Exercise and GLUT4

Marcelo Flores-Opazo[1], Sean L. McGee[2], and Mark Hargreaves[3]

[1]Laboratory of Exercise and Physical Activity Sciences, Department of Physiotherapy, University Finis Terrae, Santiago, Chile; [2]Metabolic Research Unit, School of Medicine and Institute for Mental and Physical Health and Clinical Translation (IMPACT), Deakin University, Waurn Ponds; and [3]Department of Physiology, The University of Melbourne, Melbourne, Australia

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-tubule 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

Downloaded from https://journals.lww.com/acsm-essr by BhDMf5ePHKav1zEoum1tQfN4a+kJLhEZgbsIHo4XMi0hCywCX1AWnYQp/IlQrHD3i3D0OdRyi7TvSFl4Cf3VC4/OAVpDDa8K2+Ya6H515kE= on 07/14/2020

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-tubule 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 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

Address for correspondence: Professor Mark Hargreaves, Department of Physiology, The University of Melbourne, Grattan St., Melbourne, VIC, 3010, Australia (E-mail: m.hargreaves@unimelb.edu.au).

Accepted for publication: April 20, 2020.

Associate Editor: Sandra K. Hunter, Ph.D., F.A.C.S.M.

0091-6331/4803/110–118
Exercise and Sport Sciences Reviews
DOI: 10.1249/JES.0000000000000224
Copyright © 2020 by the American College of Sports Medicine

Copyright © 2020 by the American College of Sports Medicine. Unauthorized reproduction of this article is prohibited.

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

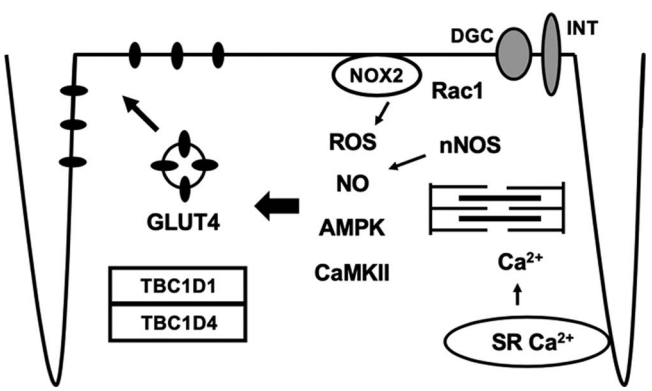

Figure 1. Summary of potential signals mediating exercise-induced GLUT4 translocation in skeletal muscle during exercise. DGC, dystroglycan glycoprotein complex; INT, integrin; nNOS, neuronal nitric oxide synthase; NOX2, NADPH oxidase 2.

main candidates are increased sarcoplasmic calcium (Ca^{2+}), secondary to sarcoplasmic reticulum (SR) Ca^{2+} release during excitation-contraction coupling and/or extracellular Ca^{2+} 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 Ca^{2+} release during excitation-contraction coupling is a potential "feedforward" regulator of GLUT4 translocation and glucose uptake. Caffeine induces SR Ca^{2+} release and increases muscle glucose transport, an effect that seems to be mediated by Ca^{2+}-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 Ca^{2+} 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 Ca^{2+} release. They could not exclude a potential role for extracellular Ca^{2+} entering via sarcolemmal Ca^{2+} 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

Copyright © 2020 by the American College of Sports Medicine. Unauthorized reproduction of this article is prohibited.

(NOS), thereby reducing NO production, results in reduced glucose uptake during exercise in humans (51) and during muscle contractions in rats (52), without any apparent effect on limb blood flow or capillary blood flow, suggesting an effect on intramuscular mechanisms, potentially GLUT4 translocation. Inhibition of NOS has been shown to reduce sarcolemmal GLUT4 levels and muscle glucose transport during contractions (53). Interestingly, there is a potential link between Rac1 and activation of NOS/NO, although this has not been studied in skeletal muscle (47). Other kinases that have been implicated in the regulation of muscle glucose uptake during 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.

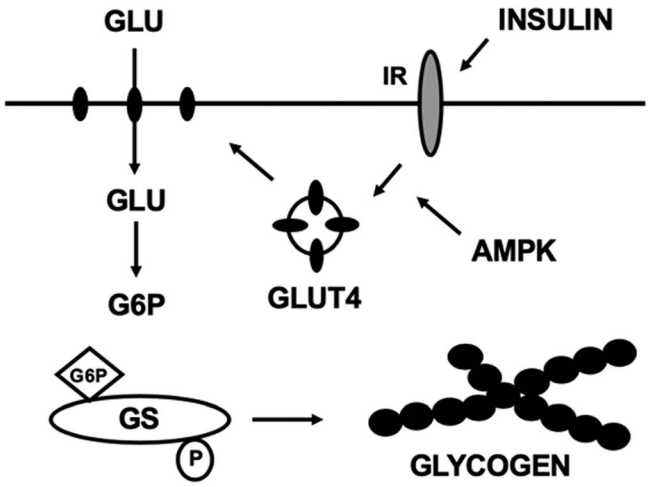

Figure 2. Intramuscular processes involved in postexercise insulin action and glycogen resynthesis. AMPK, AMP-activated protein kinase, which increases postexercise insulin sensitivity; GLU, glucose; GS, glycogen synthase, subject to allosteric regulation by G6P and covalent regulation via phosphorylation/dephosphorylation; IR, insulin receptor.

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

Copyright © 2020 by the American College of Sports Medicine. Unauthorized reproduction of this article is prohibited.

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), 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

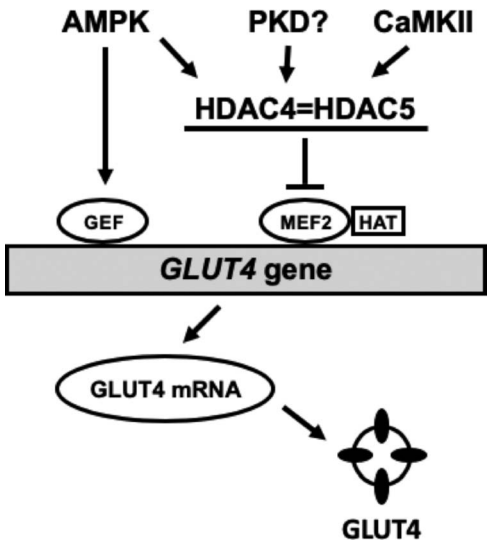

Figure 3. Summary of potential molecular mechanisms mediating exercise-induced GLUT4 transcription and expression in skeletal muscle. PKD, protein kinase D.

Copyright © 2020 by the American College of Sports Medicine. Unauthorized reproduction of this article is prohibited.

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 Ca^{2+} 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 different 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.

Acknowledgments

We acknowledge the many researchers who have worked on exercise and GLUT4 and apologize that we have not been able to cite all published work. The original work of the authors was supported by the Diabetes Australia Research Program and the National Health and Medical Research Council of Australia. Marcelo Flores-Opazo was supported by the Becas Chile scholarship program of CONYCIT, Chile.

References

1. Rose AJ, Richter EA. Skeletal muscle glucose uptake during exercise: how is it regulated? *Physiology (Bethesda)*. 2005; 20:260–70.
2. Wasserman DH, Kang L, Ayala JE, Fueger PT, Lee-Young RS. The physiological regulation of glucose flux into muscle *in vivo*. *J. Exp. Biol.* 2011; 214:254–62.
3. McConell G, Fabris S, Proietto J, Hargreaves M. Effect of carbohydrate ingestion on glucose kinetics during exercise. *J. Appl. Physiol.* 1994; 77:1537–41.
4. Zinker BA, Lacy DB, Bracy DP, Wasserman DH. Role of glucose and insulin loads to the exercising limb in increasing glucose uptake and metabolism. *J. Appl. Physiol.* 1993; 74:2915–21.
5. Klip A, McGraw TE, James DE. Thirty sweet years of GLUT4. *J. Biol. Chem.* 2019; 294:11369–81.
6. Fueger PT, Li CY, Ayala JE, et al. Glucose kinetics and exercise tolerance in mice lacking the GLUT4 glucose transporter. *J. Physiol.* 2007; 582:801–12.
7. Howlett KF, Andrikopoulos S, Proietto J, Hargreaves M. Exercise-induced muscle glucose uptake in mice with graded, muscle-specific GLUT-4 deletion. *Physiol. Rep.* 2013; 1:e00065.
8. Zisman A, Peroni OD, Abel ED, et al. Targeted disruption of the glucose transporter 4 selectively in muscle causes insulin resistance and glucose intolerance. *Nat. Med.* 2000; 6:924–8.
9. Katz A, Sahlin K, Broberg S. Regulation of glucose utilization in human skeletal muscle during moderate dynamic exercise. *Am. J. Physiol.* 1991; 260:E411–5.
10. Hirshman MF, Wallberg-Henriksson H, Wardzala LJ, Horton ED, Horton ES. Acute exercise increases the number of plasma membrane glucose transporters in rat skeletal muscle. *FEBS Lett.* 1988; 238:235–9.
11. Douen AG, Ramlal T, Klip A, Young DA, Cartee GD, Holloszy JO. Exercise-induced increase in glucose transporters in plasma membranes of rat skeletal muscle. *Endocrinology*. 1989; 124:449–54.

Copyright © 2020 by the American College of Sports Medicine. Unauthorized reproduction of this article is prohibited.

12. Fushiki T, Wells JA, Tapscott EB, Dohm GL. Changes in glucose transporters in muscle in response to exercise. *Am. J. Physiol.* 1989; 256: E580–7.
13. Goodyear LJ, Hirshman MF, Horton ES. Exercise-induced translocation of skeletal muscle glucose transporters. *Am. J. Physiol.* 1991; 261:E795–9.
14. Ploug T, van Deurs B, Ai H, Cushman SW, Ralston E. Analysis of GLUT4 distribution in whole skeletal muscle fibers: identification of distinct storage compartments that are recruited by insulin and muscle contractions. *J. Cell Biol.* 1998; 142:1429–46.
15. Rodnick KJ, Slot JW, Studelska DR, et al. Immunocytochemical and biochemical studies of GLUT4 in rat skeletal muscle. *J. Biol. Chem.* 1992; 267:6278–85.
16. Roy D, Marette A. Exercise induces the translocation of GLUT4 to transverse tubules from an intracellular pool in rat skeletal muscle. *Biochem. Biophys. Res. Commun.* 1996; 223:147–52.
17. Roy D, Jöhannsson E, Bonen A, Marette A. Electrical stimulation induces fiber type-specific translocation of GLUT-4 to T tubules in skeletal muscle. *Am. J. Physiol.* 1997; 273:E688–94.
18. Lund S, Holman GD, Schmitz O, Pedersen O. Contraction stimulates translocation of glucose transporter GLUT4 in skeletal muscle through a mechanism distinct from that of insulin. *Proc. Natl. Acad. Sci. U S A.* 1995; 92:5817–21.
19. Lauritzen HP, Galbo H, Toyoda T, Goodyear LJ. Kinetics of contraction-induced GLUT4 translocation in skeletal muscle fibers from living mice. *Diabetes.* 2010; 59:2134–44.
20. Lemieux K, Han XX, Dombrowski L, Bonen A, Marette A. The transferrin receptor defines two distinct contraction-responsive GLUT4 vesicle populations in skeletal muscle. *Diabetes.* 2000; 49:183–9.
21. Kristiansen S, Hargreaves M, Richter EA. Exercise-induced increase in glucose transport, GLUT-4 and VAMP-2 in plasma membrane from human muscle. *Am. J. Physiol.* 1996; 270:E197–201.
22. Kristiansen S, Hargreaves M, Richter EA. Progressive increase in glucose transport and GLUT4 in human sarcolemmal vesicles during moderate exercise. *Am. J. Physiol.* 1997; 272:E385–9.
23. Thorell A, Hirshman MF, Nygren J, et al. Exercise and insulin cause GLUT-4 translocation in human skeletal muscle. *Am. J. Physiol.* 1999; 277:E733–41.
24. Kennedy JW, Hirshman MF, Gervino EV, et al. Acute exercise induces GLUT4 translocation in skeletal muscle of normal human subjects and subjects with type 2 diabetes. *Diabetes.* 1999; 48:1192–7.
25. Bradley H, Shaw CS, Bendtsen C, et al. Visualization and quantitation of GLUT4 translocation in human skeletal muscle following glucose ingestion and exercise. *Physiol. Rep.* 2015; 3: e12375.
26. Gao J, Ren J, Gulve EA, Holloszy JO. Additive effect of contractions and insulin on GLUT4 translocation into the sarcolemma. *J. Appl. Physiol.* 1994; 77:1597–601.
27. Lauritzen HP. Insulin- and contraction-induced glucose transporter 4 traffic in muscle: insights from a novel imaging approach. *Exerc. Sport Sci. Rev.* 2013; 41:77–86.
28. Douen AG, Ramlal T, Rastogi S, et al. Exercise induces recruitment of the "insulin-responsive glucose transporter". Evidence for distinct intracellular insulin- and exercise-recruitable transporter pools in skeletal muscle. *J. Biol. Chem.* 1990; 265:13427–30.
29. Coderre L, Kandror KV, Vallega G, Pilch PF. Identification and characterization of an exercise-sensitive pool of glucose transporters in skeletal muscle. *J. Biol. Chem.* 1995; 270:27584–8.
30. Cartee GD. Roles of TBC1D1 and TBC1D4 in insulin- and exercise-stimulated glucose transport of skeletal muscle. *Diabetologia.* 2015; 58:19–30.
31. Stöckli J, Meoli CC, Hoffman NJ, et al. The RabGAP TBC1D1 plays a central role in exercise-regulated glucose metabolism in skeletal muscle. *Diabetes.* 2015; 64:1914–22.
32. Whitfield J, Paglialunga S, Smith BK, et al. Ablating the protein TBC1D1 impairs contraction-induced sarcolemmal glucose transporter 4 redistribution but not insulin-mediated responses in rats. *J. Biol. Chem.* 2017; 292:16653–64.
33. Sylow L, Kleinert M, Richter EA, Jensen TE. Exercise-stimulated glucose uptake—regulation and implications for glycaemic control. *Nat. Rev. Endocrinol.* 2017; 13:133–48.
34. Wright DC, Hucker KA, Holloszy JO, Han DH. Ca2+ and AMPK both mediate stimulation of glucose transport by muscle contractions. *Diabetes.* 2004; 53:330–5.
35. Witczak CA, Jessen N, Warro DM, et al. CaMKII regulates contraction- but not insulin-induced glucose uptake in mouse skeletal muscle. *Am. J. Physiol. Endocrinol Metab.* 2010; 298:E1150–60.
36. Kramer HF, Taylor EB, Witczak CA, Fujii N, Hirshman MF, Goodyear LJ. Calmodulin-binding domain of AS160 regulates contraction- but not insulin-stimulated glucose uptake in skeletal muscle. *Diabetes.* 2007; 56:2854–62.
37. Jensen TE, Sylow L, Rose AJ, et al. Contraction-stimulated glucose transport in muscle is controlled by AMPK and mechanical stress but not sarcoplasmatic reticulum Ca(2+) release. *Mol. Metab.* 2014; 3:742–53.
38. Jensen TE, Angin Y, Sylow L, Richter EA. Is contraction-stimulated glucose transport feedforward regulated by Ca^{2+}? *Exp. Physiol.* 2014; 99:1562–8.
39. Richter EA, Ruderman NB. AMPK and the biochemistry of exercise: implications for human health and disease. *Biochem. J.* 2009; 418:261–75.
40. Mu J, Brozinick JT Jr., Valladares O, Bucan M, Birnbaum MJ. A role for AMP-activated protein kinase in contraction- and hypoxia-regulated glucose transport in skeletal muscle. *Mol. Cell.* 2001; 7:1085–94.
41. Sakamoto K, McCarthy A, Smith D, et al. Deficiency of LKB1 in skeletal muscle prevents AMPK activation and glucose uptake during contraction. *EMBO J.* 2005; 24:1810–20.
42. Maarbjerg SJ, Jørgensen SB, Rose AJ, et al. Genetic impairment of AMPKalpha2 signaling does not reduce muscle glucose uptake during treadmill exercise in mice. *Am. J. Physiol. Endocrinol Metab.* 2009; 297: E924–34.
43. O'Neill HM, Maarbjerg SJ, Crane JD, et al. AMPK-activated protein kinase (AMPK) $ß_1ß_2$ muscle null mice reveal an essential role for AMPK in maintaining mitochondrial content and glucose uptake during exercise. *Proc. Natl. Acad. Sci. U S A.* 2011; 108:16092–7.
44. Kjøbsted R, Roll JLW, Jørgensen NO, et al. AMPK and TBC1D1 regulate muscle glucose uptake after, but not during, exercise and contraction. *Diabetes.* 2019; 68:1427–40.
45. Sylow L, Jensen TE, Kleinert M, et al. Rac1 is a novel regulator of contraction-stimulated glucose uptake in skeletal muscle. *Diabetes.* 2013; 62:1139–51.
46. Sylow L, Nielsen IL, Kleinert M, et al. Rac1 governs exercise-stimulated glucose uptake in skeletal muscle through regulation of GLUT4 translocation in mice. *J. Physiol.* 2016; 594:4997–5008.
47. Sylow L, Møller LL, Kleinert M, Richter EA, Jensen TE. Rac1—a novel regulator of contraction-stimulated glucose uptake in skeletal muscle. *Exp. Physiol.* 2014; 99:1574–80.
48. Henríquez-Olguin C, Knudsen JR, Raun SH, et al. Cytosolic ROS production by NADPH oxidase 2 regulates muscle glucose uptake during exercise. *Nat. Commun.* 2019; 10:4623.
49. Sandström M, Zhang SJ, Bruton J, et al. Role of reactive oxygen species in contraction-mediated glucose transport in mouse skeletal muscle. *J. Physiol.* 2006; 575:251–62.
50. Merry TL, Steinberg GR, Lynch GS, McConell GK. Skeletal muscle glucose uptake during contraction is regulated by nitric oxide and ROS independently of AMPK. *Am. J. Physiol. Endocrinol Metab.* 2010; 298:E577–85.
51. Bradley SJ, Kingwell BA, McConell GK. Nitric oxide synthase inhibition reduces leg glucose uptake but not blood flow during dynamic exercise in humans. *Diabetes.* 1999; 48:1815–21.
52. Ross RM, Wadley GD, Clark MG, Rattigan S, McConell GK. Local nitric oxide synthase inhibition reduces skeletal muscle glucose uptake but not capillary blood flow during in situ muscle contraction in rats. *Diabetes.* 2007; 56:2885–92.
53. Roberts CK, Barnard RJ, Scheck SH, Balon TW. Exercise-stimulated glucose transport in skeletal muscle is nitric oxide dependent. *Am. J. Physiol.* 1997; 273:E220–5.
54. Koh H-J, Toyoda T, Fujii N, et al. Sucrose nonfermenting AMPK-related kinase (SNARK) mediates contraction-stimulated glucose transport in mouse skeletal muscle. *Proc. Natl. Acad. Sci.* 2010; 107:15541–6.
55. Kleinert M, Parker BL, Fritzen AM, et al. Mammalian target of rapamycin complex 2 regulates muscle glucose uptake during exercise in mice. *J. Physiol.* 2017; 595:4845–55.
56. Hoffman NJ, Parker BL, Chaudhuri R, et al. Global phosphoproteomic analysis of human skeletal muscle reveals a network of exercise-regulated kinases and AMPK substrates. *Cell Metab.* 2015; 22:922–35.
57. Nelson ME, Parker BL, Burchfield JG, et al. Phosphoproteomics reveals conserved exercise-stimulated signaling and AMPK regulation of store-operated calcium entry. *EMBO J.* 2019: In press; 38:e102578.

Copyright © 2020 by the American College of Sports Medicine. Unauthorized reproduction of this article is prohibited.

58. Hespel P, Richter EA. Glucose uptake and transport in contracting, perfused rat muscle with different pre-contraction glycogen concentrations. *J. Physiol.* 1990; 427:347–59.
59. Derave W, Lund S, Holman GD, Wojtaszewski J, Pedersen O, Richter EA. Contraction-stimulated muscle glucose transport and GLUT4 surface content are dependent on glycogen content. *Am. J. Physiol.* 1999; 277:E1103–10.
60. Bergström J, Hultman E. Muscle glycogen synthesis after exercise: an enhancing factor localized to the muscle cells in man. *Nature.* 1966; 210:309–10.
61. Hingst JR, Bruhn L, Hansen MB, et al. Exercise-induced molecular mechanisms promoting glycogen supercompensation in human skeletal muscle. *Mol. Metab.* 2018; 16:24–34.
62. Ivy JL, Holloszy JO. Persistent increase in glucose uptake by rat skeletal muscle following exercise. *Am. J. Physiol.* 1981; 241:C200–3.
63. Holloszy JO. Exercise-induced increase in muscle insulin sensitivity. *J. Appl. Physiol.* 2005; 99:338–43.
64. Richter EA, Garetto LP, Goodman MN, Ruderman NB. Muscle glucose metabolism following exercise in the rat. Increased sensitivity to insulin. *J. Clin. Invest.* 1982; 69:785–93.
65. Richter EA, Mikines KJ, Galbo H, Kiens B. Effect of exercise on insulin action in human skeletal muscle. *J. Appl. Physiol.* 1989; 66:876–85.
66. Hansen PA, Nolte LA, Chen MM, Holloszy JO. Increased GLUT4 translocation mediates enhanced insulin sensitivity of muscle glucose transport after exercise. *J. Appl. Physiol.* 1998; 85:1218–22.
67. Sjøberg KA, Frøsig C, Kjøbsted R, et al. Exercise increases human skeletal muscle insulin sensitivity via coordinated increases in microvascular perfusion and molecular signaling. *Diabetes.* 2017; 66:1501–10.
68. Wojtaszewski JF, Hansen BF, Gade J, et al. Insulin signaling and insulin sensitivity after exercise in human skeletal muscle. *Diabetes.* 2000; 49:325–31.
69. Treebak JT, Frøsig C, Pehmøller C, et al. Potential role of TBC1D4 in enhanced post-exercise insulin action in human skeletal muscle. *Diabetologia.* 2009; 52:891–900.
70. McConell GK, Sjøberg KA, Ceutz F, et al. Insulin-induced membrane permeability to glucose in human muscles at rest and following exercise. *J. Physiol.* 2020; 598:303–15.
71. Knudsen JR, Steenberg DE, Hingst JR, et al. Prior exercise in humans redistributes intramuscular GLUT4 and enhances insulin-stimulated sarcolemmal and endosomal GLUT4 translocation. *Mol. Metab.* 2020; 100998 https://doi.org/10.1016/j.molmet.2020.100998.
72. Fisher JS, Gao J, Han DH, Holloszy JO, Nolte LA. Activation of AMP kinase enhances sensitivity of muscle glucose transport to insulin. *Am. J. Physiol. Endocrinol Metab.* 2002; 282:E18–23.
73. Kjøbsted R, Munk-Hansen N, Birk JB, et al. Enhanced muscle insulin sensitivity after contraction/exercise is mediated by AMPK. *Diabetes.* 2017; 66:598–612.
74. Kjøbsted R, Chadt A, Jørgensen NO, et al. TBC1D4 is necessary for enhancing muscle insulin sensitivity in response to AICAR and contraction. *Diabetes.* 2019; 68:1756–66.
75. Thong FS, Derave W, Ursø B, Kiens B, Richter EA. Prior exercise increases basal and insulin-induced p38 mitogen-activated protein kinase phosphorylation in human skeletal muscle. *J. Appl. Physiol.* 2003; 94:2337–41.
76. Geiger PC, Han DH, Wright DC, Holloszy JO. How muscle insulin sensitivity is regulated: testing of a hypothesis. *Am. J. Physiol. Endocrinol Metab.* 2006; 291:E1258–63.
77. Bogardus C, Thuillez P, Ravussin E, Vasquez B, Narimiga M, Azhar S. Effect of muscle glycogen depletion on *in vivo* insulin action in man. *J. Clin. Invest.* 1983; 72:1605–10.
78. Ivy JL, Frishberg BA, Farrell SW, Miller WJ, Sherman WM. Effects of elevated and exercise-reduced muscle glycogen levels on insulin sensitivity. *J. Appl. Physiol.* 1985; 59:154–9.
79. Kawanaka K, Han DH, Nolte LA, Hansen PA, Nakatani A, Holloszy JO. Decreased insulin-stimulated GLUT-4 translocation in glycogen-supercompensated muscles of exercised rats. *Am. J. Physiol.* 1999; 276:E907–12.
80. Derave W, Hansen BF, Lund S, Kristiansen S, Richter EA. Muscle glycogen content affects insulin-stimulated glucose transport and protein kinase B activity. *Am. J. Physiol. Endocrinol Metab.* 2000; 279:E947–55.
81. Murphy RM, Flores-Opazo M, Frankish BP, Garnham A, Stapleton D, Hargreaves M. No evidence of direct association between GLUT4 and glycogen in human skeletal muscle. *Physiol. Rep.* 2018; 6:e13917.
82. McCoy M, Proietto J, Hargreaves M. Skeletal muscle GLUT-4 and postexercise muscle glycogen storage in humans. *J. Appl. Physiol.* 1996; 80:411–5.
83. Hickner RC, Fisher JS, Hansen PA, et al. Muscle glycogen accumulation after endurance exercise in trained and untrained individuals. *J. Appl. Physiol.* 1997; 83:897–903.
84. Greiwe JS, Hickner RC, Hansen PA, Racette SB, Chen MM, Holloszy JO. Effects of endurance exercise training on muscle glycogen accumulation in humans. *J. Appl. Physiol.* 1999; 87:222–6.
85. Henriksen EJ, Bourey RE, Rodnick KJ, Koranyi L, Permutt MA, Holloszy JO. Glucose transporter protein content and glucose transport capacity in rat skeletal muscles. *Am. J. Physiol.* 1990; 259:E593–8.
86. Kern M, Wells JA, Stephens JM, et al. Insulin responsiveness in skeletal muscle is determined by glucose transporter (Glut4) protein level. *Biochem. J.* 1990; 270:397–400.
87. Koranyi LI, Bourey RE, Vuorinen-Markkola H, et al. Level of skeletal muscle glucose transporter protein correlates with insulin-stimulated whole body glucose disposal in man. *Diabetologia.* 1991; 34:763–5.
88. Kristiansen S, Gade J, Wojtaszewski JF, Kiens B, Richter EA. Glucose uptake is increased in trained vs. untrained muscle during heavy exercise. *J. Appl. Physiol.* 2000; 89:1151–8.
89. Ren J-M, Marshall BA, Mueckler MM, McCaleb M, Amatruda JM, Shulman GI. Overexpression of Glut4 protein in muscle increases basal and insulin-stimulated whole body glucose disposal in conscious mice. *J. Clin. Invest.* 1995; 95:429–32.
90. Hansen PA, Gulve EA, Marshall BA, et al. Skeletal muscle glucose transport and metabolism are enhanced in transgenic mice overexpressing the Glut4 glucose transporter. *J. Biol. Chem.* 1995; 270:1679–84.
91. Tsao T-S, Burcelin R, Katz EB, Huang L, Charron MJ. Enhanced insulin action due to targeted GLUT4 overexpression exclusively in muscle. *Diabetes.* 1996; 45:28–36.
92. Goodyear LJ, Hirshman MF, Smith RJ, Horton ES. Glucose transporter number, activity and isoform content in plasma membranes of red and white skeletal muscle. *Am. J. Physiol.* 1991; 261:E556–61.
93. Marette A, Richardson JM, Ramlal T, et al. Abundance, localization, and insulin-induced translocation of glucose transporters in red and white muscle. *Am. J. Physiol.* 1992; 263:C443–52.
94. Megeney LA, Neufer PD, Dohm GL, et al. Effects of muscle activity and fiber composition on glucose transport and GLUT-4. *Am. J. Physiol.* 1993; 264:E583–93.
95. Daugaard JR, Nielsen JN, Kristiansen S, Andersen JL, Hargreaves M, Richter EA. Fiber type-specific expression of GLUT4 in human skeletal muscle: influence of exercise training. *Diabetes.* 2000; 49:1092–5.
96. Flores-Opazo M, Boland E, Garnham A, Murphy RM, McGee SL, Hargreaves M. Exercise and GLUT4 in human subcutaneous adipose tissue. *Physiol. Rep.* 2018; 6:e13918.
97. Ploug T, Stallknecht BM, Pedersen O, et al. Effect of endurance training on glucose transport capacity and glucose transporter expression in rat skeletal muscle. *Am. J. Physiol.* 1990; 259:E778–86.
98. Rodnick KJ, Holloszy JO, Mondon CE, James DE. Effects of exercise training on insulin-regulatable glucose-transporter protein levels in rat skeletal muscle. *Diabetes.* 1990; 39:1425–9.
99. Rodnick KJ, Henriksen EJ, James DE, Holloszy JO. Exercise training, glucose transporters, and glucose transport in rat skeletal muscles. *Am. J. Physiol.* 1992; 262:C9–14.
100. Neufer PD, Shinebarger MH, Dohm GL. Effect of training and detraining on skeletal muscle glucose transporter (GLUT4) content in rats. *Can. J. Physiol. Pharmacol.* 1992; 70:1286–90.
101. Goodyear LJ, Hirshman MF, Valyou PM, Horton ES. Glucose transporter number, function, and subcellular distribution in rat skeletal muscle after exercise training. *Diabetes.* 1992; 41:1091–9.
102. Dela F, Handberg A, Mikines KJ, Vinten J, Galbo H. GLUT4 and insulin receptor binding and kinase activity in trained human muscle. *J. Physiol.* 1993; 469:615–24.
103. Houmard JA, Shinebarger MH, Dolan PL, et al. Exercise training increases GLUT-4 protein concentration in previously sedentary middle-aged men. *Am. J. Physiol.* 1993; 264:E896–901.
104. Houmard JA, Hickey MS, Tyndall GL, Gavigan KE, Dohm GL. Seven days of exercise increase GLUT-4 protein content in human skeletal muscle. *J. Appl. Physiol.* 1995; 79:1936–8.
105. Gulve EA, Spina RJ. Effect of 7–10 days of cycle ergometer exercise on skeletal muscle GLUT-4 protein content. *J. Appl. Physiol.* 1995; 79:1562–6.

Copyright © 2020 by the American College of Sports Medicine. Unauthorized reproduction of this article is prohibited.

106. McCoy M, Proietto J, Hargreves M. Effect of detraining on GLUT-4 protein in human skeletal muscle. *J. Appl. Physiol.* 1994; 77:1532–6.
107. Phillips SM, Han X-X, Green HJ, Bonen A. Increments in skeletal muscle GLUT-1 and GLUT-4 after endurance training in humans. *Am. J. Physiol.* 1996; 270:E456–62.
108. Kraniou GN, Cameron-Smith D, Hargreaves M. Effect of short-term training on GLUT-4 mRNA and protein expression in human skeletal muscle. *Exp. Physiol.* 2004; 89:559–63.
109. Burgomaster KA, Cermak NM, Phillips SM, Benton CR, Bonen A, Gibala MJ. Divergent response of metabolite transport proteins in human skeletal muscle after sprint interval training and detraining. *Am. J. Physiol. Integr Comp Physiol.* 2007; 292:R1970–6.
110. Dela F, Ploug T, Handberg A, et al. Physical training increases muscle GLUT4 protein and mRNA in patients with NIDDM. *Diabetes.* 1994; 43:862–5.
111. Hussey SE, McGee SL, Garnham A, Wentworth JM, Jeukendrup AE, Hargreaves M. Exercise training increases adipose tissue GLUT4 expression in patients with type 2 diabetes. *Diab. Obes. Metab.* 2011; 13:959–62.
112. Little JP, Gillen JB, Percival ME, et al. Low-volume high-intensity interval training reduces hyperglycemia and increases muscle mitochondrial capacity in patients with type 2 diabetes. *J. Appl. Physiol.* 2011; 111:1554–60.
113. Holten MK, Zacho M, Gaster M, Juel C, Wojtaszewski JF, Dela F. Strength training increases insulin-mediated glucose uptake, GLUT4 content, and insulin signaling in skeletal muscle in patients with type 2 diabetes. *Diabetes.* 2004; 53:294–305.
114. Asp S, Daugaard JR, Richter ER. Eccentric exercise decreases glucose transporter GLUT4 protein content in human skeletal muscle. *J. Physiol.* 1995; 482:705–12.
115. Kristiansen S, Jones J, Handberg A, Dohm GL, Richter EA. Eccentric contractions decrease glucose transporter transcription rate, mRNA, and protein in skeletal muscle. *Am. J. Physiol.* 1997; 272:C1734–8.
116. Vukovich MD, Arciero PJ, Kohrt WM, Racette SB, Hansen PA, Holloszy JO. Changes in insulin action and GLUT-4 with 6 days of inactivity in endurance runners. *J. Appl. Physiol.* 1996; 80:240–4.
117. Biensø RS, Ringholm S, Kiilerich K, et al. GLUT4 and glycogen synthase are key players in bed rest–induced insulin resistance. *Diabetes.* 2012; 61:1090–9.
118. Megeney LA, Michel RN, Boudreau CS, et al. Regulation of muscle glucose transport and GLUT-4 by nerve-derived factors and activity-related processes. *Am. J. Physiol.* 1995; 269:R1148–53.
119. Fogt DL, Slentz MJ, Tischler ME, Henriksen EJ. GLUT-4 protein and citrate synthase activity in distally or proximally denervated rat soleus muscle. *Am. J. Physiol.* 1997; 272:R429–32.
120. Neufer PD, Dohm GL. Exercise induces a transient increase in transcription of the GLUT-4 gene in skeletal muscle. *Am. J. Physiol.* 1993; 265:C1597–603.
121. MacLean PS, Zheng D, Jones JP, Olson AL, Dohm GL. Exercise-induced transcription of the muscle glucose transporter (GLUT4) gene. *Biochem. Biophys. Res. Comms.* 2002; 292:409–14.
122. Kuo C-H, Browning KS, Ivy JL. Regulation of GLUT4 protein expression and glycogen storage after prolonged exercise. *Acta Physiol. Scand.* 1999; 165:193–201.
123. Kraniou Y, Cameron-Smith D, Misso M, Collier G, Hargreaves M. Effects of exercise on GLUT-4 and glycogenin gene expression in human skeletal muscle. *J. Appl. Physiol.* 2000; 88:794–6.
124. Kraniou GN, Cameron-Smith D, Hargreaves M. Acute exercise and GLUT4 expression in human skeletal muscle: influence of exercise intensity. *J. Appl. Physiol.* 2006; 101:934–7.
125. McGee SL, Hargreaves M. Exercise and myocyte enhancer factor 2 regulation in human skeletal muscle. *Diabetes.* 2004; 53:1208–14.
126. Leick L, Plomgaard P, Grønløkke L, Al-Abaiji F, Wojtaszewski JF, Pilegaard H. Endurance exercise induces mRNA expression of oxidative enzymes in human skeletal muscle late in recovery. *Scand. J. Med. Sci. Sports.* 2010; 20:593–9.
127. Hussey SE, McGee SL, Garnham A, McConell GK, Hargreaves M. Exercise increases skeletal muscle GLUT4 gene expression in patients with type 2 diabetes. *Diabetes Obes. Metab.* 2012; 14:768–71.
128. Gurley JM, Griesel BA, Olson AL. Increased skeletal muscle GLUT4 expression in obese mice after voluntary wheel running exercise is posttranscriptional. *Diabetes.* 2016; 65:2911–9.
129. Knight JB, Eyster CA, Griesel BA, Olson AL. Regulation of the human GLUT4 gene promoter: interaction between a transcriptional activator and myocyte enhancer factor 2A. *Proc. Natl. Acad. Sci. U S A.* 2003; 100:14725–30.
130. McGee SL, Sparling D, Olson AL, Hargreaves M. Exercise increases MEF2- and GEF DNA-binding activity in human skeletal muscle. *FASEB J.* 2006; 20:348–9.
131. Holmes BF, Sparling DP, Olson AL, Winder WW, Dohm GL. Regulation of muscle GLUT4 enhancer factor and myocyte enhancer factor 2 by AMP-activated protein kinase. *Am. J. Physiol. Endocrinol Metab.* 2005; 289:E1071–6.
132. McGee SL, van Denderen BJ, Howlett KF, et al. AMP-activated protein kinase regulates GLUT4 transcription by phosphorylating histone deacetylase 5. *Diabetes.* 2008; 57:860–7.
133. Niu Y, Wang T, Liu S, Yuan H, Li H, Fu L. Exercise-induced GLUT4 transcription via inactivation of HDAC4/5 in mouse skeletal muscle in an AMPKα2-dependent manner. *Biochim Biophys Acta Mol Basis Dis.* 1863; 2017:2372–81.
134. Backs J, Backs T, Bezprozvannaya S, McKinsey TA, Olson EN. Histone deacetylase 5 acquires calcium/calmodulin-dependent kinase II responsiveness by oligomerization with histone deacetylase 4. *Mol. Cell. Biol.* 2008; 28:3437–45.
135. Garcia-Roves PM, Jones TE, Otani K, Han DH, Holloszy JO. Calcineurin does not mediate exercise-induced increase in muscle GLUT4. *Diabetes.* 2005; 54:624–8.
136. Murgia M, Jensen TE, Cusinato M, Garcia M, Richter EA, Schiaffino S. Multiple signalling pathways redundantly control glucose transporter GLUT4 gene transcription in skeletal muscle. *J. Physiol.* 2009; 587:4319–27.
137. McGee SL, Swinton C, Morrison S, et al. Compensatory regulation of HDAC5 in muscle maintains metabolic adaptive responses and metabolism in response to energetic stress. *FASEB J.* 2014; 28:3384–95.
138. Smith JA, Kohn TA, Chetty AK, Ojuka EO. CaMK activation during exercise is required for histone hyperacetylation and MEF2A binding at the MEF2 site on the Glut4 gene. *Am. J. Physiol. Endocrinol Metab.* 2008; 295: E698–704.
139. Joseph JS, Ayeleso AO, Mukwevho E. Exercise increases hyper-acetylation of histones on the *Cis*-element of NRF-1 binding to the *Mef2a* promoter: implication on type 2 diabetes. *Biochem. Biophys. Res. Comms.* 2017; 486:83–7.
140. Baar K, Song Z, Semenkovich CF, et al. Skeletal muscle overexpression of nuclear respiratory factor 1 increases glucose transport capacity. *FASEB J.* 2003; 17:1666–73.
141. Koh J-H, Hancock CR, Han DH, Holloszy JO, Nair KS, Dasari S. AMPK and PPARβ positive feedback loop regulates endurance exercise training-mediated GLUT4 expression in skeletal muscle. *Am. J. Physiol. Endocrinol Metab.* 2019; 316:E931–9.
142. Herman MA, Kahn BB. Glucose transport and sensing in the maintenance of glucose homeostasis and metabolic harmony. *J. Clin. Invest.* 2006; 116:1767–75.
143. Abel ED, Peroni O, Kim JK, et al. Adipose-selective targeting of the GLUT4 gene impairs insulin action in muscle and liver. *Nature.* 2001; 409:729–33.
144. Sinha MK, Raineri-Maldonado C, Buchanan C, et al. Adipose tissue glucose transporters in NIDDM: decreased levels of muscle/fat isoform. *Diabetes.* 1991; 40:472–7.
145. Rodnick KJ, Haskell WL, Swislocki ALM, Foley JE, Reaven GM. Improved insulin action in muscle, liver, and adipose tissue in physically trained human subjects. *Am. J. Physiol.* 1987; 253:E489–95.
146. Hirshman MF, Goodyear LJ, Horton ED, Wardzala LJ, Horton ES. Exercise training increases GLUT-4 protein in rat adipose cells. *Am. J. Physiol.* 1993; 264:E882–9.
147. Stallknecht B, Andersen PH, Vinten J, et al. Effect of physical training on glucose transporter protein and mRNA levels in rat adipocytes. *Am. J. Physiol.* 1993; 265:E128–34.
148. Ferrara CM, Reynolds TH, Zarnowski MJ, Brozinick JT Jr., Cushman SW. Short-term exercise enhances insulin-stimulated GLUT4 translocation and glucose transport in adipose cells. *J. Appl. Physiol.* 1998; 85:2106–11.

Copyright © 2020 by the American College of Sports Medicine. Unauthorized reproduction of this article is prohibited.

149. Graham TE, Yang Q, Blüher M, et al. Retinol-binding protein 4 and insulin resistance in lean, obese, and diabetic subjects. *N. Engl. J. Med.* 2006; 354:2552–63.
150. Sparling DP, Griesel BA, Weems J, Olson AL. GLUT4 enhancer factor (GEF) interacts with MEF2A and HDAC5 to regulate the GLUT4 promoter in adipocytes. *J. Biol. Chem.* 2008; 283:7429–37.
151. Dalen KT, Ulven SM, Bamberg K, Gustafsson JA, Nebb HI. Expression of the insulin-responsive glucose transporter GLUT4 in adipocytes is dependent on liver X receptor alpha. *J. Biol. Chem.* 2003; 278:48283–91.
152. Ngarmukos C, Baur EL, Kumagai AK. Co-localization of GLUT1 and GLUT4 in the blood-brain barrier of the rat ventromedial hypothalamus. *Brain Res.* 2001; 900:1–8.
153. Ren H, Yan S, Zhang B, lu TY, Arancio O, Accili D. Glut4 expression defines an insulin-sensitive hypothalamic neuronal population. *Mol. Metab.* 2014; 3:452–9.
154. Reno CM, Puente EC, Sheng Z, et al. Brain GLUT4 knockout mice have impaired glucose tolerance, decreased insulin sensitivity and impaired hypoglycemic counterregulation. *Diabetes.* 2017; 66:587–97.

Copyright © 2020 by the American College of Sports Medicine. Unauthorized reproduction of this article is prohibited.