proposed to form meshlike structures beneath the sarcolemma (31). Rearrangement of the actin cytoskeleton is necessary for insulin to induce GLUT4 translocation in L6 myotubes (140, 161, 302) and mouse gastrocnemius muscle (303), and the small Rho family GTPase Rac1 plays an important role in this respect (43, 140, 141, 161, 302, 303). In accordance, actin filament disrupting agents such as latrunculin B impair GLUT4 translocation and glucose transport stimulated by insulin in cells (296) and in incubated rat epitrochlearis muscle (31) and in mouse soleus and EDL muscle (291a). Recently, it was reported that exercise in mice and humans increases GTP loading (activation) of Rac1 in muscle, and in addition, it was shown that chemical inhibition of Rac1 as well as KO of Rac1 in muscle partly impaired contraction-induced glucose uptake in mouse muscle (292). Other parts of the cytoskeleton may also be involved in GLUT4 translocation elicited by muscle contractions. Thus Myo1c is an actin-based motor protein that has been shown to be involved in GLUT4 translocation in 3T3-L1 adipocytes. Myo1c was recently shown to be expressed in mouse muscle, and expression of a mutated form in muscle by electroporation was shown to attenuate the contraction-induced muscle glucose uptake (297). Taken together, there is increasing evidence for a role of regulation of cytoskeletal components in contraction-induced glucose uptake in muscle.

4. Nitric oxide

Nitric oxide synthase (NOS) is expressed in skeletal muscle cells, and NOS activity (253) and nitric oxide (NO) production (20) are increased in muscle during exercise/contraction. In rodents there is equivocal evidence regarding the involvement of NOS in contraction-stimulated glucose transport in skeletal muscle. Some studies find that inhibition of NOS does not decrease contraction-induced glucose uptake (65, 119, 263), while others show a decrease (20, 211, 254, 288). However, NOS inhibition only decreased contraction-induced glucose uptake in fast-twitch extensor digitorum longus (EDL) muscle and not in the more slow-twitch soleus muscle (211), indicating a fiber type specific influence of NO on glucose uptake in contracting muscle. This may be related to higher NOS expression in EDL than...
soleus muscle (211). Interestingly, in humans, inhibition of NOS by infusion of l-NMMA leads to reduced glucose uptake without affecting total blood flow across the working limb of patients with type 2 diabetes as well as in healthy subjects (29, 162). In anesthetized rats, infusion of the NOS inhibitor l-NMMA blunted the contraction-induced glucose uptake in the lower leg muscles (primarily fast-twitch muscle) without any effect on microvascular perfusion (262). Taken together, there is now substantial evidence for a role of NOS in the control of contraction-induced glucose uptake, but this effect seems to be limited to fast-twitch muscle.

5. Reactive oxygen species

Reactive oxygen species (ROS) have also been suggested to activate glucose uptake in contracting muscle. The production of ROS increases in muscle during exercise (242), and incubation of skeletal muscle with H2O2 increases glucose uptake (147). Incubation of mouse EDL muscle with the unspecific ROS scavenger N-acetylcysteine (NAC) decreased contraction-induced glucose uptake (211, 274), and since the effect was equally pronounced in wild-type and AMPK kinase dead muscles, it was concluded that the effect of NAC is independent of AMPK. However, in vitro stimulation with harsh protocols leads to rapid reduction in contraction force (211, 274), and this type of stimulation hardly reflects physiological contractions. With the use of milder stimulation in the perfused rat hindlimb, NAC was ineffective in reducing muscle glucose uptake despite positive evidence of decreased ROS production (210). Furthermore, infusion of NAC in humans did not decrease exercise-induced glucose uptake (212). Taken together, the available evidence indicates that ROS participate in regulation of muscle glucose uptake during very intense electrical stimulation in vitro but that importance of ROS during physiological exercise is unlikely.

6. Signaling related to energy charge of muscle

During muscle contraction, the energy charge of the muscle is more or less decreased depending on the intensity and duration of exercise. This leads to decreased concentration of creatine phosphate, and during intense or prolonged exercise also of ATP, while the concentrations of creatine and AMP increase (30). Such changes lead to activation of the cellular energy sensor AMP-activated protein kinase (AMPK) (108). AMPK is a heterotrimeric enzyme composed of a catalytic α-subunit and regulatory β- and γ-subunits. The α- and β-subunits each exist in two isoforms (α1; α2 and β1; β2), and the γ-subunit in three isoforms (γ1, γ2, and γ3).

Physiological activation of AMPK occurs in skeletal muscle during exercise likely in response to increased binding of AMP and ADP and decreased binding of ATP to the γ-subunit. The first observations of activation in rodent skeletal muscle were reported by Winder and Hardie (321), and activation in human muscle during exercise was shown in three independent studies published in 2000 (42, 80, 332). In human skeletal muscle, the trimeric composition of AMPK is restricted to three complexes, where the αβ2γ1 and αβ2γ2 complexes comprise ~80% of the total pool, and αβ2γ3 complexes comprise the remaining ~20% (325). Interestingly, γ3 complexes are unique to skeletal muscle (24, 325), and the αβ2γ3 complexes are predominantly activated during exercise in humans (24). Only when exercise is prolonged are αβ2γ1 complexes activated (299). α1-Containing complexes are usually only slightly or not at all activated during exercise in humans (289, 299).

AMP binding to the γ-subunit can stimulate AMPK allosterically, but this has only a moderate activating effect (~10-fold) (34). More importantly, AMP binding leads to increased AMPK phosphorylation at Thr-172 of the α-subunit which can enhance AMPK activity more than 100-fold (109). The increased phosphorylation of AMPK is apparently due to inhibition of AMPK phosphatases by both AMP and ADP (225, 273, 291). Thus the major upstream kinase of AMPK, LKB-1, is constitutively active in muscle, and in the basal state, AMPK is continuously phosphorylated and dephosphorylated in a futile cycle.

Activation of AMPK by AICAR in resting muscle results in increased glucose uptake (209), and this effect is lost when α2- or γ3-AMPK subunits are deficient (21, 152, 216). Therefore, the increase in muscle glucose uptake during exercise could be assumed to be secondary to AMPK activation during exercise. However, the influence of AMPK is not settled, because a partial deficiency of AMPK, such as occurs in germline LKB1 KO mice (152) and γ3 KO mice (21) is associated with a normal rate of glucose uptake during electrically induced muscle contractions (152). In contrast, in mice overexpressing a dominant negative α2AMPK construct in muscle, glucose uptake during electrical stimulation of muscle is impaired in most studies (1, 146, 216, 280) but not in all (81, 211). In the muscle specific LKB1 KO mice in which α2 AMPK KO (152) and γ3 KO mice (21) is associated with a normal rate of glucose uptake during electrically induced muscle contractions (152). In contrast, in mice overexpressing a dominant negative α2AMPK construct in muscle, glucose uptake during electrical stimulation of muscle is impaired in most studies (1, 146, 216, 280) but not in all (81, 211). In the muscle specific LKB1 KO mice in which α2 AMPK activation is completely blunted during electrical stimulation, glucose uptake is also severely blunted (166, 271), but this could as well be due to impaired activation of the AMPK-related kinase SNARK which has been shown to be involved in contraction-induced glucose uptake (167) as discussed below. However, the role of LKB-1 in muscle glucose uptake during exercise/contraction has been questioned in a recent report (148) as discussed below. In α1 AMPK KO mice, glucose uptake during twitch contractions was shown to be decreased compared with wild type (147), in agreement with previous studies in which a modest decrease in glucose uptake during tetanic contractions was observed in the soleus muscle of α1 AMPK KO mice (152; although this was not discussed in the paper). In a recent study in which both β-subunits of AMPK were knocked out in a muscle specific fashion, glu-
cose uptake during contractions in vitro as well as during running exercise was markedly reduced along with both α1 and α2 AMPK activity (224). This suggests that when total AMPK activity is reduced to negligible levels, muscle glucose transport during contractile activity is also markedly reduced. Still, it cannot be ruled out that the loss of β-subunits has other effects than impairing the formation of AMPK trimeric complexes and that this might also influence glucose uptake. Notably, the β1β2 dKO mice had normal GLUT4 protein expression but were more exercise intolerant than other partially AMPK-deficient mice as the AMPK kinase dead, the β2 KO mice, the α2 KO mice, and the LKB1 KO mice (224). Taken together, the available data from mice with genetic ablation of one or more AMPK subunits or overexpression of kinase dead subunits indicate that AMPK partially mediates the increase in glucose uptake during electrical stimulation of muscle. At this point, it is not entirely clear what the reason is for the decreased exercise tolerance in the various AMPK-deficient models, but decreased mitochondrial enzyme activity has been demonstrated in several of the models and the biggest decrease in running ability has so far been demonstrated in the β1β2 dKO mice which also seem to have the largest decrease in mitochondrial enzyme activity (224).

The fact that AMPK influences many transcriptional processes in muscle can complicate interpretation of results obtained in KO models, since metabolic effects could be due to chronic transcriptional effects rather than to acute AMPK deficiency. To circumvent this problem, chemical inhibitors can be used, although there always remains questions regarding their specificity. As an example, the AMPK inhibitor compound C has been shown to partially inhibit AMPK phosphorylation, TBC1D1 phosphorylation, and glucose uptake in electrically stimulated incubated rat epitrochlearsis muscle (84), suggesting that acute inhibition of AMPK decreases contraction-induced muscle glucose uptake. Still, compound C has been shown to inhibit a wide variety of kinases (19), and therefore, results obtained with this inhibitor should be interpreted with caution and its use as AMPK inhibitor has actually been discouraged (19).

Results obtained with reductionist models like electrical stimulation of incubated muscle in vitro do not necessarily reflect how glucose uptake is regulated during exercise in vivo when muscle recruitment patterns, blood flow, hormonal changes among others also influence muscle glucose uptake. As an example, in mice overexpressing a dominant negative α2 AMPK construct, glucose uptake measured in vivo during treadmill running was normal (192) despite that several groups have shown that these mice have decreased muscle glucose uptake during electrical stimulation of muscles in vitro (1, 146, 216, 280). It is noteworthy that in the exact same dominant negative α2 AMPK construct mouse model, another study in fact found decreased muscle glucose uptake during treadmill exercise compared with WT mice (185). In that study, however, it was argued that the decrease in muscle glucose uptake during exercise was due to decreased glucose delivery rather than decreased membrane transport across the sarcolemma (185). Recent results further support that exercise in vivo may elicit different results than when stimulating muscles electrically in vitro. Thus it was recently found that in LKB-1 KO mice, in which muscle glucose uptake previously was found to be decreased compared with WT controls during harsh electrical stimulation (166, 271) glucose uptake during treadmill running was similar if not higher in LKB-1 KO mice than in WT controls (148). Only when muscle from LKB-1 KO mice were stimulated with the same intense stimulation protocol as used previously was a decrease in contraction-induced glucose uptake found in LKB-1 KO muscle compared with WT (148). It might be speculated why the results on muscle glucose uptake obtained in vivo and in vitro differ. Likely the rate-limiting step in glucose uptake is different during the two conditions. In mice, there is evidence that glucose phosphorylation rather than transport can be rate limiting during treadmill running as discussed previously (76, 77, 79, 104), whereas glucose transport is likely rate limiting in vitro since overexpressing HKII did not increase muscle glucose transport in incubated muscle during stimulation with insulin (106). Exercise in vivo is a complicated process taxing both cardiovascular, metabolic, and neuroendocrine systems as well as coordination, motor control, and motivation. While exercise in vivo therefore is more difficult to evaluate, it remains the more physiological exercise type compared with electrical stimulation in vitro.

Interestingly, in the β1β2 dKO mice, muscle glucose uptake during treadmill exercise increased less than in the WT mice when exercise in the two groups was carried out at the same relative exercise intensity (224), perhaps indicating that when AMPK activity is virtually totally ablated, then muscle glucose uptake is compromised during exercise in vivo as well as during electrically induced contractions.

AMPK belongs to a family of AMPK-related kinases all of which are activated by LKB1 and several members are expressed in skeletal muscle. Of these QSK, QIK, MARK2/3, and MARK4 do not appear to be activated during electrically induced muscle contractions (269). However, SNARK/NUA1K2 is activated by muscle contractions, and it was recently shown that in mice heterozygous KO of SNARK as well as electroporation of a mutated SNARK construct in mouse muscle are accompanied by decreased contraction-induced muscle glucose uptake (167), indicating that SNARK in part mediates contraction-induced glucose uptake.

7. Downstream targets affecting glucose transport during contractions

In muscle, the proximal insulin signaling pathway is not activated during muscle contractions, except perhaps during very intense contractions where a minor and transient
increase in phosphorylation of Akt Ser-473 and activity of Akt1, -2, and -3 has been described (270). Muscle contractions also are able to increase glucose uptake normally in muscle devoid of the insulin receptor (326). However, recent developments in the downstream signaling beyond Akt have revealed converging signaling between the insulin and the contraction pathway in skeletal muscle. Such convergence points are, e.g., members of the Tre-2, BUB2, CDC16, 1 domain family (TBC1). In skeletal muscle, these members are Akt substrate of 160 kDa (AS160), which today is often referred to as TBC1D4, and its family member TBC1D1. AS160 was initially identified as a signaling molecule downstream of Akt linking insulin signaling to GLUT4 trafficking in adipocytes (154, 275). The link between TBC1D4 and D1 and GLUT4 translocation is thought to involve Rab (ras homologous from brain) proteins. Rab proteins are members of the Ras small GTPases superfamily (319). Rab GTPases can switch between a cytosolic inactive state when binding GDP to an active GTP-bound state anchored to the membrane. Rab 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 (153, 319). Since TBC1D1/4 have in vitro GAP (GTPase activating protein) activity towards a number of Rabs (82, 214, 252), it is thought that this GTPase activity is an important regulator of GLUT4 translocation.

Phosphorylation of specific TBC1D1 and TBC1D4 residues inhibits the Rab-GAP function, which then leads to GTP loading and activation of target Rabs in turn promoting GLUT4 translocation (35). The mechanistic link between TBC1D1/TBC1D4 phosphorylation and subsequent Rab protein activation seems to involve interaction between TBC1D1/TBC1D4 phosphor motifs and 14-3-3 proteins, the latter sequestering TBC1D1 and D4 and thereby relieving the Rab proteins from the GTPase activity of TBC1D1 and -4 (40, 41, 90, 238).

Rab2A, Rab8A, Rab10, Rab11, and Rab14 were detected in immunopurified GLUT4 vesicles isolated from adipocytes (180, 214) and have been shown to be in vitro substrates for both TBC1D4 and TBC1D1 (214, 252). They can thus be expected to play a role in GLUT4 translocation stimulated by insulin (134, 136) at least in adipocytes. Evidence for the importance of three of these Rabs was provided by Ishikura et al. (134), who demonstrated that the defect in GLUT4 translocation caused by mutating TBC1D4 on four phosphorylation sites (the 4P mutation) could be reversed by overexpressing Rabs 8A and 14 in L6 myotubes. Furthermore, expression of constitutive active AS160 lowered GLUT4 at the surface membrane in L6 myotubes, and this effect could be counteracted by overexpressing Rab13 and 8A (290).

As regards Rabs downstream of TBC1D1, there is evidence from knockdown experiments in myotubes that Rab 8A and Rab14 are downstream targets for TBC1D1 (135), but it is currently unsettled which specific Rabs may be downstream targets for TBC1D1 in mature muscle during contractions. Interestingly, Rab4, which was not detected in GLUT4 vesicles from adipocytes, has been detected in GLUT4 containing vesicles from mature rat muscle (279). The various Rabs may play different roles in different tissues, and it is likely that some Rabs have specific roles during insulin-induced but not contraction-induced GLUT4 translocation and vice versa. Furthermore, expression of TBC1D1 and TBC1D4 varies between tissues and also between muscle fiber types (293). As discussed above, muscle contraction most likely involves decreased endocytosis in combination with increased exocytosis of GLUT4, and therefore other Rabs than those that can be immunoprecipitated with GLUT4 (and therefore reside in the same storage vesicles as GLUT4) may be involved in GLUT4 trafficking. This could include Rab5 which has been shown to be involved in insulin-stimulated decreased rate of GLUT4 internalization in 3T3-L1 adipocytes (128). However, if Rab5 is involved in GLUT4 translocation in muscle during contractions is not known.

Some data from skeletal muscle suggest a role of TBC1D4 and TBC1D1 in contraction-induced glucose uptake. Several important features are shared by TBC1D4 and TBC1D1. These include a calmodulin-binding domain (CBD) and two phosphotyrosine-binding domains (PTB). Apparently, the Rab-GAP function of both TBC1D1 and TBC1D4 may be involved in regulation of GLUT4 translocation and glucose uptake in response to both contractions and insulin (6, 172, 306), although the involvement in contraction-induced glucose uptake is equivocal as discussed below. However, TBC1D1 and TBC1D4 also display several differences such as the expression pattern in various tissues and species (36, 293, 300). Second, TBC1D4 and TBC1D1 have different phosphorylation sites that are targeted by different kinases (40, 90, 231, 300) allowing for different regulation of the two paralog proteins. It should be realized that the two proteins have similar size and because they are both recognized by the phospho Akt substrate (PAS) antibody, interpreting data generated with the PAS antibody may lead to confusion about which protein is in fact measured if TBC1D1 and TBC1D4 are not immunoprecipitated before western blotting with the PAS antibody. In particular, such confusion can arise when blotting glycolytic (EDL) and more oxidative (soleus) mouse muscle since the expression of TBC1D1 is much higher in EDL than soleus while the expression of TBC1D4 is much higher in soleus than in EDL (293). However, in the rat, there is no relationship between muscle fiber type as determined by myosin heavy chain expression and protein expression of TBC1D1 and TBC1D4 (36).
TBC1D1/4 during muscle contractions. In accordance and perhaps influences the function and importance of calcium binding protein calmodulin becomes activated during muscle contractions.

Both TBC1D4 and TBC1D1 have a CBD. As muscle contractions are elicited via increased Ca^{2+} release from the sarcoplasmic reticulum, it would be predicted that the calcium binding protein calmodulin becomes activated and perhaps influences the function and importance of TBC1D1/4 during muscle contractions. In accordance with this assumption, mutations that block calmodulin binding of the TBC1D4 CBD reduce contraction- but not insulin-induced glucose uptake in muscle of ~40% (169). A TBC1D4 mutant additionally containing a deactivating mutation in the Rab-GAP domain restored contraction-induced glucose uptake. This suggests that the CBD via deactivation of the Rab-GAP function of TBC1D4 can induce glucose uptake. Whether this is also the case for TBC1D1 remains to be established.

IV. EXERCISE AND SKELETAL MUSCLE GLUT4 EXPRESSION

In the late 1980s, GLUT4 was identified as the key glucose transporter isoform responsible for insulin- and contraction-stimulated glucose transport in skeletal muscle (25, 38, 138). Since overexpression of GLUT4 in skeletal muscle is associated with enhanced glucose disposal and insulin action (105, 243, 298, 301), there has been considerable interest in the regulation of skeletal muscle GLUT4 expression and in therapeutic strategies to increase GLUT4 expression in various metabolic disorders characterized by skeletal muscle insulin resistance. In rodent skeletal muscle, there are quite marked differences in GLUT4 expression between the various skeletal muscle fiber types, with GLUT4 expression higher in the type I oxidative fibers compared with the more glycolytic, type II fibers (36, 95, 116, 160, 168, 195, 207). These differences in GLUT4 expression are thought to reflect the differences in oxidative capacity and activity patterns between the respective fibers (207). Denervation results in reduced GLUT4 expression in muscle (27, 48, 70, 208), with a relatively greater decline in oxidative muscle. The reduction in GLUT4 expression appears to be related to the decline in both neural activity and the influence of neurotrophic factors released from the nerve (70, 208), and there is a greater decline in GLUT4 expression following denervation compared with tetrodotoxin treatment that only blocks nerve activity but does not sever the nerve from the muscle (206). Interestingly, results from cross-innervation experiments suggest that GLUT4 expression is more related to the oxidative capacity of the muscle than the twitch-velocity characteristics (150). The differences in GLUT4 expression between muscle fiber types are much smaller in humans, but there is generally a higher GLUT4 expression in type I fibers in the order of 20–35% (FIGURE 5; Refs. 53, 54, 88). That said, such differences were not observed in all muscles, with relatively little difference between fiber types observed in soleus and triceps muscles (55). This could reflect differences in habitual activity levels (55).

A. Molecular Regulation of Skeletal Muscle GLUT4 Expression

Analysis of the human GLUT4 promoter identified 2.4 kb of the 5'-flanking region that contains the necessary ele-
ments to ensure appropriate tissue-specific GLUT4 expression and regulation by alterations in hormone and substrate levels induced by fasting (189). A further series of experiments involving 5' deletions mapped the important regulatory components to 895 bp upstream from the transcription initiation site (294). Subsequent analysis identified two highly conserved areas: one furthest from the transcription initiation site was termed domain 1 and did not appear to possess a binding site for any known transcription factors. The second, nearer to the transcription initiation site, contained a binding site for the myocyte enhancer factor 2 (MEF2) family of transcription factors, termed the MEF2 domain, that was necessary, but not sufficient, for full GLUT4 expression (294). With the use of specific antibodies and electrophoretic mobility shift assays, it was demonstrated that the MEF2A and MEF2D isoforms bind to this GLUT4-MEF2 domain (294) and that the MEF2A-MEF2D heterodimer is involved in the hormonal regulation of the GLUT4 gene (215). In relation to domain 1, a novel binding protein was identified and termed GLUT4 enhancer factor (228). In human tissues, there is a somewhat restricted pattern of glucose enhancer factor (GEF) expression that only overlaps with MEF2A in tissues with high GLUT4 expression (165). Furthermore, in cell culture experiments, whereas GEF and MEF2A alone did not activate GLUT4 promoter activity, their coexpression enhanced GLUT4 promoter activity four- to fivefold (165). Collectively, these various studies indicate that both domain 1 and the MEF2 domain, and their associated binding factors, are necessary for full GLUT4 expression in skeletal muscle. Interestingly, the decreased GLUT4 expression following denervation appears to be mediated by factors other than GEF and MEF2 (142). There are indeed other factors that interact with MEF2 in influencing skeletal muscle GLUT4 expression. Santalucia et al. (276) demonstrated that MyoD and thyroid receptor-α function cooperatively with MEF2 to modulate GLUT4 expression in L6E9 cells. In addition, the Krüppel-like factor KLF15 acts in synergy with MEF2A to activate the GLUT4 promoter and, based on coimmunoprecipitation analyses, specifically interacts with MEF2A (98). The transcriptional coactivator peroxisome proliferator-activated receptor (PPAR) γ coactivator 1α (PGC-1α) has a key role in the regulation of mitochondrial biogenesis, but has also been shown to control GLUT4 expression in myocytes by binding and activating MEF2C (213). Of note, skeletal muscle overexpression of nuclear respiratory factor 1 (NRF-1), a downstream target of PGC-1α, also increases GLUT4 expression and glucose transport capacity (17). Recently, it has been shown that treatment of L6E9 and C2C12 myocytes with recombinant neuregulin, a growth factor structurally related to epidermal growth factor, increased oxidative capacity and GLUT4 levels, secondary to increased PGC-1α expression (33). Such interactions may explain the close association between skeletal muscle oxidative capacity and GLUT4 expression.

MEF2 is subject to transcriptional repression by the class II histone deacetylases (HDAC) (205). These enzymes are involved in the balance of acetylation and deacetylation of key residues on histone proteins associated with chromatin. Acetylation of these residues generally results in greater access of key factors to promoter regions and transcriptional activation. Rodent studies have demonstrated that the class IIa HDACs, comprising isoforms 4, 5, 7, and 9, play a key role in determining the muscle phenotype (236). They are regulated by phosphorylation-dependent nuclear export (205) and proteasomal degradation (236), both of which remove their repressive function. The expression of HDACs 4, 5, and 7 is lower in type I oxidative muscles (236) which may be important for the higher oxidative capacity and GLUT4 expression in these muscles. Two key upstream kinases that phosphorylate the class II HDACs are CaMK and AMPK. Caffeine treatment of C2C12 myocytes results in reduced nuclear HDAC5 abundance, hyper-acetylation of histone H3 close to the MEF2 binding site on the GLUT4 promoter, and increased MEF2A binding to the GLUT4 gene (217). HDAC5 is not normally thought to be a substrate for CaMK but acquires CaMK responsiveness via dimerization with HDAC4, a known CaMK target (18). These data elaborate the previous observation that repeated exposure of L6 myotubes to caffeine increases GLUT4 expression via activation of CaMK and the involvement of MEF2A and MEF2D (226). Similarly, it has been shown that AMPK is an HDAC5 kinase and that the effects of AICAR-induced AMPK activation on GLUT4 expression (226, 227) are mediated via phosphorylation of HDAC5 (204). AMPK-mediated GLUT4 transcription is dependent on response elements within 895 bp proximal to the human...
GLUT4 promoter (342) and involves increases in both GEF and MEF2 binding to the GLUT4 promoter (124). Another enzyme, this time a phosphatase, that can influence GLUT4 expression via its effects on MEF2 is calcineurin. Calcineurin can activate MEF2 either directly via dephosphorylation (337) or indirectly via NFAT dephosphorylation. It has been demonstrated that skeletal muscle GLUT4 levels are increased in transgenic mice overexpressing activated calcineurin (264). To examine the interaction between these various signaling pathways, Murgia et al. (218) utilized a genetic approach involving mice that expressed a kinase-dead form of AMPK, in combination with transfection of plasmids expressing specific peptide inhibitors of the CaMKII and calcineurin signaling pathways. They showed that calcineurin played the dominant role in type II tibialis anterior muscle, whereas there was redundancy in the type I soleus muscle since at least two pathways had to be inhibited to reduce GLUT4 reporter activity (218). Experiments in primary human skeletal muscle cells in culture have also demonstrated redundancy. Caffeine and AICAR individually increase GLUT4 mRNA, but in combination their effects are not additive (McGee and Hargreaves, unpublished data), suggesting that these kinases target the same residues on HDAC4/5. Finally, although less studied, the degradation of GLUT4 also influences steady-state expression levels. It has been demonstrated that GLUT4 is degraded by calpain-2 and that overexpression of calpastatin, an endogenous calpain inhibitor, increases skeletal muscle GLUT4 levels (229).

B. Exercise Effects on GLUT4 Expression

Regular exercise training results in enhanced insulin- and contraction-stimulated glucose transport capacity. A fundamental adaptation to exercise training is an increase in skeletal muscle GLUT4 levels which has been observed in humans (FIGURES 5 AND 6; Refs. 53, 58, 75, 102, 127, 173, 232) and rodents (96, 158, 221, 245, 255, 256, 282). The increase in skeletal muscle GLUT4 often occurs rapidly in response to an exercise stimulus (99, 101, 174, 179, 244). Similarly, there is a rapid decline in skeletal muscle GLUT4 with cessation of training or inactivity (FIGURE 6; Refs. 126, 199, 309). In contrast, eccentric exercise that produces muscle damage results in a transient reduction in skeletal muscle GLUT4 levels (13, 14, 178) and impaired insulin action (12, 15, 16, 178).

A single exercise bout in rats increases GLUT4 transcription (220) and polysomal-associated GLUT4 mRNA (179) and increased GLUT4 protein expression in some (179), but not all (85, 107), studies. In human skeletal muscle, a single exercise bout results in increased skeletal muscle GLUT4 mRNA immediately after exercise (173, 174, 201), and it remains elevated for several hours after exercise (173, 174), but appears to return to preexercise levels within 24 h (173). Although alterations in mRNA stability cannot be completely excluded, the increase in GLUT4 mRNA is most likely due to increased GLUT4 transcription. The increase in GLUT4 mRNA is often associated with increased GLUT4 protein expression 3–24 h after exercise (174). However, there are also studies in which no increase in GLUT4 protein expression was observed in this time period (74, 186, 287, 329). This perhaps reflects the potentially large intersubject variability in the initial GLUT4 protein response to a single exercise bout. Indeed, with repeated exercise bouts (training), although all studies demonstrate increased skeletal muscle GLUT4 expression, there is variation in individual responses (127).

The increases in skeletal muscle GLUT4 mRNA following a single bout of exercise have led to the hypothesis that training-induced increases in GLUT4 levels result from the repeated, transient increases in GLUT4 transcription (and mRNA) following single exercise bouts, that translate to an increase in steady-state GLUT4 protein expression (194). There is some empirical evidence in support of such a suggestion (173). Accordingly, this has resulted in efforts to understand the molecular regulation of the exercise-induced increase in GLUT4 transcription (mRNA). The increase in transcription of the human GLUT4 gene in response to exercise is mediated by response elements within −895 bp of the promoter (194), again implicating domain I and the MEF2 domain and the transcription factors GEF and MEF2. Exercise increases histone hyperacetylation at the MEF2 site and MEF2A binding to the GLUT4 promoter in rodent muscle (283, 284), responses that were dependent upon CaMK activation (284). In human skeletal muscle, it has been shown that a single exercise bout reduces the nuclear abundance of HDAC4 (200), HDAC5 (200, 201), and MEF2-associated HDAC5 (201), with a concomitant increase in GLUT4 mRNA (FIGURE 7) (201). In addition, there were increases in MEF2-associated PGC-1α and p38 MAPK-mediated phosphorylation of MEF2 (201) which,
together with the removal of HDAC5 repression, would have increased MEF2 transcriptional activity. The abovementioned changes were associated with nuclear translocation of the α2 subunit of AMPK (203), activation of both CaMK and AMPK (200), and increased MEF2 and GEF DNA binding (202). Just as has been shown in resting muscle (218), there is likely to be some degree of redundancy in the signaling pathways that mediate the exercise-induced increase in skeletal muscle GLUT4 expression. The exercise-induced increase in GLUT4 mRNA is preserved in transgenic mice expressing a dominant negative AMPK (123), and inhibition of calcineurin does not attenuate the exercise-induced increase in GLUT4 (87). The increase in skeletal muscle GLUT4 following exercise in humans was unaffected by adrenergic receptor blockade (101). It could be hypothesized that high-intensity exercise, by activating both calcium-dependent signaling pathways and AMPK, would increase skeletal muscle GLUT4 expression to a greater extent than lower intensity exercise. However, this was not the case with single bouts of exercise of differing intensity, but matched for total energy expenditure, which produced similar increases in GLUT4 mRNA and protein

![FIGURE 7](https://physrev.physiology.org/)  
**FIGURE 7.** Total and nuclear HDAC5 abundance, MEF2-associated HDAC5, and GLUT4 mRNA before and after 60 min of exercise at ~70% VO2 peak (n = 7). Symbols denote significant differences compared with rest. [From McGee and Hargreaves (201).]

![FIGURE 8](https://physrev.physiology.org/)  
**FIGURE 8.** Schematic of molecular signaling involved in contraction-induced GLUT4 gene activation.
levels (174). Of note is the observation that high-intensity intermittent exercise training and more traditional endurance exercise training produce similar increases in skeletal muscle GLUT4 levels (91), albeit with a much lower total energy expenditure with the former. Exercise training remains the most potent stimulus to increase skeletal muscle GLUT4 expression, an effect that contributes to improved insulin action and glucose disposal and enhanced muscle glycogen storage in the trained state (75, 100, 198). The well-described increase in skeletal muscle insulin sensitivity in the hours following a single exercise bout appears to be less dependent on GLUT4 expression given the above-mentioned variability in the GLUT4 protein response to acute exercise, the observation that increased GLUT4 translocation, rather than expression, mediates enhanced postexercise insulin action (107) and the finding that inhibition of protein synthesis did not prevent the post-exercise-induced increase in muscle insulin sensitivity (69). Our current understanding of the molecular mechanisms that regulate exercise-induced alterations in skeletal muscle GLUT4 expression is summarized in Figure 8.

V. CONCLUSIONS

Glucose is an important fuel for contracting skeletal muscle during prolonged, strenuous exercise, with muscle glucose uptake determined primarily by exercise intensity, duration, and glucose supply. During exercise, coordinated increases in skeletal muscle blood flow, capillary recruitment, GLUT4 translocation to the sarcolemma and T-tubules, and metabolism are all important for glucose uptake and oxidation. Which of these steps are limiting for glucose uptake during exercise depends on the actual exercise conditions. The translocation of GLUT4 to the sarcolemma and T-tubules is fundamental for skeletal muscle glucose uptake and involves the regulated trafficking of GLUT4 from intracellular storage sites. The actual docking and fusion of the GLUT4 vesicles to the surface membrane is not completely understood but seems to require complex interactions between SNARE and Rab proteins, Rab GTPases, and the actin cytoskeleton. Upstream signaling pathways that ultimately may lead to GLUT4 translocation may include AMPK, CaMKII, NOS, and ROS; however, their relative importance remains to be fully elucidated and there is considerable redundancy. Furthermore, the contraction and insulin signaling pathways to glucose transport are distinct in their proximal course, but several convergence points between the insulin and the contraction pathway have recently been discovered thereby perhaps explaining the additive effects of these stimuli on glucose transport and the benefits of exercise on skeletal muscle insulin action. While acute regulation of muscle glucose uptake relies on GLUT4 translocation, glucose uptake also depends on muscle GLUT4 expression which is increased following exercise. Again, AMPK and CaMKII are key signaling kinases that appear to regulate GLUT4 expression via the HDAC4/5-MEF2 axis and MEF2-GEF interactions. Nuclear export of HDAC4/5 results in histone hyperacylation on the GLUT4 promoter and increased GLUT4 transcriptional activity following exercise. Exercise training remains the most potent stimulus to increase skeletal muscle GLUT4 expression, an effect that may partly contribute to improved insulin action and glucose disposal and enhanced muscle glycogen storage following exercise training in health and disease.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

REFERENCES

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