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Journal of Nutritional Biochemistry 16 (2005) 267–271

Journal of Nutritional Biochemistry

RESEARCH ARTICLES

Effect of postexercise carbohydrate supplementation on glucose uptake-associated gene expression in the human skeletal muscle

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Abstract

We previously found that the exercise-induced elevation in GLUT4 mRNA of rat muscle can be rapidly down-regulated when glucose is given immediately following exercise. The purpose of this study was to determine the effect of postexercise carbohydrate diet on GLUT4 and hexokinase (HK) II mRNA levels in the human skeletal muscle. Eight untrained male subjects (age, 20.7 ± 3.1 years) exercised for 60 min on a cycle ergometer at a 70–75% maximal oxygen consumption. The postexercise dietary treatment was performed in a crossover design. Immediately after the exercise, a diet with 70% carbohydrate content (1 g per kilogram of body weight; 356 ± 19.8 kcal) was given to half of the subjects (eaten in 10 min) followed by a 3-h recovery, while the control subjects remained unfed for 3 h. Biopsies were performed on the deep portion of the vastus lateralis muscle of all subjects immediately after the exercise and 3 h after the carbohydrate ingestion. Blood glucose and serum insulin concentrations were measured every 30 min for 3 h. At the end of the 3-h recovery, blood glucose and serum insulin levels were not different from control levels, indicating that the oral carbohydrate was mostly disposed in the body within 3 h. In addition, GLUT4 and HK II mRNA levels were significantly lowered in the exercised human skeletal muscle in subjects receiving the carbohydrate diet. In conclusion, the present study demonstrates that GLUT4 mRNA and HK II mRNA in the exercised human skeletal muscle were significantly lowered by a high-carbohydrate diet.

Keywords: Exercise; Carbohydrate; GLUT4; Hexokinase II

1. Introduction

The skeletal muscle plays an important role in postprandial glucose uptake in humans [1]. Numerous researchers have found that exercise can increase expression of the proteins that govern glucose uptake in the skeletal muscle including GLUT4 protein and hexokinase (HK) II [2–5]. This molecular adaptation has been thought, to some extent, to be associated with the improvement in insulin sensitivity in normal individuals and diabetes patients [3]. The GLUT4 protein is the predominant isoform of glucose transporter protein expressed in the skeletal muscle, which translocates to plasma membrane upon insulin stimulation to facilitate glucose transporting across the plasma membrane [6,7]. On the other hand, the function of HK II is to phosphorylate the intracellular glucose, and, as a result, it prevents the countertransport of glucose out of the cell membrane. Therefore, HK II and GLUT4 protein work collaboratively to maintain a continuous glucose disposal in the skeletal muscle after a diet.

It was reported that an acute bout of exercise significantly elevates GLUT4 mRNA and HK II mRNA in rat skeletal muscle [8,9]. This has been confirmed in the human skeletal muscle by the study of Koval et al. [4], where moderate intensity treadmill exercise transiently increased the mRNA levels of GLUT4 protein and HK II. In rat muscle, this molecular response is followed by a lingering increase in GLUT4 protein [8,10]. We previously determined that the exercise-induced elevation in GLUT4 mRNA of rat muscle

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doi:10.1016/j.jnutbio.2004.12.006

can be rapidly down-regulated when glucose is given immediately following exercise [10]. In addition, Garcia-Roves et al. [11] demonstrated that the increase in GLUT4 mRNA by exercise remains elevated for at least 42 h in rats fed with a carbohydrate-free diet. These results suggest that postexercise dietary carbohydrate accessibility regulates the level of GLUT4 mRNA in rat skeletal muscle. It is currently unknown whether supplementation of a normal high-carbohydrate diet after exercise can also affect the mRNA levels of GLUT4 and HK II in the human skeletal muscle. Therefore, the purpose of the study was to determine the effect of a high-carbohydrate diet on GLUT4 and HK II mRNA levels in the exercised human skeletal muscle.

2. Methods

2.1. Subjects

Vastus lateralis muscle biopsies were obtained from eight healthy male volunteers (age, 20.7 ± 3.1 years). The Changhua Christian Hospital Ethics Committee approved this protocol, and the nature, purpose and possible risks were explained to each subject before written consent was obtained. This work was conducted in accordance with the guidelines in the Declaration of Helsinki.

2.2. Exercise

Maximal oxygen consumption ($V_{O_2}max$) of all the subjects was measured 2 days before the exercise. On the day of the trial, the subjects reported to the laboratory at 8:00 a.m. after an overnight fast. Subjects were asked to warm up for 5 min and performed a 60-min cycling exercise at a 70–75% $V_{O_2}max$ while drinking water was supplied ad libitum during and after the exercise. Immediately after the exercise, the control group (Control, $n=4\times2$) remained fasted for 3 h, whereas the carbohydrate-supplemented group (CHO, $n=4\times2$) provided a diet containing 70% carbohydrate content for a 3-h recovery. The same experimental trial was performed again 7 days later to determine the consistency of the results, but both treatments were crossed over for all subjects. Muscle biopsy samples were obtained immediately after the exercise and 3 h after the carbohydrate diets for RT-PCR analysis.

2.3. Diet

Immediately after the exercise, toasted bread (moderate GI diet, 1 g carbohydrate per kilogram of body weight) was given to the CHO group and water ad libitum for all subjects. The average energy intake of the diet for the CHO subjects was 356 ± 19.8 kcal (carbohydrate, 70.8 ± 3.9 g; protein, 11.7 ± 0.6 g; fat, 2.8 ± 0.1 g). The diet was consumed within 10 min.

2.4. Muscle tissue collection

Muscle biopsy was performed under local anesthesia (2% lignocaine with adrenaline). An incision of 10 mm length and depth was made in the skin and muscle fascia at about 20 cm above the knee using an aseptic technique. Vastus lateralis biopsies (about 50 mg) from the right quadriceps femoris muscle were obtained using the percutaneous biopsy technique of Bergstrom [12]. Samples were blotted dry and grossly dissected free of fat and connective tissue, frozen in liquid nitrogen and stored at $-80\,^{\circ}C$ before RT-PCR analysis.

2.5. Blood glucose and serum insulin

After the exercise, a 20-G polyethylene catheter (Jelco, Tampa, FL, USA) was placed in an antecubital vein for blood sampling. Blood samples were then taken before and during the postexercise recovery following the carbohydrate supplementation. The catheter was kept clean by flushing with a small amount of saline solution containing heparin (10 IU/ml) following each sample collection. The Control and CHO subjects remained sedentary for 3 h before the second muscle biopsy. During recovery, blood samples were collected every 30 min for 180 min. Blood samples were collected into fluoride heparin and serum tubes. Serum was obtained after centrifuging at $4\,^{\circ}C$ for 15 min at 6000 rpm and was stored at $-70\,^{\circ}C$ before insulin analysis. Blood glucose was determined by an automated glucose analyzer (YSI Life Sciences, Yellow Springs, OH, USA). Serum insulin levels were determined by using the radioimmunoassay method with a commercial kit (Baylor Diagnostics, Tarrytown, NY, USA) according to the manufacturer's instruction.

2.6. RT-PCR for GLUT4 mRNA and HK II mRNA analyses

Total RNA from the vastus lateralis muscle samples was extracted using Tri-Reagent (Molecular Research Center, Cincinnati, OH, USA), and RT-PCR analyses were performed afterward. The quantitative RT-PCR was accomplished by using a recombinant RNA (rcRNA) template as an internal standard (IS) to quantitate mRNA expression according to a method described previously [13]. The procedure for generating an rcRNA template for IS was followed by a procedure as described by Vanden Heuvel et al. [14]. Of total RNA and IS rcRNA, 0.1–0.25 μg was reverse transcribed with M-MMLV reverse transcriptase (Gibco-BRL, Gaithersburg, MD, USA) in a 20 μl final volume of the reaction buffer consisting of 25 mM Tris–HCl (pH 8.3 at $25\,^{\circ}C$), 50 mM $(NH_4)_2SO_4$, 0.3% β-mercaptoethanol, 0.1 mg/ml bovine serum albumin, 5 mM $MgCl_2$, 1 mM of each deoxynucleotide triphosphate, 2.5 U RNase inhibitor and 2.5 mM oligo $(dT)_{16}$ (Promega Corporation, Phoenix, AZ, USA). The reaction mixtures were incubated for 15 min at $45\,^{\circ}C$, and the reactions were then stopped by heat denaturing at $99\,^{\circ}C$ for 5 min. For amplification reaction, the oligonucleotide primer sequences of both GLUT4 and HK II genes were selected by using the computer software Primer Select (DNASTAR, Madison, WI, USA) and were synthesized by MDBio (Taipei, Taiwan, ROC). The primer sequences for the GLUT4 gene are 5′-GGGGCCTACGTETTC-3′ (forward) and 5′-CACGGCCAAACCACAACACAT-3′ (reverse). The primer sequences for HK II are 5′-ATGGTGGAAGGCGATGAGG-

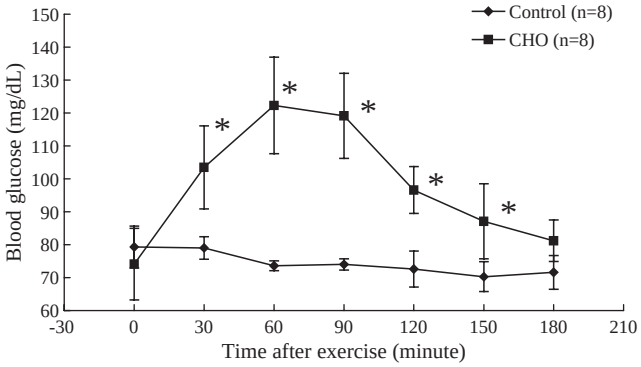

Fig. 1. Blood glucose concentrations following a postexercise high-carbohydrate supplementation. Asterisk indicates significant difference versus control group, $P<.05$.

3′ (forward) and 5′-CTGGCGGAGCGTGTGGAC-3′ (reverse). A PCR master mix containing 4 mM $MgCl_2$, 2.5 U Taq polymerase and 6 pmol forward and reverse primers was added to the newly synthesized complementary DNA samples to a total volume of 50 μl. The reactions for PCR amplification were heated to 94 °C for 3 min and followed by a reannealing step at 55 °C. The elongation step was performed at 72 °C for 60 s. The denaturing–annealing–elongation cycle was repeated 32 times. A 5-min elongation step at 72 °C was carried out after the last cycle. The amplified PCR products of the IS and target mRNA were then separated by 2.5% NuSieve/agarose (3:1 w/w) gel electrophoresis and visualized by ethidium bromide staining. Gels were then photographed and quantified by densitometric analysis with a Zero-Dscan (Scanalytics, FairFax, VA, USA).

2.7. Statistical analysis

Mean difference of both GLUT4 and HK II mRNA levels before and 3 h after carbohydrate supplementation was compared by using the paired *t* test. Comparison of pre- and post-GLUT4 and HK II mRNA levels in fasted and CHO-fed subjects was conducted by independent Student's *t* test. A level of $P<.05$ for Type I error was set for significance for all tests. Data are presented as the mean ± S.E. Power estimation was calculated to determine sample size. A total of six subjects would have been required for 80% power. We initially had eight subjects in this study and thus met the sample size necessary to detect statistical differences.

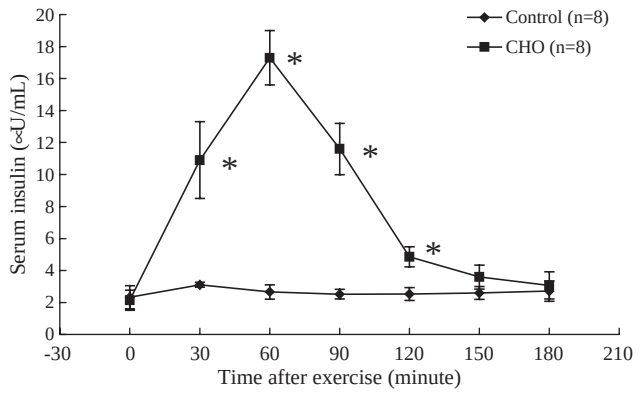

Fig. 2. Serum insulin concentrations following a postexercise high carbohydrate supplementation. Asterisk indicates significant difference versus control group, $P<.05$.

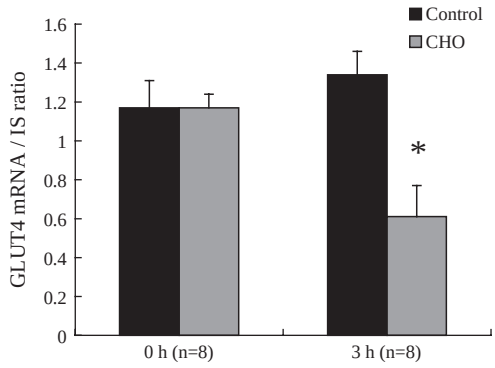

Fig. 3. GLUT4 mRNA level in the deep portion of the vastus lateralis muscle immediately following exercise and 3 h after a postexercise high-carbohydrate supplementation. Asterisk indicates significant difference versus control group, $P<.05$.

3. Results

The blood glucose and serum insulin levels before and during a 3-h recovery following carbohydrate supplementation are shown in Figs. 1 and 2. The blood glucose and serum insulin concentrations of the CHO group peaked at 60 min following the postexercise carbohydrate supplementation and were significantly greater than those of the control group ($P<.05$). At the end of the 3-h recovery period, both glucose and insulin levels in the CHO group returned to a level that was not significantly different from that in the control group.

Fig. 3 displays the result of GLUT4 mRNA from RT-PCR analysis. In the control group, no significant difference in the GLUT4 mRNA levels was observed between the immediate postexercise value and the 3-h postexercise value. In the CHO group, the GLUT4 mRNA level was significantly lower 3 h after the postexercise carbohydrate supplementation compared with the immediate postexercise

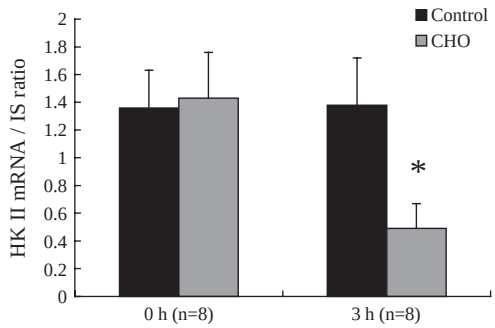

Fig. 4. HK II mRNA level in the deep portion of the vastus lateralis muscle immediately following exercise and 3 h after a postexercise high-carbohydrate supplementation. Asterisk indicates significant difference versus control group, $P<.05$.

level ($P<.05$). The GLUT4 mRNA level in the CHO group was also significantly lower than that in the control group at 3 h postexercise ($P<.05$).

The result for HK II mRNA is shown in Fig. 4. Similarly, no significant difference was found in the HK II mRNA levels between the immediate postexercise value and the 3-h postexercise value in the control group. In the CHO group, the HK II mRNA level was significantly lower at 3 h postexercise compared with the immediate postexercise level and was significantly lower than that in the control group at 3 h postexercise.

4. Discussion

To our best knowledge, this is the first study to demonstrate that postexercise GLUT4 and HK II mRNA levels in the human skeletal muscle can be suppressed by supplementation of a high-carbohydrate diet. In our previous work, we demonstrated that GLUT4 mRNA in rat muscle can be elevated transiently by a prolonged exercise, but maintenance at a high level appears to be dependent on carbohydrate availability during recovery [15]. GLUT4 mRNA levels were still elevated 16 h postexercise in rats intubated with glucose postexercise albeit at lower levels than those increased in postexercise fasted rats. More specifically, postexercise GLUT4 mRNA was reduced dramatically by glucose intubation within 90 min, when glycogen was returned to the preexercised level [10]. These results implicate an interactive effect of carbohydrate intake on the expression and regulation of proteins governing muscle glycogen storage. However, whether the same effect can also influence the HK II mRNA level, another glucose uptake-related gene product that can be induced by exercise, was unknown. In the present study, both GLUT4 and HK II mRNA levels in the human skeletal muscle were reduced by a high-carbohydrate diet compared with the immediate postexercise state. The present result confirms our hypothesis that high-carbohydrate dietary intake immediately after exercise suppresses the mRNA levels for proteins related to glucose uptake in the human skeletal muscle, specifically GLUT4 and HK II.

Similar to the GLUT4 protein, HK II is primarily found in insulin-sensitive tissues such as skeletal muscle and adipose tissue [16], which functions to catalyze the phosphorylation of the intracellular glucose molecule after glucose is transported from outside the cell. This process generates a concentration gradient across the plasma membrane and ensures that the transported glucose is not countertransported out of the cell. Previously, HK II along with GLUT4 has been shown to be elevated after a single bout of exercise both in rats and in humans [4,8,9], implying that both glucose transport and glucose uptake processes were regulated in concert by exercise.

We must note that down-regulation of GLUT4 mRNA and HK II mRNA by carbohydrate supplementation does not mean that the levels of these proteins were suppressed after exercise. GLUT4 and HK II protein levels are generally known to be elevated by both an acute bout of exercise and exercise training [3]. In our previous study, postexercise glucose intubation in exercised rats down-regulated muscle GLUT4 mRNA within 90 min, but the overall GLUT4 mRNA associated with the polysomes in the exercised muscle was significantly increased during the 16-h recovery period [10]. Polysome-associated GLUT4 mRNA is considered to be translationally active mRNA. The translational activation of GLUT4 mRNA by carbohydrate supplementation explains the linear increase in GLUT4 protein level in rat muscle during the recovery period. Conversely, the postexercise fasted condition sustained a high GLUT4 mRNA level in rat skeletal muscle, but, at the same time, the exercised rat muscle exhibited poor translational efficiency [15]. Apparently, the increase in GLUT4 protein synthesis does not require high levels of total GLUT4 mRNA in the muscle but is more closely related to the association of mRNA to polysomes. The influence of mRNA levels, mRNA bound to polysomes and protein expression of HK II was not examined and, therefore, it is unknown whether HK II responds in a similar manner as GLUT4. The current result points to a possibility that HK II may be regulated in the same manner as GLUT4 in response to different dietary conditions after exercise.

The underlying mechanism of subsequent mRNA degradation for GLUT4 and HK II by carbohydrate supplementation during recovery is not clear. The current finding that postexercise GLUT4 mRNA and HK II mRNA in the human muscle were suppressed by postexercise carbohydrate supplementation implies a possibility that the glycogen status in the skeletal muscle plays a role for regulating the glucose uptake process of the skeletal muscle. Ingestion of a high-carbohydrate diet after a glycogen-depleting exercise normally results in a large increase in muscle glycogen, above normal fed levels, and both GLUT4 protein and HK II are involved with the process of glycogen synthesis. In this study, both GLUT4 and HK II mRNA levels remained suppressed until the time when postprandial blood glucose was completely disposed in the body, suggesting that the refilling status of muscle glycogen may be a feedback signal for the regulation of the gene related to glucose uptake. To support this hypothesis, an early study by Jensen et al. [17] found that both basal glucose uptake and insulin-stimulated glucose uptake are inversely correlated with glycogen concentration. In addition, recent studies have also found that feeding rats with a high-carbohydrate diet after exercise, with muscle glycogen overload, results in a decrease in muscle insulin responsiveness such that the effect of an exercise-induced increase in GLUT4 on insulin-stimulated glucose transport is completely eliminated [11,18,19]. In contrast, the current study clearly demonstrated that the postexercise fasted state sustained a higher expressional level of GLUT4 and HK II than did the carbohydrate-fed condition. These results suggest that the carbohydrate fuel status and the molecular machinery for

glucose uptake operate interactively to prevent either insufficient glycogen storage or glycogen overload in the exercised human muscle.

Alternatively, the rapid down-regulation of GLUT4 mRNA and HK II mRNA that occurred during the insulin surge after carbohydrate supplementation suggests that insulin may play a role in the regulation of both gene transcripts in the exercised muscle. In rats, the down-regulation of GLUT4 mRNA lasted no longer than 16 h after the carbohydrate supplementation [10], corresponding to the time when the exercise-induced insulin sensitivity subsides [20]. This result suggests that insulin action on muscle may have been the cause for GLUT4 mRNA and HK II mRNA down-regulation in the exercised muscle. In the current study, we also observed similar results in which GLUT4 mRNA and HK II mRNA were reduced until insulin returned to normal (Fig. 2). It has been reported that insulin can down-regulate GLUT4 mRNA by both inhibiting GLUT4 gene transcription and reducing GLUT4 mRNA stability in mammalian cells [21]. This evidence suggests that insulin and not carbohydrate itself serves as the primary signal for regulating GLUT4 and/or HK II mRNA levels.

In summary, previous studies in rats demonstrated that the levels of GLUT4 protein and HK II in the skeletal muscle can be elevated by an acute bout of exercise, but glucose intubation down-regulated GLUT4 mRNA with greater increase in GLUT4 protein due to accelerated translation compared with exercise-fasted rats. The present study further demonstrated that the postexercise carbohydrate supplementation also down-regulated the GLUT4 mRNA and HK II mRNA in the human skeletal muscle at the time when postprandial blood glucose was completely disposed in the body. The current study implicates the existence of a negative feedback mechanism for carbohydrate storage by regulating the genes related to glucose uptake in the exercised human muscle.

Acknowledgments

This work was supported by the National Science Council (NSC91-2413-H-030-011; Taipei, Taiwan, ROC).

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