Exercise Effects on Muscle Insulin Signaling and Action
Invited Review: Regulation of skeletal muscle GLUT-4 expression by exercise

G. LYNIS DOHM
Department of Biochemistry, Brody School of Medicine,
East Carolina University, Greenville, North Carolina 27858

Dohm, G. Lynis. Invited Review: Regulation of skeletal muscle GLUT-4 expression by exercise. J Appl Physiol 93: 782–787, 2002; 10.1152/japplphysiol.01266.2001.—The amount of GLUT-4 protein is a primary factor in determining the maximal rate of glucose transport into skeletal muscle. Therefore, it is important that we understand how exercise regulates GLUT-4 expression so that therapeutic strategies can be designed to increase muscle glucose disposal as a treatment for diabetes. Muscle contraction increases the rates of GLUT-4 transcription and translation. Transcriptional control likely requires at least two DNA binding proteins, myocyte enhancer factor-2 and GLUT-4 enhancer factor, which bind to the promoter. Increased GLUT-4 expression may be mediated by the enzyme AMP-activated kinase, which is activated during exercise and has been demonstrated to increase GLUT-4 transcription. Further research needs to be done to investigate whether AMP-activated kinase activates myocyte enhancer factor-2 and GLUT-4 enhancer factor to increase transcription of the GLUT-4 gene.

glucose transporter-4 promoter; myocyte enhancer factor-2; adenosine 5'-monophosphate kinase; transgenic mice

GLUCOSE TRANSPORT INTO SKELETAL muscle is primarily mediated by a membrane-associated glucose transport protein termed GLUT-4. Under normal resting conditions, most of the GLUT-4 molecules reside in membrane vesicles inside the muscle cell. In response to insulin or muscle contraction, GLUT-4 translocates to the cell membrane where it inserts to increase glucose transport.

The amount of GLUT-4 protein is a primary factor in determining the maximal rate of glucose transport into skeletal muscle (32). Because skeletal muscle is an important tissue in maintaining postprandial glucose homeostasis, increasing GLUT-4 protein is a potential method to treat the hyperglycemia associated with diabetes. We and others (6, 15, 18, 50, 53) have shown that exercise can increase the content of muscle GLUT-4. Therefore, it is important that we understand how exercise regulates GLUT-4 expression so that therapeutic strategies might be designed to help increase muscle glucose disposal as a treatment for diabetes. In this review, the mechanisms that may be involved in regulating GLUT-4 expression are examined.

CHANGES IN MUSCLE GLUT-4 IN RESPONSE TO STIMULI

Changes in GLUT-4 expression in response to exercise can be rather rapid. In rats, 1 day after swimming for 6 h, there was a twofold increase in GLUT-4 mRNA and a 1.5-fold increase in GLUT-4 protein (51). On the second day after swimming, muscle GLUT-4 protein was twofold higher than in sedentary controls. After termination of swim training, rat muscle GLUT-4 protein returned to control levels by 40 h (22) or 90 h (31). These studies suggest that GLUT-4 has a short half-life, and its expression can be changed rapidly. GLUT-4 expression can be induced by short-term, high-intensity, intermittent training (fourteen 20-s exercise bouts/day for 8 days) or low-intensity, prolonged exercise training (total exercise time of 360 min/day for 8
days) (55). Like exercise training, electrical stimulation also increases muscle GLUT-4 content (13, 20). In contrast to swimming exercise, GLUT-4 content was not elevated until 5 days of electrical stimulation (20). Several studies have confirmed that muscle GLUT-4 is increased by exercise training in humans (23, 26, 37, 38). Seven days of strenuous cycling resulted in a threefold increase in muscle GLUT-4 (24). There is some controversy as to how long the elevated GLUT-4 persists after training is terminated (25, 61).

Paradoxically, eccentric exercise has been shown to decrease muscle GLUT-4 in both men (1) and rats (2). Eccentric contractions caused GLUT-4 to decrease, whereas concentric contractions and passive stretch of the muscle had no effect (33). Unaccustomed eccentric contractions produce muscle damage and soreness. Repair of the damaged tissue leads to a much different response in the regulation of GLUT-4 expression than does normal exercise (34).

In contrast to exercise, forced chronic physical inactivity (16) or limb immobilization (12, 49) in rats causes muscle GLUT-4 to be decreased. Immobilization of human vastus muscle as a result of casting also decreased muscle GLUT-4 by 50% after 1 wk of inactivity, and the level remained decreased at 6 wk of casting (4). Surprisingly, inactivity caused by hindlimb suspension of rats caused the opposite effect with increased GLUT-4 (expressed per milligram of total protein) in the atrophied muscle (19, 27).

Denervation by sectioning of a nerve, such as the sciatic or peroneal, is often used as a model for muscle inactivity. Muscle GLUT-4 protein and mRNA are decreased in denervated rat (5) and rabbit (8) muscle. Direct electrical stimulation of rabbit tibialis anterior muscle protected GLUT-4 mRNA levels against the effect of denervation (8). An interesting study was performed by Chilibeck et al. (9) to confirm these findings in human subjects. They performed functional electrical stimulation cycle ergometer training on individuals with motor-complete spinal cord injury. GLUT-4 protein was increased by 72% with training.

The results of Megeney et al. (39, 41) argue that the denervation effect is not simply due to the lack of muscle activity. GLUT-4 was measured at different time points after the sciatic nerve was sectioned at different locations. GLUT-4 decrements occurred more rapidly in denervated muscles with a short nerve stump than in those with a long nerve stump, suggesting that neurogenic factor(s) released from the nerve influences GLUT-4 expression. In a second study, they treated muscles with tetrodotoxin, which blocks the propagation of action potentials along the sciatic nerve, but axonal transport is maintained. GLUT-4 mRNA was depressed in the denervated muscle but not in the tetrodotoxin-treated muscle, suggesting again that neurogenic factors were regulating GLUT-4 expression.

In rat muscle, GLUT-4 protein and glucose transport are markedly higher in red-oxidative (type I and IIA fibers) muscle fibers than in white-glycolytic fibers (type IIB) (32, 40). This might be an important factor in the adaptation to exercise, because endurance training results in a shift from type IIB to type IIA fibers. However, in human skeletal muscle, there is a much smaller difference in GLUT-4 expression in different muscle fiber types (10, 17). Daugaard et al. (10) isolated individual muscle fibers and typed them according to myosin isofrom. GLUT-4 was ~20% higher in fibers expressing myosin heavy chain I than in those expressing myosin heavy chain IIA or IIX. No difference could be detected between IIA and IIX fibers. After 2 wk of exercise training, GLUT-4 was increased by ~23% in type I muscles, but there were no changes in type IIA or IIX. However, the low-intensity exercise that was used is known to recruit primarily type I fibers.

Hormones also regulate muscle glucose transporter protein concentration. GLUT-4 expression was increased by insulin (28) and thyroid hormone (57) and decreased by elevated cAMP (28, 60). Regulation of GLUT-4 expression by contractile activity is independent of hormonal regulation, because treadmill running increased GLUT-4 in streptozotocin diabetic rats (30). The effects of insulin-deficiency and denervation on GLUT-4 concentrations were additive. Physical training also increases muscle GLUT-4 protein and mRNA in patients with Type 2 diabetes (11). These results suggest that muscle contractile activity directly modulates muscle GLUT-4 expression, independent of insulin action.

REGULATION OF MUSCLE GLUT-4 EXPRESSION

To understand the mechanisms that increase muscle GLUT-4 mRNA and protein in response to exercise, we investigated whether the rate of GLUT-4 transcription was increased. Nuclei were isolated from muscle of rats after a single bout of exercise or after the last bout of treadmill training, and GLUT-4 transcription was determined by nuclear run-on analysis. Transcription was unaltered 30 min after the last exercise bout, was increased 1.8-fold after 3 h, and returned to control values by 24 h (44). This suggests that there is a rapid but transient activation of gene transcription. Further evidence that GLUT-4 gene transcription is increased by exercise was provided from studies of transgenic mice carrying the human GLUT-4 (hGLUT-4) promoter linked to a chloramphenicol acetyl transferase (CAT) reporter gene (36). Transgenic mice were treadmill exercised, and CAT mRNA was increased 12 h after the end of the exercise bout. These results demonstrate that there is an “exercise response element” in the GLUT-4 promoter that drives gene transcription in response to exercise.

Kuo et al. (35) provided evidence that translation of the GLUT-4 message is also regulated by exercise. They found that polysome-associated GLUT-4 mRNA was increased immediately after exercise and remained significantly increased for the first 5 h of recovery. Polysome-associated mRNA is considered to be translationally active mRNA. Therefore, GLUT-4 ex-
pression may be regulated at both the transcriptional and translational level.

Transcription of a gene is usually regulated by DNA sequences in a promoter that is 5’ upstream of the initiation site. With the use of muscle cell culture experiments, it was recently reported (54) that the rat promoter has an 82-bp muscle-specific enhancer (between −502 and −420 bp from the initiation start site) that is active in both cardiac and skeletal muscle. The GLUT-4 enhancer relies on the concerted interaction among the transcription factors MyoD and myocyte enhancer factor-2 (MEF-2) and the thyroid receptor-α1 to produce full expression.

Transgenic mice with various portions of the hGLUT-4 promoter fused to a reporter gene, such as the CAT gene, have been used to identify DNA sequences in the promoter of the GLUT-4 gene that regulate transcription. When active portions of the GLUT-4 promoter are present, the mice express reporter mRNA. By using this approach, it was found that −895 bp of the hGLUT-4 promoter were sufficient to provide tissue-specific expression, i.e., GLUT-4 was expressed only in adipose tissue, heart, and skeletal muscle. In addition, this promoter construct demonstrated normal regulation in diabetic animals (insulin deficient). A deletion or mutation of the MEF-2 binding domain of the GLUT-4 promoter ablated transgene expression in all tissues (46, 56). By using isoform-specific antibodies in electrophoretic mobility shift assays, MEF-2A and MEF-2D isoforms were shown to bind the GLUT-4 MEF-2 binding domain in skeletal muscle (46, 56). Mora and Pessin (43) demonstrated that the MEF-2A-MEF-2D heterodimer is responsible for hormonally regulated expression of the GLUT-4 gene. Recently, Michael et al. (42) reported that expression of the transcriptional regulator peroxisome proliferator-activated receptor-γ coactivator-1 (PGC-1) in L6 cells causes the total restoration of GLUT-4 mRNA levels to those observed in vivo. Because PGC-1 is a coactivator of MEF-2C, it seems likely that regulation may require the concerted action of transcription factors and coactivators.

Oshel et al. (47) described another putative transcription factor, called GLUT-4 enhancing factor (GEF), that is important in the regulation of GLUT-4 gene expression. GEF binds to DNA sequences in a region designated domain 1 (−724 bp to −712 bp from the initiation start site). Deletion of domain 1 completely eliminated expression of GLUT-4 in muscle. These results demonstrate that MEF-2 and GEF binding domains must function together for proper expression and regulation of the GLUT-4 gene. Oshel et al. speculated that regulated and tissue-specific expression of the GLUT-4 gene occurs only in tissues in which sufficient GEF binding activity and MEF-2 binding activity coincide. Regulation of the GLUT-4 gene under an insulin-deficient condition may reflect the levels of either GEF binding activity, or MEF-2 binding activity, or an interaction of the two sites.

Utilizing transgenic mice carrying the hGLUT-4/CAT gene, we investigated regulation of the GLUT-4 promoter in muscle during exercise. A single bout of exercise increased endogenous muscle GLUT-4 mRNA with a peak at 12 h after the end of exercise (36). Mice with −895 bp of the hGLUT-4 promoter demonstrated an increase in CAT mRNA in muscle that also peaked at 12 h after exercise. In contrast, CAT was not increased by exercise in mice with −730 bp of the GLUT-4 promoter. This regulation is very similar to that reported by Oshel et al. (47) and suggests that MEF-2 and GEF may be involved in upregulating the GLUT-4 gene in response to exercise. Interestingly, denervation caused GLUT-4 and CAT mRNAs to be depressed in mice with the −730-bp promoter construct (29), demonstrating that regulation of expression in denervated muscle is different than that for exercise (or lack of exercise).

Ezaki (14) investigated regulation of the mouse GLUT-4 promoter. He found that 1,000 bp of the promoter provided the DNA sequences required for the appropriate tissue-specific and metabolic regulation of GLUT-4 expression. In contrast to results with the hGLUT-4 promoter, he found that the MEF-2 binding site was not necessary for expression of GLUT-4 in murine muscle. The exercise response element was found to be between −1,000 bp and −423 bp from the start site (58, 59). In agreement with the results with the human promoter (36), the DNA sequences required for GLUT-4 regulation by denervation were different from those for exercise (59) and lay between −423 bp and the initiation start site. A representation of the regulatory elements within the hGLUT-4 promoter are shown in Fig. 1.

**Signals for Regulation of GLUT-4 Expression during Exercise**

To understand regulation of GLUT-4 expression, we must know the changes during exercise that activate transcription factors to turn on the muscle GLUT-4 gene. One signaling candidate is muscle high-energy phosphates, which decline during exercise. Evidence for the role of energy charge in transcriptional regulation came from a study in which GLUT-4 protein and high-energy phosphate levels were shown to be inversely correlated in electrically stimulated muscles (64). Depleting muscle creatine phosphate by feeding rats a creatine analog also caused muscle GLUT-4 protein to be increased (52), further suggesting a relationship between a decrease in high-energy phosphates and increased GLUT-4 expression.

A likely link between energy charge and regulation of gene transcription is the enzyme AMP-activated protein kinase (AMPK). AMPK is activated during exercise and has been termed a master metabolic switch because it phosphorylates key target proteins that control flux through metabolic pathways (62). It is also related to SNR1 protein in yeast, which is known to regulate gene expression. Chronic activation of AMPK by injection of the adenosine analog 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR) caused GLUT-4 protein to be increased in muscle (21). Interest-
The content of GLUT-4 protein in muscle is an important determinant of glucose homeostasis, and the effectiveness of exercise in the treatment of diabetes may relate to the increase in muscle GLUT-4. Therefore, research needs to be done to discover the mechanisms that regulate muscle GLUT-4.

One fertile area of investigation is the signal that turns on the GLUT-4 gene. Hormones, such as insulin and thyroid hormone, induce gene expression, but their concentrations do not change in concert with the changes in GLUT-4 during exercise. In addition, muscle contraction and one-legged exercise both increase muscle GLUT-4 without changes in hormones. Thus the signal is most logically a substance that has altered concentration in muscle during exercise. As discussed above, \( \text{Ca}^{2+} \) and AMP are good candidates for signaling during exercise, because their intracellular concentrations increase during exercise. In addition, both molecules are activators of kinases and/or phosphatases, which would provide a pathway for the regulation of transcription factors. One approach to the study of these signaling pathways will be to utilize transgenic and knockout mice that overexpress or ablate the \( \text{Ca}^{2+} \) - and AMP-related kinases and phosphatases.

Another area of research interest is the transcription factors that are "turned on" by the signals generated in muscle during exercise. As indicated above, MEF-2 and GEF are candidates that have been identified so far. However, the involvement of coactivators, such as PGC-1, and the interaction of other transcription factors, such as myoD and thyroid hormone receptors, may make understanding the regulation of GLUT-4 expression a challenge.

**FUTURE RESEARCH QUESTIONS**

The greatest increase was found in white muscle fibers, and there was no GLUT-4 induction by AICAR in soleus muscle (7, 65). AICAR treatment also reversed the decline in GLUT-4 protein in all denervated muscles except the soleus (48). In vitro incubation of epitrochlearis muscles with AICAR also increased GLUT-4 expression, demonstrating that the effect was a direct activation of expression in the muscle (45).

Using transgenic mice carrying the hGLUT-4 promoter/CAT gene, we demonstrated that AICAR increases GLUT-4 transcription (65). As with exercise, the transgene with −895 bp of the promoter regulated in a manner similar to that of the endogenous gene, but this regulation was lost with the −730-bp promoter. This suggests that exercise and AICAR activate the same transcription factors to increase GLUT-4 expression. Because MEF-2 and GEF are known to be important transcription factors for the regulation of the GLUT-4 gene, we measured the effect of AICAR on the DNA binding of these two proteins. AICAR increased MEF-2 binding, whereas GEF binding to DNA was slightly reduced, suggesting that these transcription factors may be involved in regulating GLUT-4 gene transcription in response to exercise and AMP activation.

It has recently been shown that chronic activation of AMP kinase also leads to activation of DNA binding by the transcription factor called nuclear respiratory factor-1 (NRF-1) (3). NRF-1 is important in the regulation of mitochondrial biogenesis and transcription of respiratory enzymes. Because the expression of mitochondrial enzymes and GLUT-4 is increased by exercise and AICAR treatment, it is possible that the NRF-1 might also play a role in GLUT-4 expression.

Another attractive signaling candidate for regulation of GLUT-4 gene expression during exercise is calcium, which is released during the contraction cycle. Wu et al. (63) recently demonstrated that MEF-2 is activated in muscle by a calcineurin-dependent pathway. Calcineurin is a calcium-activated phosphatase, and its activity correlates with expression of endogenous genes that are transcriptionally activated by muscle contractions. It could be speculated that GLUT-4 expression may be regulated by calcineurin activation of MEF-2.

**Fig. 1.** Representation of the important response elements in the human GLUT-4 promoter. GEF, binding site for the GLUT-4 enhancer factor; MEF-2, binding site for myocyte enhancer factor-2; TRE, binding site for thyroid hormone receptor-α. Promoter sequences within −895 bp of the start site contain sufficient information for the correct tissue expression of GLUT-4 and for regulation by exercise, insulin, 5-aminimidazole-4-carboxamide-ribonucleoside (AICAR), and denervation. If either the MEF-2 or GEF binding site is mutated, there is no GLUT-4 expression in muscle. Deletion of DNA between −526 and −712 bp did not alter GLUT-4 expression or regulation. The −730-bp promoter (contains a partial GEF binding site) demonstrates low-level expression of GLUT-4 in muscle with regulation by denervation, but not by exercise, insulin, or AICAR.
It is important that we continue to study the regulation of muscle GLUT-4 expression. If we understand how exercise regulates the gene, we may then be able to devise pharmaceutical interventions that would increase muscle GLUT-4 and provide a treatment for the hyperglycemia of diabetes.

REFERENCES