Exercise and GLUT4

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FLORES-OPAZO, M., S.L. McGEE, and M. HARGREAVES. Exercise and GLUT4. Exerc. Sport Sci. Rev., Vol. 48, No. 3, pp. 110–118, 2020. The glucose transporter GLUT4 is critical for skeletal muscle glucose uptake in response to insulin and muscle contraction/exercise. Exercise increases GLUT4 translocation to the sarcolemma and t-tubules and, over the longer term, total GLUT4 protein content. Here, we review key aspects of GLUT4 biology in relation to exercise, with a focus on exercise-induced GLUT4 translocation, postexercise metabolism and muscle insulin sensitivity, and exercise effects on GLUT4 expression. Key Words: exercise, GLUT4, insulin sensitivity, skeletal muscle, muscle, glucose contraction and increased muscle glucose metabolism, is insufficient to increase muscle glucose uptake (4). The rapid translocation of glucose transporters to the sarcolemma and transverse tubules effectively removes membrane glucose transport as a rate-limiting step for muscle glucose uptake during exercise (2). The identification and cloning of the insulin- and contraction/exercise-responsive facilitative glucose transporter GLUT4 by multiple research teams 30 yr ago have stimulated an enormous body of research, leading to an enhanced understanding of the regulation of glucose transport and uptake in fat and muscle in response to both insulin and contraction/exercise [see (5) for review]. The critical importance of GLUT4 during exercise is emphasized by the almost complete attenuation of muscle glucose uptake during contraction/exercise when this transporter isoform is selectively deleted from skeletal muscle (6–8). Phosphorylation of glucose to glucose 6-phosphate (G6P) by hexokinase is the first step in the intracellular metabolism of glucose, and this step seems to be a key determinant of muscle glucose uptake during the early stages of exercise (2,9). The progressive increase in muscle glucose uptake during exercise is associated with a reduction in muscle G6P levels as the rate of glycogenolysis slows (9). This perspective summarizes key aspects of GLUT4 biology in relation to exercise, with a focus on exercise-induced GLUT4 translocation, postexercise metabolism and muscle insulin sensitivity, and exercise effects on GLUT4 expression.

EXERCISE-INDUCED GLUT4 TRANSLOCATION

Translocation of GLUT4 from intracellular depots to the sarcolemma and t-tubules is a fundamental event facilitating muscle glucose uptake during exercise. Early studies in rodents demonstrated increased cytochalasin-B binding in plasma membranes obtained after exercise (10–12). Because this method cannot distinguish between different glucose transporter isoforms, it required the generation of specific antibodies

KEY POINTS

- Skeletal muscle GLUT4 content correlates with glucose transport capacity in response to insulin and muscle contractions/exercise.
- GLUT4 translocation to the sarcolemma and t-tubules is a fundamental event for increased muscle glucose uptake during exercise.
- Enhanced postexercise skeletal muscle insulin sensitivity is characterized by greater insulin-induced GLUT4 translocation.
- Exercise training increases skeletal muscle GLUT4 content.

INTRODUCTION

The increased glucose uptake in insulin (fat and muscle) and contraction/exercise (muscle) responsive cells occurs by facilitated diffusion and, during exercise, is subject to physiological regulation by glucose delivery, membrane permeability to glucose, and the intracellular metabolism of glucose (1,2). The marked hyperemia and increased capillary blood flow during exercise facilitate glucose delivery to contracting skeletal muscle. Hepatic glucose output has the major role of maintaining, or even increasing, plasma glucose during exercise (2), and raising the plasma glucose concentration further by ingesting carbohydrate increases glucose uptake during exercise (3). However, an increased glucose delivery alone, in the absence of muscle

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and reagents to examine the isoform-specific effects of muscle contraction/exercise. GLUT1 is largely responsible for basal glucose transport, and there are no changes in its membrane distribution with exercise (13). In contrast, GLUT4 is absent from the sarcolemma and t-tubules under basal conditions (14,15); however, with muscle contraction/exercise, there is translocation from intracellular storage sites to the sarcolemma and t-tubules, as seen in studies using membrane fractionation and biochemical analyses (13,16,17), immunocytochemistry (14,15), exofacial photolabeling (18), and intravital imaging (19). It is possible that separate pools of GLUT4 are targeted to the sarcolemma and t-tubules (20). Exercise-induced GLUT4 translocation also has been observed in human skeletal muscle (21–25). Based on differences between measured rates of membrane glucose transport and GLUT4 content, it has been suggested that there may be an increase in GLUT4 intrinsic activity (a measure of the number of molecules of glucose transported per unit of GLUT4 protein). This may reflect methodological challenges, because other studies indicate that GLUT4 translocation can fully account for the increase in membrane glucose transport with muscle contraction (15,18). That said, whether exercise alters GLUT4 intrinsic activity remains somewhat of an open question. Insulin and contraction/exercise have distinct and additive effects on muscle glucose transport and GLUT4 translocation (18,26), which likely are due to differences in the upstream signaling pathways, separate GLUT4 kinetics and compartmentalization (27), and/or translocation of different pools of glucose transporters (14,28,29). The observation of preserved contraction/exercise-induced GLUT4 translocation in insulin-resistant states has catalyzed considerable effort in identifying the molecular mechanisms underlying this GLUT4 translocation (Fig. 1). Such understanding may lead to the development of novel therapeutic strategies to enhance muscle glucose uptake in metabolic diseases characterized by muscle insulin resistance.

It is attractive to link key intramuscular signals generated during exercise to increased GLUT4 translocation and membrane glucose transport. These signals seem to converge on the Rab GTPase-activating proteins Tre-2/BUB2/cdc 1 domain family (TBC1D) 1 and TBC1D4 [also known as Akt substrate of 160 kd (AS160)] that regulate Rab GTPases and Rab proteins involved in the trafficking of GLUT4 vesicles (5,30–32). The main candidates are increased sarcoplasmic calcium (Ca2+), secondary to sarcoplasmic reticulum (SR) Ca2+ release during excitation-contraction coupling and/or extracellular Ca2+ entry, elevated AMP-activated protein kinase (AMPK) activity in response to metabolic perturbations within contracting muscle, and mechanical stress. Other potential signals include reactive oxygen species (ROS) and nitric oxide (NO). The mechanisms regulating muscle glucose uptake during exercise have been extensively reviewed recently (33), and they are complex with considerable redundancy, perhaps not surprising given the importance of glucose homeostasis during exercise. We will briefly review some of the key observations. SR Ca2+ release during excitation-contraction coupling is a potential “feedforward” regulator of GLUT4 translocation and glucose uptake. Caffeine induces SR Ca2+ release and increases muscle glucose transport, an effect that seems to be mediated by Ca2+-calmodulin–dependent protein kinase II (CaMKII), the major CaMK isoform in skeletal muscle (34,35) and calmodulin regulation of AS160 (36). In contrast, Jensen et al. (37) reported that SR Ca2+ release was not sufficient to stimulate glucose transport in the absence of muscle contraction feedback signals and that the full contraction glucose transport response could be recapitulated with stimulation of AMPK and mechanical stress, without need for SR Ca2+ release. They could not exclude a potential role for extracellular Ca2+ entering via sarcoplasmic Ca2+ channels during muscle contraction (38).

AMPK is considered an important sensor of cellular energy status and has been implicated in many of the metabolic and molecular responses to exercise [see (39) for a review]. Despite the appeal of a link between muscle energy status, AMPK activation, and glucose transport during contraction/exercise, numerous studies over the years, including those in mice using an inactive AMPK mutant (40) and selective knockout of the upstream kinase LKB1 (41) and specific AMPK isoforms (42,43), have failed to clearly define a role for AMPK in regulating muscle glucose uptake. Recently, it has been proposed that AMPK and TBC1D1 regulate insulin-independent muscle glucose uptake after, but not during, exercise (44). In a comprehensive review of the literature, these authors further proposed that previous differences in the interpretation of the role of AMPK could be reconciled by whether glucose uptake was measured during or after exercise, with only the latter being AMPK dependent (44).

Ras-related C3 botulinum toxin substrate 1 (Rac1) is a small Rho family GTPase that is involved in regulation of the actin cytoskeleton, vesicle trafficking, and ROS production via nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. Exercise has been shown to increase Rac1 activation and phosphorylation of its downstream target p21-activated protein kinase (PAK1) in murine and human skeletal muscle (45). Rac1 inhibition and muscle-specific deletion reduced muscle glucose uptake (45) and sarcolemmal GLUT4 translocation (46) during exercise. The membrane dystroglycan glycoprotein complex associates with Rac1 during muscle contraction, thereby potentially linking mechanical stress with glucose uptake (47). Rac1 also has a role in stimulating ROS production via NADPH oxidase 2, which in turn increases GLUT4 translocation and glucose uptake during exercise (48), consistent with the previously reported role of ROS in mediating muscle glucose uptake (49,50). Inhibition of nitric oxide synthase

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**Figure 1.** Summary of potential signals mediating exercise-induced GLUT4 translocation in skeletal muscle during exercise. DGC, dystroglycan glycoprotein complex; INT, integrin; nNOS, neuronal nitric oxide synthase; NOX2, NADPH oxidase 2; GLU84 translocation in skeletal muscle during exercise.
exercise include the AMPK-related kinase SNARK (54) and mammalian target of rapamycin complex 2 (55). Of note, phosphoproteomic analysis of human (56) and rat (57) muscle samples after exercise/contraction has identified >1000 exercise-regulated phosphosites on >550 proteins, many of unknown function. Thus, there may be many other potential signaling pathways that regulate the metabolic and molecular responses to exercise, including GLUT4 translocation and muscle glucose uptake. Finally, although muscle glycogen metabolism can influence muscle glucose uptake via effects on intracellular glucose metabolism (9), glycogen availability also has been shown to modulate muscle glucose transport and GLUT4 translocation during muscle contractions (58,59). Whether this is due to effects on the abovementioned signaling pathways or direct effects on GLUT4 and its trafficking remains somewhat unresolved.

**POSTEXERCISE METABOLISM AND GLUT4**

After glycogen-lowering exercise, a key metabolic process during recovery is resynthesis of muscle glycogen, facilitated by carbohydrate ingestion and increased muscle glucose uptake. The classic study of Bergström and Hultman (60) demonstrated increased glycogen synthesis in the exercised limb with dietary carbohydrate intake over 3 d. They concluded “that exercise with glycogen depletion enhances the resynthesis of glycogen. … It could be that a stimulation of one or more of the factors directly involved in glycogen synthesis takes place or that an effect is provided on the cell membrane, stimulating glucose uptake.” In an elegant reexamination of this phenomenon utilizing contemporary techniques, it was demonstrated that the key regulators of glycogen “supercompensation” after glycogen-depleting exercise were sustained activation of AMPK and glycogen synthase and increased expression of GLUT1, GLUT4, and hexokinase II (61). During the early recovery period, there is a persistent increase in muscle glucose uptake in the absence of insulin (62), followed by a second phase of enhanced muscle insulin sensitivity (63). The exercise-induced increase in muscle insulin sensitivity was first demonstrated in rats by Richter et al. (64) and confirmed in human skeletal muscle (65). This increased muscle insulin sensitivity is due to enhanced translocation of GLUT4 to the cell surface in response to insulin (66); however, the underlying mechanisms, be they related to insulin signaling or GLUT4 trafficking processes, remain to be fully elucidated (63). Studies in human skeletal muscle have demonstrated that prior exercise increases insulin-stimulated microvascular perfusion and activation of glycogen synthase (67), but it does not alter activation of the proximal insulin signaling pathway (68). There is, however, greater activation of TBC1D4 (69), a ~2-fold increase in insulin-induced membrane permeability (70), and enhanced insulin-stimulated sarcolemmal and endosomal GLUT4 translocation (71) in human skeletal muscle. Thus, all steps in the glucose uptake process (i.e., delivery, transport, and metabolism) are affected by prior exercise. Activation of AMPK seems to be an important prerequisite of enhanced postexercise insulin sensitivity (72,73), potentially via activation of TBC1D4 (74,75). Prior exercise also increases insulin-stimulated p38 mitogen-activated protein kinase (75), which has been implicated in greater GLUT4 intrinsic activity. That said, whether exercise has any effect on GLUT4 intrinsic activity is equivocal (63). An interesting hypothesis is that any stimulus that increases cell surface GLUT4 results in greater sensitivity of GLUT4 translocation to a subsequent stimulus (76). The mechanisms have not been identified, but it may reflect effects on GLUT4 localization and trafficking.

The postexercise increase in skeletal muscle insulin sensitivity is associated with muscle glycogen depletion during exercise (77,78). It has been shown that insulin-stimulated glucose transport is influenced by muscle glycogen content (79,80), and it has been suggested that GLUT4 vesicles may directly associate with glycogen (29). However, in a recent study, we were unable to provide biochemical evidence of such an association (81), suggesting that the influence of muscle glycogen is mediated by mechanisms other than direct glycogen-GLUT4 interaction. There is a correlation between muscle GLUT4 content and postexercise glycogen resynthesis (82), and higher muscle GLUT4 expression contributes to enhanced postexercise glycogen accumulation in trained individuals (83,84). The intramuscular processes involved in enhanced postexercise insulin action and glycogen resynthesis are summarized in Figure 2.

**EXERCISE AND GLUT4 EXPRESSION**

There is a correlation between skeletal muscle GLUT4 content and glucose transport capacity in response to insulin (85–87) and contractions/exercise (85,88). Overexpression of GLUT4 in murine skeletal muscle is associated with enhanced insulin action, increased glucose transport, and greater muscle glycogen content (89–91). Skeletal muscle fiber type differences
in GLUT4 expression have been reported. In rodents, a much higher expression has been observed in the type I compared with the type II fibers (85,92–94), due to differences in oxidative capacity and activity levels between the fibers (94). In contrast, the GLUT4 levels in human skeletal muscle fibers are similar or only slightly (~20%–30%) higher in type I fibers (95,96). A key adaptation to exercise training is increased skeletal muscle GLUT expression, observed in both rodents (97–101) and humans (102–109). Importantly, increased muscle GLUT4 protein content is observed in patients with type 2 diabetes after endurance (110,111), high-intensity interval (112), and strength (113) training. In contrast, eccentric exercise results in a transient reduction in muscle GLUT4 protein levels (114,115). Skeletal muscle GLUT4 content is reduced with detraining and bed rest (106,116,117). Denervation also has been reported to reduce GLUT4 expression because of diminished neural activity and neurotrophic factors released from the nerve (118,119).

A single bout of exercise has been shown to increase GLUT4 transcription (120), mRNA (121), and polysomal-associated GLUT4 mRNA (122) in rodent skeletal muscle and GLUT4 mRNA in human skeletal muscle (123–126). Of note, exercise increases GLUT4 mRNA in skeletal muscle of patients with type 2 diabetes (127). These increases are transient, returning to pre-exercise levels within 18–24 h. Thus, the more prolonged increases in GLUT4 protein with exercise training are thought to be the result of the cumulative effects of these transient increases in GLUT4 mRNA, which in turn stimulate GLUT4 protein synthesis in the longer term (108,122). Of course, posttranscriptional regulation of GLUT4 protein synthesis, stability, or degradation is also likely to be important (128) but has been less studied. The expression of GLUT4 in skeletal muscle seems to be critically dependent on the binding of the transcriptional factors myocyte enhancer factor 2 (MEF2) and GLUT4 enhancer factor (GEF) to the GLUT4 promoter (129). In human skeletal muscle, we have shown that exercise increases the DNA binding of both MEF2 and GEF (130), an effect that may be partly mediated by AMPK activation (131). In addition, exercise reduced the association of histone deacetylase 5 (HDAC5), a protein involved in transcriptional repression, with MEF2 and increased p38 mitogen-activated protein kinase specific MEF2 phosphorylation (125). We subsequently demonstrated that AMPK is an HDAC5 kinase (132), because phosphorylation of HDAC5 mediates its dissociation from MEF2 and nuclear export. In mice, exercise increases skeletal muscle GLUT4 transcription via an AMPK-dependent inactivation of HDAC4/5 (133). CaMKII phosphorylates HDAC4 and in so doing also targets HDAC5, given the complex that forms between these two HDAC isoforms (134). In addition to the removal of HDAC inhibition, the recruitment of coactivators and histone acetyl transferases (HAT) is required for histone acetylation on key promoters. Two such coactivators are calcineurin/nuclear factor of activated T cells (NFAT) and peroxisome proliferator-activated receptor γ coactivator 1α (PGC1α). Although we did not observe any nuclear translocation of NFAT with exercise in human skeletal muscle, we did see an increase in MEF2-associated PGC1α (125). The former observation is consistent with the suggestion that calcineurin may not play a role in enhancing GLUT4 expression after exercise (135). That said, there is redundancy in the control of skeletal muscle GLUT4 expression, which allows for compensation, such as increased protein kinase D activation, if a particular kinase/signaling pathway is inactivated (136,137). As mentioned in a previous section, there are potentially many, as yet unidentified, kinases that could mediate the effects of exercise on GLUT4 expression (56). The specific HAT involved in skeletal muscle GLUT4 expression remains to be elucidated, but p300 is a potential candidate. Exercise results in histone hyperacetylation at the MEF2 site on the Glut4 gene in rats, an effect mediated via CaMKII activation (138). It also has been shown that exercise increased histone acetylation surrounding the nuclear respiratory factor 1 (NRF-1) binding sequence of the Mef2a promoter and that this was associated with increased MEF2-NRF-1 binding to this region (139), consistent with observations that both MEF2 and GLUT4 expressions are higher in NRF-1 transgenic mice (140). The NRF-1/MEF2A pathway is also regulated by cooperation between AMPK and peroxisome proliferator-activated receptor β/δ (PPARβ/δ), which enhances the exercise-induced increase in skeletal muscle GLUT4 expression (141). Thus, there are numerous pathways by which exercise can increase skeletal muscle GLUT4 expression (Fig. 3).

The other major tissue expressing GLUT4 is adipose tissue, and there has been considerable interest in this tissue given the importance of adipose tissue GLUT4 expression for whole-body glucose metabolism and insulin action (142). Adipose tissue–specific deletion of GLUT4 results in impaired insulin action (143), and patients with type 2 diabetes have lower adipose tissue GLUT4 content (144). Exercise training is associated with enhanced insulin action in adipose tissue in humans (145), an effect that could be mediated by altered GLUT4 expression, although this has not been well studied. In rodents, exercise training increases adipose tissue GLUT4 expression (146–148). In contrast, we did not observe an increase in adipose tissue GLUT4 content after a single bout of exercise or after 10 d of exercise training in healthy subjects (96). We had previously observed increased adipose tissue GLUT4 in patients...
with type 2 diabetes following 4 wk of exercise training (111), and although GLUT4 content was not measured directly, an increase was inferred from the exercise-training-induced reduction in serum retinol binding protein 4 (RBP4) levels and the inverse relationship between serum RBP4 and adipocyte GLUT4 expression (149). In addition to GEF and MEF2 regulation of adipose tissue GLUT4 expression (150), the liver X receptor α appears to have a role in GLUT4 expression (151), although to our knowledge, exercise effects on these factors have not been studied.

Finally, GLUT4 has been detected in the brain, notably in areas of the hypothalamus that are associated with glucoregulation (152,153). Brain knockout of GLUT4 results in dysregulated glucose metabolism and impaired insulin action (154). Exercise training is associated with improved glucoregulation, which could be associated with altered GLUT4 expression, although this has never been studied.

FUTURE PERSPECTIVES

GLUT4 translocation to the sarcolemma and t-tubule is a fundamental event for increased muscle glucose uptake during exercise. Further elucidation of the molecular and cellular mechanisms underlying this process could optimize exercise prescription and facilitate development of novel therapeutic strategies in metabolic diseases characterized by skeletal muscle insulin resistance. There seems to be considerable redundancy in the regulation of GLUT4 translocation during muscle contraction, with the main candidates having been Ca²⁺ and metabolic disturbances as key signals via CaMKII and AMPK. More recently, ROS have emerged as key stimuli. Another interesting point is the apparent difference in results obtained using in vitro versus in vivo rodent models, and that has obvious implications for translation to human relevance. The differential regulation of GLUT4 trafficking by insulin and muscle contractions requires further investigation. Different signaling pathways have been identified, converging on TBC1D1 and TBC1D4 and GLUT4 trafficking. Whether there are differential pools of insulin- and contraction-responsive GLUT4 vesicles, or differential stimulation of the same GLUT4 vesicle pool, still remains somewhat of an open question.

Enhanced postexercise skeletal muscle insulin sensitivity is characterized by greater insulin-induced GLUT4 translocation. Is this primarily due to improved insulin signaling to GLUT4 trafficking processes or does increased GLUT4 expression contribute in any way? Currently, the weight of evidence supports the former. Enhanced postexercise muscle insulin sensitivity has been observed in the absence of any change in muscle GLUT4 protein content (66), and cycloheximide inhibition of protein synthesis in the hours after exercise does not prevent the increase in insulin sensitivity observed in vitro (72). Whether the increase in muscle GLUT4 content that has been observed in vivo in some studies is important remains to be determined. Perhaps, it is of greater significance for the longer-term exercise- and training-induced adaptations in insulin action and muscle glycogen storage. In relation to the latter process, the relative importance of GLUT4 and glycogen synthase for enhancing postexercise muscle glycogen storage, especially in the trained state, remains worthy of ongoing investigation. There is, of course, a close link between the two given the important role of G6P in the allosteric activation of glycogen synthase.

Exercise training increases skeletal muscle GLUT4 expression even though GLUT4 deletion studies suggest that GLUT4 expression in the untrained state may be sufficient for exercise-induced muscle glucose uptake. Is the increase in GLUT4 essential for the increase in muscle glucose transport capacity with exercise training and does this provide a “safety factor” that better preserves metabolic homeostasis in response to exercise in the trained state? Are there differential effects of exercise training on the expression and localization of GLUT4 vesicles and/or different pools of GLUT4 vesicles?

Adipose tissue GLUT4 abundance influences adipokine secretion and whole-body glucose metabolism and insulin action. This may be particularly relevant in obesity and type 2 diabetes, conditions in which adipose tissue GLUT4 expression is lower than that in age-matched, healthy subjects. Exercise training has been shown to increase adipose tissue GLUT4 content in type 2 diabetes, but whether it increases adipose tissue GLUT4 expression in healthy humans and contributes to improved whole-body insulin action remains an open question. Exercise effects on other tissues expressing GLUT4 (e.g., hypothalamus) are currently not known but worthy of investigation.

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