Rapid upregulation of GLUT-4 and MCT-4 expression during 16 h of heavy intermittent cycle exercise


Department of Kinesiology, University of Waterloo, Waterloo, Ontario, Canada

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Green HJ, Duhamel TA, Holloway GP, Moule JW, Ranney DW, Tupling AR, Ouyang J. Rapid upregulation of GLUT-4 and MCT-4 expression during 16 h of heavy intermittent cycle exercise. Am J Physiol Regul Integr Comp Physiol 294: R594–R600, 2008. First published December 5, 2007; doi:10.1152/ajpregu.00699.2007.—In this study, we have investigated the hypothesis that an exercise protocol designed to repeatedly induce a large dependence on carbohydrate and large increases in glycolytic flux rate would result in rapid increases in the principal glucose and lactate transporters in working muscle, glucose transporter (GLUT)-4 and monocarboxylate transporter (MCT)-4, respectively, and in activity of hexokinase (Hex), the enzyme used to phosphorylate glucose. Transporter abundance and Hex activity were assessed in homogenates by Western blotting and quantitative chemiluminescence and fluorometric techniques, respectively, in samples of tissue obtained from the vastus lateralis in 12 untrained volunteers [peak aerobic power (V\textsubscript{O\textsubscript{2peak}}) = 44.3 ± 2.3 ml·kg\textsuperscript{-1}·min\textsuperscript{-1}] before cycle exercise at repetitions 1 (R1), 2 (R2), 9 (R9), and 16 (R16). The 16 repetitions of the exercise were performed 6 min at ~90% V\textsubscript{O\textsubscript{2peak}} once per hour. Compared with R1, GLUT-4 increased (P < 0.05) by 28% at R2 and remained elevated (P < 0.05) at R9 and R16. For MCT-4, increases (P < 0.05) of 24% were first observed at R9 and persisted at R16. No changes were observed in GLUT-1 and MCT-1 or in Hex activity. The ~17- to 24-fold increase (P < 0.05) in muscle lactate observed at R1 and R2 was reduced (P < 0.05) to an 11-fold increase at R9 and R16. It is concluded that an exercise protocol designed to strain muscle carbohydrate reserves and to result in large increases in lactic acid results in a rapid upregulation of both GLUT-4 and MCT-4.

This is best illustrated for carbohydrates (CHO), the principal fuel used by the muscle in energy metabolism. This fuel is provided in the form of endogenous glycogen deposited in the muscle cell and in the form of blood glucose delivered to the muscle from the liver, where it is generated by glycolysis and gluconeogenesis (9). In muscle during exercise, the activation of phosphorylase results in hydrolysis of glycogen and the formation of glucose-1-phosphate, which then enters the glycolytic pathway. In contrast, blood glucose after crossing the plasma membrane is phosphorylated to glucose-6-phosphate by the activation of hexokinase (Hex) before entering the glycolytic pathway.

Glucose entry into the cell can potentially represent a rate-limiting site in the availability of glucose as a cellular substrate. This problem has been addressed by a family of proteins called glucose transporters (GLUT), of which GLUT-4 and GLUT-1 represent the major and minor forms in skeletal muscle, respectively, that allow for the facilitated diffusion of this substrate (27). It is known that both contractile activity and insulin result in the translocation of GLUT-4 to the plasma membrane from intracellular storage sites, allowing increased entry of glucose for use as a substrate during contractile activity or during recovery from contractile activity to replenish the glycogen stores (27).

Glycolysis also results in the formation of lactate and hydrogen ions, potential inhibitory constituents of cell function if the accumulation is not regulated. Regulation is mediated by a special family of proteins called monocarboxylate transporters (MCT), of which MCT-4 and MCT-1 represent the major and minor forms in muscle, respectively (20, 33). These transporters are specialized for the facilitated cotransport of both the lactate anion and the hydrogen proton across membranes for redistribution to other sites both inside and outside the cell.

It is now recognized that regular contractile activity can induce an extensive remodeling of the cell resulting in the altered abundance of a wide range of proteins and isoforms involved in a variety of processes (46). Numerous studies have documented the changes that occur in the enzymes of the metabolic pathways to increased usage, the pattern of change depending on the intensity and duration of the exercise and consequently the strain imposed on the pathways involved in ATP resynthesis (48). It is clear that these changes are accompanied by increases in the proteins involved in both glucose (10, 62) and lactate (20, 33) transport. Collectively these adaptations appear to promote a tighter metabolic control resulting in less of a disturbance in phosphorylation potential to achieve a given level of oxidative phosphorylation.
A controversial area is the rate at which these adaptations can occur after the onset of training and whether the proteins and processes involved in addressing a common problem all change in a coordinated fashion.

There is evidence to suggest that GLUT-4 can be dramatically upregulated (27), with initial increases observed within the first few sessions of regular exercise (31, 36, 50, 53). Although there is evidence that GLUT-1 can also increase with training, the time frame for response seems to be considerably longer (50). Hex has also been shown to be a readily adaptable enzyme, capable of responding with just a few exercise sessions (26, 44, 45, 50). Such also appears the case for the lactate transporters MCT-1 and MCT-4 (3, 7, 17, 33, 50).

Although these studies underline the adaptable nature of the wide range of proteins involved in substrate delivery, metabolism, and by-product distribution, it is not clear how rapidly the adaptations occur and whether or not the expressions of the various adaptations have a similar time frame. Since most studies have employed submaximal aerobic-based exercise, it is possible that strain imposed on the different processes involved in substrate utilization and the management of the metabolic by-product, lactic acid, may not have been sufficient to promote optimal adaptation. We have addressed this issue by using a 16-h model of heavy intermittent exercise designed to repeatedly strain the processes involved in both excitation and contraction and the metabolic pathways involved in OXPHOS and glycolysis. Using this model, we have been able to demonstrate early adaptive responses in muscle performance (14), the Na+/K+-ATPase (15), sarcoplasmic reticulum Ca2+ cycling (29), and metabolic behavior (16) in the working vastus lateralis muscle. In this article, we report on the responses of proteins involved in glucose and lactate transport.

The purpose of this study was to investigate the effects of repetitive heavy exercise on the expression of the glucose transporters GLUT-1 and GLUT-4, the lactate transporters MCT-1 and MCT-4, and Hex in working muscle. We have rationalized that the use of an intermittent protocol of heavy exercise designed to induce a large and repetitive activation of muscle (24, 27, 28).

Experimental Design

The design employed involved the performance of 16 bouts of cycle exercise at ~91% V\textsubscript{O\textsubscript{2peak}}, each bout lasting for 6 min, over 16 h. This protocol allowed for a 54-min period of recovery between exercise bouts, sufficient to allow for restoration of muscle high-energy phosphates and the return of lactate to near-resting levels (16). Tissue was extracted by needle biopsy from the vastus lateralis (2) before and after exercise at repetitions 1 (R1), 2 (R2), 9 (R9), and 16 (R16). For the measurement of GLUT, MCT, and Hex, only the tissue extracted before exercise at R1, R2, R9, and R16 was used for analyses. This tissue was immediately placed in liquid N\textsubscript{2} and stored at −80°C until the measurements were made. To reduce the number of biopsies that would have to be performed in the 16-h period, we administered R1 and R2 ~4 wk before the date when the total 16-h protocol was performed. The procedures for the entire protocol were as detailed above, with the exception that no tissue was harvested at R1 and R2. The biopsies were alternated between legs at sites that had been prepared before the start of exercise and from regions previously described (29).

On each experimental day, the participants ingested a can of Ensure (250 kcal) meal replacement consisting of 9.4 g of protein, 6.7 g of fat, and 38 g of CHO (Ross Products Division, Saint-Laurent, QC, Canada). The Ensure was ingested after reporting to the laboratory in the morning and ~2 h before exercise. We routinely use this protocol to standardize nutrient intake before exercise. During the 16-h protocol, dietary supplements and water were only allowed after the first two repetitions of the exercise. After the first two repetitions, water was allowed ad libitum and the consumption of vegetables, fruits, and Gatorade bars was provided on a regulated basis. The details of the dietary composition and the energy intake appear in an earlier publication (14). For 1 wk before the intermittent work protocol, all participants were instructed to follow their normal diet, to avoid alcohol, and not to engage in vigorous activity.

Analytical Procedures

Transports. For the measurement of concentrations of GLUT and MCT, electrophoresis and Western blotting were performed on homogenates extracted from the vastus lateralis. For the determination of GLUT-1 and GLUT-4, frozen tissue (20–30 mg) was diluted 1:20 with homogenizing buffer consisting of 10 mM HEPES pH 7.4, 250 mM sucrose, 2 mM EGTA and a protease inhibitor cocktail (Roche Diagnostics, Mannheim, Germany). The homogenate was prepared with a Polytron 2100 homogenizer (2 × 15 s at a setting of 7). A pellet was isolated by centrifugation (230,000 g for 75 min at 4°C) and homogenized in 1–2 ml of buffer (10 mM Tris base, 1 mM EDTA, pH 7.4) with the Polytron homogenizer. The homogenate (50 μg in 2% SDS buffer) was applied to a 10% polyacrylamide gel, separated with standard SDS-PAGE protocols (38), and transferred to polyvinylidene difluoride (PVDF) membranes. After blocking with a 5% skim milk suspension for 1 h at 22°C, the membranes were incubated for 16 h at 4°C with anti-GLUT polyclonal antiserum (CBL242 and CBL243 for GLUT-1 and GLUT-4, respectively) obtained from Chemicon International. Dilution was 1:200 for each serum.

After being washed in Tris·HCl pH 7.5, 150 mM NaCl, 0.1% Tween (Tris-buffered saline-0.1% Tween), the membranes were treated with horseradish peroxidase-conjugated anti-rabbit secondary antibody (Santa Cruz Biotechnology) for 1 h. Membranes were then washed in Tris-buffered saline-0.1% Tween. Isoform detection was accomplished with an enhanced chemiluminescence procedure (Amersham, Little Chalfont, UK). Blots were analyzed with a Chemi Genius 2 model bioimaging system (Syngene, Frederick, MD) with Syngene software version 1.0.

The measurement of MCT-1 and MCT-4 followed the same procedures. However, in the case of these proteins, dilution was 1:400 in polyclonal antiserum (AB3353P and AB3316P for MCT-1 and MCT-4, respectively; Chemicon International).
We have verified the linearity of measurement over the range of protein levels used for each of the transporters measured. All samples were run in duplicate on separate gels. Protein was measured by the Bio-Rad assay, in which detergent was present.

For all transporter measurements, the individual values were originally expressed as a percentage of a standard (α-actin content) and then as a percentage of the initial (R1) value. The preexercise value was set at 100%.

**Heat shock protein 70.** We also examined for changes in muscle heat shock protein (HSP70) since this protein is known to be involved in stabilizing a wide range of proteins that may be altered by the stress of exercise (41). Measurement of HSP70 was also made by Western blot and immunodetection, also using the general procedures described for the measurement of the GLUT and MCT transporters. For this protein, 10 μg of homogenate in 2% SDS buffer was applied to a 10% polyacrylamide gel, separated with standard SDS-PAGE protocols, and trans-ferred to PVDF membranes. After blocking with a 10% skim milk suspension, the membranes were incubated for 16 h at 4°C with the anti-HSP70 monoclonal antibody SPA-810 (Stressgen Biotechnologies). After being washed in Tris-buffered saline-0.1% Tween, the membranes were treated with horseradish peroxidase-conjugated anti-mouse secondary antibody (Santa Cruz Biotechnol-ogy) for 1 h and washed in Tris-buffered saline-0.1% Tween, the signals were detected, and the densitometric analysis performed as described for the GLUT and MCT transporters. All samples were run in duplicate.

**Hexokinase activity.** For the measurement of Hex activity, the frozen tissue samples (20–25 g) were hand-homogenized in a phos-photase buffer (pH 7.4) containing 0.02% bovine serum albumin (BSA), 5 mercaptoethanol, and 0.5 mM EDTA. As with the homogenates used for the measurements of other properties, care was taken to remove visible connective tissue. Homogenates were then diluted in 20 mM imidazole buffer with 0.02 BSA. Measurement of Hex was performed as previously described by others (25) and by our labora-tory (50). In brief, 80 μg of muscle was added to 100 μl of Tris-HCl buffer (pH 8.1) containing (mM) 5 glucose, 5 ATP, 2 MgCl2, and 0.5 NAD±, with 0.5% Triton X-100, 0.5% BSA, and glucose-6-phos-photase dehydrogenase (2 U/ml). The reaction was allowed to proceed for 1 h at room temperature (23–24°C) until addition of HCl and incubation at 95°C for 3 min to stop the reaction. After the reaction was stopped, 1 ml of 50 mM imidazole buffer (pH 7.0), 100 μM NADP, 30 mM ammonium acetate, 5 mM MgCl2, 1 mM EDTA, and phosphogluconate dehydrogenase (0.1 U/ml) were added and allowed to stand at room temperature for 15 min. Hex was determined by the amount of NADPH fluorescence measured against standards for glucose-6-phosphate.

For all properties, the samples for a given individual and for a given property were assessed during the same analytical session.

**Data analyses.** One-way ANOVA procedures for repeated mea-sures were used to examine the effect of the number of repetitions of the exercise on muscle GLUT and MCT isoforms, Hex, and HSP70. Where significance was found, the Newman-Keuls technique was applied to determine which means were significantly different. The probability for statistical significance was set at P < 0.05. Data are presented as means ± SE. Where differences are indicated in the text, significance is implied.

**RESULTS**

**Transporters**

We found that the repetitive sessions of heavy exercise resulted in an increase in GLUT-4 but not GLUT-1 (Fig. 1). For GLUT-4, the initial increase was observed before exercise at R2. The increase observed at R2 persisted at both R9 and R16.

Our exercise protocol also resulted in rapid increases in MCT-4 (Fig. 2). The increase, which amounted to 24%, was first observed at R9. No further increase in MCT-4 was found at R16. Although there was a trend for MCT-1 to increase at R2, the change was not significant. Further repetitions of the exercise also failed to induce an increase in MCT-1.

**Hexokinase Activity**

Measurements of Hex performed before exercise did not result in an altered activity regardless of the number of repetitions performed (Fig. 3).

**Heat Shock Protein**

HSP70 was elevated by the intermittent exercise protocol, but not until R9 (Fig. 4). At R16, an increase of 16% was found. At R16, the change in HSP70 was not different from R1.

**DISCUSSION**

This study has produced several novel results. As hypothe-sized, we have found that both GLUT-4 and MCT-4, the principal glucose and lactate transporters in skeletal muscle respectively, can be rapidly upregulated. In contrast, we failed...
to find an increase in the abundance of either GLUT-1 or MCT-1. We also failed to find an upregulation in Hex with our exercise challenge as proposed. The fact that Hex did not change and that the increases in GLUT-4 and MCT-4 occurred at different times during our repetitive exercise protocol would also suggest that the responses are not coupled as suggested.

This study confirms what has been reported on many previous occasions, namely, that exercise is a potent stimulus for the upregulation in GLUT-4 (10, 27). Our results also confirm the results of others in that the increase in GLUT-4 is an early adaptive event occurring soon after the initiation of regular contractile activity in both human (19, 50) and rat (23, 31, 53) skeletal muscle. In fact, it has been demonstrated that an increase in GLUT-4 can occur after exercise in rat muscle with 3–6 h of swimming (37, 1, 53) and in human muscle with 27–60 min of cycle exercise (18, 35). What is unique about our study is that we have been able to demonstrate that the increase in GLUT-4 can occur with just one 6-min session of heavy exercise. Although there was a suggestion that continued performance of the intermittent exercise would result in further increases in GLUT-4, as indicated by an additional 24% increase by the ninth repetition of the exercise, the difference was not significant.

Interestingly, few studies have examined the effect of exercise on GLUT-1, which is also expressed in skeletal muscle but to a minor extent (27). We have reported (50) that increases in GLUT-1 in human vastus lateralis can be induced with prolonged cycle exercise but not as an early response to training.

The effect of exercise on GLUT-1 expression in muscle appears controversial in rodents, with some studies reporting increases (27, 52) while others have not (13, 54). This uncertainty may be a reflection of differences in training stimulus and/or the fiber type composition of the muscle studied. Although the increases in GLUT-4 observed in our study as well as others can be explained by a combination of altered degradation and/or synthesis rates, it is generally acknowledged that increased transcription is the dominant mechanism (27, 42). It was reported previously that a single session of exercise can result in an approximately twofold increase in mRNA synthesis immediately after exercise (35) and at 3 h of recovery (35, 43), with a return to baseline at 24 h of recovery (43). There is increasing evidence that the signaling mechanism promoting the increase in GLUT-4 mRNA with contrac-
tile activity is mediated by reductions in the phosphorylation status of the cell. According to current theory, the signaling molecule is AMP-activated protein kinase (AMPK), which is very sensitive to disturbances in cellular energy homeostasis (21, 61). Recently, it was reported that nitric oxide, which increases with contractile activity, can increase GLUT-4 expression via mechanisms that include AMPK signaling (39). There is also evidence to indicate that increases in cytosolic free calcium (Ca$^{2+}$) may act via second messenger systems to increase transcription of GLUT-4 (27).

Although we have not measured the changes in GLUT-4 mRNA in this study, there is reason to believe that it would increase given the pronounced effect that our intermittent heavy exercise protocol had on the energy status of the cell (16) and the reported cycle of changes in Ca$^{2+}$ (29). However, studies remain to be completed examining the role of both transcriptional and posttranscriptional mechanisms in the effects observed.

We have also hypothesized that the increase in GLUT-4 would be accompanied by parallel increases in Hex, the enzyme used to phosphorylate glucose to glucose-6-phosphate on entry into the cell. This theory was based on previous studies demonstrating increases in both GLUT-4 and Hex in skeletal muscle with just a few sessions of exercise (26, 31, 36, 50, 53). Moreover, studies employing 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR) to study the upregulation in GLUT-4 have also reported pronounced increases in Hex (30). Additional exercise-based studies have also shown that a single exercise session results in increases in both Hex activity and mRNA, suggesting that the increase in activity is mediated by increased protein, secondary to increased transcription (44, 45). The coordinated increases in GLUT-4 and Hex would not appear to apply to this study, since we found no change in Hex activity regardless of the number of exercise repetitions completed. In fact, there was a tendency (P = 0.10) for a decrease to occur by the 16th repetition of the exercise. It should be emphasized that not all studies report parallel increases in both GLUT-4 and Hex (43).

There is evidence to indicate that CHO intake in the recovery period and the glycogen repletion status of the exercised muscle can affect both the persistence of the increase in GLUT-4 that occurs with the exercise (4–6, 31) and the response of Hex (4). In the presence of CHO, the changes that occur in these properties are more rapidly reversed (4–6). In this study, we allowed nutrient intake to occur on a regulated basis after 2 h of the exercise protocol. The kilocalories consumed for each 4 h of the protocol ranged between 465 and 644, with total intake amounting to 2,339 ± 195 (14). The percent of total kilocalories from CHO averaged 78.4 ± 1.3. The CHO intake was apparently insufficient to avoid glycogen depletion, since by the end of the exercise only 10% remained in the working vastus lateralis (16). It is possible that the response of both GLUT-4 and Hex could have been altered by the CHO feeding.

With the exercise protocol that was utilized, and the large demand for CHO given the intensity of the exercise and the large increase in glycolytic flux rate that occurred, pronounced reductions in endogenous muscle glycogen concentration were both expected and observed (16). Blood glucose could be an important source of substrate in muscle for glycolysis both during the repeated bouts of exercise (34) and for glycogenogenesis during the 54 min of recovery between exercise bouts (60), during which time nutrients were allowed. In this regard, the increase in GLUT-4 is important during both exercise and recovery in making glucose available to the muscle cell (27).

As expected given the numerous other studies in both rodents (7, 47) and humans (3, 12, 17, 51), our intermittent exercise protocol also resulted in increases in MCT-4. Increases in MCT-1 are indicated, as suggested by the 20% increase by the second repetition of the exercise, but the change was not significant (P = 0.10). It is accepted that MCT-1, which only exists in minor concentrations in muscle, can increase if the training stimulus is appropriate (3), which could happen with just a single session of the exercise (7, 17). Current evidence suggests that the MCTs exist only in the plasmalemma and are not translocated to the plasmalemma from intracellular sites with contractile activity (20, 33). The rate of cotransport of lactate and hydrogen ion by facilitated diffusion is directly related to the abundance of MCT-4 in the plasmalemma (20, 33). We showed earlier in humans (49), as have others (11) using radioisotopes, that lactate clearance is increased with training. In our study, we found that the increased clearance occurs with the first few days of training onset (49). It is inviting to speculate that the dramatic reduction in lactate concentration that we reported in the muscle at the end of the ninth repetition of the exercise in an associated paper (16) was to some degree mediated by enhanced transport out of the cell. In the absence of measurements of lactate transport, we cannot rule out the importance of a reduction in glycolysis or the use of pyruvate (57, 58) or lactate in mitochondrial respiration (12). As with GLUT-4, it is not clear whether the increase in MCT-4 was mediated by altered rates of synthesis and/or degradation. Increases in transcription are believed to occur in response to increases in muscle lactate (33, 51). Interestingly, the exercise used in this study results in large increases in muscle lactate (16). A recent study has reported that in L6 cells, high lactate results in increases in MCT-1 but not MCT-4 (22).

**Perspectives and Significance**

In summary, we have found that a protocol of heavy intermittent exercise designed to repeatedly challenge CHO and lactic acid homeostasis results in a rapid increase in the principal transporters involved in the facilitated diffusion of glucose and lactic acid across the muscle plasmalemma. These results indicate that GLUT-4 and MCT-4 belong to a class of proteins designed to respond quickly to the imposition of substrate and metabolic strain. The increase in strain occurring as a result of the intermittent exercise protocol can be appreciated by the early increase in HSP70 that also occurs. The increase in HSP70 is known to respond to a variety of perturbations, including exercise (40, 41, 56). The increase in HSP70 is also known to be involved in the protection of a range of proteins involved in such things as mitochondrial function (55) and sarcoplasmic reticulum Ca$^{2+}$ cycling (59).

This paper represents one of a series of papers in which we have examined the adaptive responses in a wide range of properties to 16 repetitions of heavy exercise performed once per hour. Each 6-min bout resulted in increases in OXPHOS to levels near VO$_2$ peak (14) and a major activation of glycolysis as indicated by the large increase in muscle lactate (16). Under
these conditions, providing for increased glucose delivery to the muscle while decreasing lactate and hydrogen ion accumulation would appear to be desirable adaptations. The increases that we have observed in GLUT-4 and MCT-4 may represent an early and important response in the management of muscle carbohydrate and lactic acid.

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