Regulation of skeletal muscle GLUT-4 expression by exercise and nutritional stimuli

Kazuhiko Higashida¹, Izumi Tabata², Mitsuru Higuchi¹ and Shin Terada³*

1 Faculty of Sport Sciences, Waseda University, 2-579-15 Mikajima, Tokorozawa, Saitama 359-1192, Japan
2 Faculty of Sport and Health Science, Ritsumeikan University, 1-1-1 Nojihigashi, Kusatsu, Shiga 525-8577, Japan
3 Department of Life Sciences, Graduate School or Arts and Sciences, The University of Tokyo, 3-8-1 Komaba, Meguro-ku, Tokyo 153-8902, Japan

Received: July 5, 2013 / Accepted: July 16, 2013

Abstract  Skeletal muscle is the primary site of glucose uptake in humans. Glucose transport activity, which is the rate-limiting step in muscle glucose metabolism, is linearly related to the content of the GLUT-4 isoform of the glucose transporter. Therefore, the level of GLUT-4 in skeletal muscle may be an important determinant of whole-body glucose disposal. It has been well documented that long-term, low- to moderate-intensity endurance exercise training induces an increase in muscle GLUT-4 content. However, emerging evidence suggests that an adaptive increase in GLUT-4 occurs even after a single acute bout of exercise or high-intensity intermittent exercise training. Recent findings also indicate that nutritional status affects GLUT-4 expression in skeletal muscle. This review provides an overview of the effects of exercise and nutritional status on GLUT-4 content in skeletal muscle, and summarizes recent progress in elucidating the molecular regulation of muscle GLUT-4 gene expression by exercise and nutritional stimuli.

Keywords: GLUT-4, skeletal muscle, exercise training, alternate-day fasting, high-fat diet

Introduction

In healthy individuals, the blood glucose level rapidly returns to normal after a meal. Skeletal muscle accounts for approximately 40% of total body mass and is the largest tissue for insulin-stimulated glucose disposal. DeFronzo et al. clearly demonstrated that skeletal muscle is responsible for at least 80% of insulin-stimulated glucose disposal, and that muscle glucose uptake is severely impaired in noninsulin-dependent diabetic (type 2 diabetic) patients¹. Under most physiological conditions, glucose transport is the rate-limiting step in skeletal muscle glucose metabolism². These observations highlight the importance of the glucose transport capacity in skeletal muscle for preventing insulin resistance and type 2 diabetes.

Muscle glycogen is essential for prolonged, strenuous exercise. When muscle glycogen stores are depleted, muscle fatigue develops and vigorous exercise cannot be continued. Thus, raising muscle glycogen before competitions improves the ability to perform prolonged, vigorous exercise³. Skeletal muscle glycogen accumulation has been shown to be limited by glucose transport as well as glycogen synthase activity⁴, suggesting that enhancing the muscle glucose transport capacity can lead to the improvement of endurance exercise performance through elevated muscle glycogen concentration.

As indicated above, glucose transport into skeletal muscle is a key step in glucose metabolism for improving health and exercise performance. Glucose is carried across the cell membrane by a family of transporter proteins, called GLUTs. The protein content of glucose transporter-4 (GLUT-4), a predominant isoform of glucose transporter in skeletal muscle, is linearly related to the maximal insulin- and contraction-stimulated glucose transport activity⁵, providing evidence that GLUT-4 protein content determines whole-body glucose disposal as well as muscle glucose transport capacity. This review provides a brief overview of the effects of exercise and nutritional status on GLUT-4 content in skeletal muscle and the current thinking on the molecular regulatory mechanisms of GLUT-4 gene expression by exercise and nutritional stimuli.

Adaptive response of muscle GLUT-4 to exercise training

Two isoforms of the facilitative glucose transporter family are expressed in skeletal muscle, GLUT-4 and GLUT-1. In an unstimulated state, the GLUT-4 isoform is located in an intracellular pool, and then it is translocated to the sarcolemma by the actions of insulin and muscle contraction. This represents the major mechanism by which
insulin and contraction stimulate glucose transport into the skeletal muscle. The much less abundant GLUT-1 isoform appears to reside primarily in the plasma membrane, where it likely plays a role in basal, but not in insulin- or contraction-stimulated glucose transport.

It has been well documented in numerous studies that low- to moderate-intensity endurance exercise training results in a remarkable adaptive increase in GLUT-4 protein content in rodent skeletal muscle. An adaptive increase in GLUT-4 is associated with a proportional increase in maximal insulin-stimulated muscle glucose transport activity. Exercise training-induced adaptation in GLUT-4 is also observed in humans (e.g. Ref 9). This is one reason why exercise is widely used as an effective tool for prevention and treatment of type 2 diabetes. In contrast to GLUT-4, exercise training has less effect on GLUT-1 content in skeletal muscle.

Studies of the effects of exercise on muscle GLUT-4 content have involved long-term exercise training, usually consisting of a 6 to 12-week program of running or swimming because of the general belief that skeletal muscle adapts to exercise relatively slowly. However, to provide a survival advantage, an adaptation must occur rapidly enough to enhance the organ’s capacity to respond to the environmental stimulus to which it is adapting. Ren et al. therefore performed a study to test the hypothesis that the adaptive increase in GLUT-4 occurs rapidly in response to an adequate adaptive stimulus. Consequently, it was found that GLUT-4 protein content was increased by about 50% in rat skeletal muscle 16 hours after exercise, rats exhibited a 2-fold increase in GLUT-4 protein content in swimming-trained rat skeletal muscle. In this study, rats bearing a weight equivalent to 14 - 16% of their body weight repeated a 20-second swimming bout 14 times separated by a rest between exercise bouts (net exercise time was only 280 s/day). After 8 days of the high-intensity intermittent training, GLUT-4 protein content in rat skeletal muscle was increased 2-fold compared to that of sedentary control rats. Furthermore, this high-intensity intermittent training-induced increase in GLUT-4 was comparable to that induced by 6-h low-intensity prolonged exercise training, which is regarded as the maximal stimulus related to exercise training (Fig. 1). These findings suggest that a high-intensity intermittent exercise training could be used to improve skeletal muscle glucose metabolism. However, the protocol used in the above-mentioned study is extremely strenuous and exhaustive, and may not be suitable for exercise oriented toward health promotion. In a subsequent study, Fujimoto et al. showed that only 3 intermittent bouts of high-intensity exercise/day for 5 days could increase GLUT-4 protein content in the muscle to a level that is not statistically different from that observed after exhaustive training consisting of 14 bouts of intermittent exercise. These results indicate that non-exhaustive, short-term intermittent exercise training could be a valuable tool for healthy humans in terms of preventing type 2 diabetes safely.

**Mechanisms underlying exercise-induced GLUT-4 biogenesis**

Although the discovery that exercise training increases muscle GLUT-4 protein was reported in the early 1990s, the molecular mechanisms by which exercise stimulates GLUT-4 biogenesis in skeletal muscle has not been fully elucidated. The first breakthrough in elucidation of how muscle GLUT-4 is regulated was the paper by Liu...
et al. showing that there is a myocyte enhancer factor 2 (MEF2)-binding site in the GLUT4 promoter that is essential for GLUT4 expression in C2C12 myotubes\(^{15}\). That study also found that the MEF2 binding site is necessary, but not sufficient, for transcriptional activation. A subsequent study identified a 30-bps regulatory element, located upstream of the MEF2-binding site. The protein that binds to the sequence was cloned and named GLUT-4 enhancer factor (GEF)\(^{16}\). Both MEF2 and GEF proteins binding to their recognition sites are shown to be necessary for normal GLUT-4 mRNA expression.

The second breakthrough was the discovery of an inducible coactivator, the peroxisome proliferator-activated receptor gamma coactivator-1α (PGC-1α), which docks on and activates many transcriptional factors, and thus enhances the expressions of its target genes\(^{17}\). PGC-1α was cloned from a differentiated brown fat cell line, and it is now widely accepted as a key regulator of adaptive mitochondrial biogenesis in skeletal muscle. It has been reported that overexpression of PGC-1α triggers a significant increase in GLUT-4 mRNA and protein content in cultured myotubes and mice skeletal muscle\(^{18,19}\). These findings provide evidence that PGC-1α plays an important role in the regulation of GLUT-4 as well as mitochondrial biogenesis in skeletal muscle.

Exercise causes numerous disturbances in cellular homeostasis in skeletal muscle, making it difficult to determine which of the many signals generated during exercise are responsible for inducing the increase in GLUT-4. However, previous studies have suggested that one group of signaling candidates that induce GLUT-4 biogenesis are muscle high-energy phosphates, which decline in exercising muscle. Another candidate is the increase in cytosolic Ca\(^{2+}\) concentration.

Depleting muscle creatine phosphate by feeding rats a creatine analog, β-guanidinopropionic acid, causes muscle GLUT-4 protein to increase\(^{20}\), 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 the flux through metabolic pathways. Chronic activation of AMPK by injection of the adenine analog 5-aminomidazole-4-carboxamide ribonucleotide (AICAR) into rats results in an increase in GLUT-4 protein in vivo\(^{21}\); the same pattern occurs when isolated rat muscles are exposed to AICAR in vitro\(^{22}\). Furthermore, AMPK phosphorylates GEF\(^{23}\) and the transcription repressor histone deacetylase 5 (HDAC5)\(^{24}\). These modifications by AMPK result in the activation of GEF and release of MEF2 from repression by HDAC5, which lead to increased GLUT-4 gene expression. An acute bout of high-intensity intermittent exercise has been shown to substantially increase AMPK activity in rat skeletal muscle\(^{25}\). This higher activation of AMPK might be involved in the above-mentioned high-intensity training-induced increase in GLUT-4 content in skeletal muscle.

Muscle contraction increases the concentration of intracellular Ca\(^{2+}\), and results in the activation of calcium/calcmodulin-dependent protein kinase (CaMK). Ojuka et al. reported that raising cytosolic Ca\(^{2+}\) by exposing L6 myotubes to caffeine, which release Ca\(^{2+}\) from the sarcoplasmic reticulum, induces increases in GLUT-4\(^{26}\). This increase in GLUT-4 in myotubes probably results from enhanced expression of MEF2A and MEF2D, which were shown to form heterodimer and activate GLUT-4 gene transcription in striated muscle\(^{27}\). In addition, transgenic expression of CAMKIV in skeletal muscle increases the amount of GLUT-4 mRNA compared with that in wild-type mice\(^{28}\). These findings provide evidence that CaMK, as well as AMPK signaling, mediates the exercise-induced GLUT-4 biogenesis in skeletal muscle.

Shortly after the discovery of PGC-1α, Terada et al. reported for the first time that a single bout of endurance exercise induces rapid increases in PGC-1α mRNA and protein in rat skeletal muscle\(^{29,30}\). They also demonstrated that incubation of the rat epitrochlearis muscle with AICAR induced an increase in PGC-1α mRNA and protein content. Furthermore, overexpression of the constitutively active form of AMPK was shown to increase PGC-1α protein content in mice skeletal muscle\(^{31}\). On the other hand, overexpression of CAMKIV in skeletal muscle results in an increase in PGC-1α expression\(^{32}\). Furthermore, inhibition of CaMKII activity prevents the Ca\(^{2+}\)-induced increase in PGC-1α expression in L6 myotubes and the rat epitrochlearis muscle\(^{33,34}\). PGC-1α binds to and activates MEF2 and also increases MEF2 protein expression by activating nuclear respiratory factor-1 (NRF-1)\(^{35}\). Taken together, these findings suggest that enhanced expression and activation of PGC-1α is involved in the exercise-induced upregulation of GLUT-4 expression, possibly through AMPK and CaMK signaling. Fig. 2 shows the hypothetical mechanisms that regulate GLUT-4 gene transcription in response to exercise.

**Effect of nutritional status on GLUT-4 content in skeletal muscle**

As mentioned above, exercise training has been well recognized as a strong inducer of GLUT-4 in skeletal muscle. Emerging evidence suggests that nutritional status also regulates GLUT-4 expression in skeletal muscle.

Reducing the energy intake by alternate-day fasting, which consists of a period (most often 24 h) of ad libitum feeding alternating with a period of fasting, has recently received considerable attention as a new diet strategy to help obese individuals lose weight and fat mass. Since the alternate-day fasting has been shown to induce mitochondrial biogenesis in the heart\(^{36}\), which usually occurs in parallel with an increase in GLUT-4\(^{38}\), we assessed
the effect of alternate-day fasting on muscle GLUT-4 expression in rats. Body weight and total intra-abdominal fat mass substantially decreased in rats exposed to 6-wk alternate-day fasting. However, contrary to expectation, we found that the GLUT-4 protein content in rat skeletal muscle is decreased after 6-wk alternate-day fasting, while 1-wk endurance exercise training induced a significant increase in muscle GLUT-4 content (Fig. 3)\(^7\). This result seems to contrast with previous studies showing that the restriction of daily food intake to 60 - 75% of ad libitum enhanced insulin-stimulated glucose transport in rat skeletal muscle without affecting the total GLUT-4 content\(^3\). It was also reported that 15-day alternate-day fasting increased insulin-mediated glucose uptake rates in humans without affecting muscle GLUT-4 content when subjects ate sufficient food on the non-fasting days to maintain their body weight throughout the intervention\(^3\). On the basis of these findings, it is possible that calorie restriction and alternate-day fasting with/without maintaining body weight exerts different effects on GLUT-4 content and glucose metabolism in skeletal muscle.

Recently, we reported that 4-week high-fat, high-calorie diet feeding, which is an experimental model frequently used to induce obesity and muscle insulin resistance, causes increases in PGC-1α protein content and mitochondrial enzyme activity in rat skeletal muscle\(^4\). As mentioned above, PGC-1α plays an important role in the regulation of GLUT-4 gene expression in skeletal muscle. It therefore seems plausible that GLUT-4 protein content might be elevated in the skeletal muscle of rats fed a high-fat diet. However, it was found that a high-fat diet has no effects on muscle GLUT-4 protein content, and results in a decrease in GLUT-4 mRNA expression despite an increase in PGC-1α\(^4\). The dissociation between PGC-1α and GLUT-4 protein content, in rats fed a high-fat diet, suggests that a substantial increase in PGC-1α protein is not sufficient to activate GLUT-4 gene transcription, and that other factors activated by a high-fat diet are involved in the decrease in transcription and/or cause the degradation of GLUT-4 mRNA.

Raising blood FFA by high-fat diet feeding has been shown to activate the nuclear receptor peroxisome proliferator-activated receptor (PPAR) delta (δ), which directly binds to DNA and regulates genes involved in fatty acid uptake and catabolism in skeletal muscle\(^4\). We therefore evaluated the effect of activation of PPARδ on GLUT-4 expression, and found that treatment by GW501516, a specific activator of PPARδ, significantly reduced the GLUT-4 mRNA and protein content in L6 myotubes\(^4\). This result suggests that the high-fat diet-induced activation of PPARδ downregulates GLUT-4 expression at a transcriptional level. However, no significant decrease in GLUT-4 protein content was observed in the skeletal muscle of rats fed a high-fat diet, despite a 30% decrease in muscle GLUT-4 mRNA expression\(^4\). Based on these findings, it is likely that a posttranslational regulatory mechanism compensates for the decreased GLUT-4 mRNA induced by PPARδ activation, resulting in the preservation of muscle GLUT-4 protein content in rats fed a high-fat diet.

Recently, Gan et al. reported a novel function of PPARδ as a transcriptional coactivator\(^2\). They reported that GLUT-4 protein content is upregulated in the skeletal muscle of mice with PPARδ overexpression. In addition, they indicated that PPARδ has the capacity to coactivate MEF2 with AMPK without binding DNA in a ligand-independent manner. Taken together, it is likely that activation of PPARδ, by its ligand, and increased PPARδ
expression induce opposite effects on GLUT-4 gene expression in skeletal muscle.

**Future prospects**

Although exercise can be used as a treatment to ameliorate diabetes, the development of a dietary or pharmacological intervention that mimics the adaption to exercise is necessary for individuals who are unable to exercise vigorously enough to induce significant adaptation because of disease or old age. GLUT-4 is a potential target for therapy oriented to enhancing glucose disposal by skeletal muscle. For that purpose, future extensive studies are required to understand the precise mechanisms that control GLUT-4 transcription, translation and post-translational steps.

**References**


