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Effects of exercise on GLUT-4 and glycogenin gene expression in human skeletal muscle

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Kraniou, Yorgos, David Cameron-Smith, Marie Misso, Greg Collier, and Mark Hargreaves. Effects of exercise on GLUT-4 and glycogenin gene expression in human skeletal muscle. *J. Appl. Physiol.* 88: 794–796, 2000.—To investigate the effect of exercise on GLUT-4, hexokinase, and glycogenin gene expression in human skeletal muscle, 10 untrained subjects (6 women and 4 men, 21.4 ± 1.2 yr, 66.3 ± 5.0 kg, peak oxygen consumption = 2.30 ± 0.19 l/min) exercised for 60 min on a cycle ergometer at a power output requiring $73 \pm 4\%$ peak oxygen consumption. Muscle samples were obtained by needle biopsy before, immediately after, and 3 h after exercise. Gene expression was quantified, relative to 29S ribosomal protein cDNA, by RT-PCR. GLUT-4 gene expression was increased immediately after exercise (1.7 ± 0.4 vs. 0.9 ± 0.3 arbitrary units; $P < 0.05$) and remained significantly higher than baseline 3 h after the end of exercise (2.2 ± 0.4 vs. 0.9 ± 0.3 arbitrary units; $P < 0.05$). Hexokinase II gene expression was significantly higher than the resting value 3 h after the end of exercise (2.9 ± 0.4 vs. 1.3 ± 0.3 arbitrary units; $P < 0.05$). Exercise increased glycogenin mRNA more than twofold (2.8 ± 0.6 vs. 1.2 ± 0.2 arbitrary units; $P < 0.05$) 3 h after the end of exercise. For the first time, we report that a single bout of exercise is sufficient to cause upregulation of GLUT-4 and glycogenin gene expression in human skeletal muscle. Whether these increases, together with the associated increase in hexokinase II gene expression, lead to increased expression of these key proteins in skeletal muscle and contribute to the enhanced skeletal muscle glucose uptake, glycogen synthesis, and insulin action observed following exercise remains to be determined.

hexokinase; glycogen; insulin action

EXERCISE ACTIVATES A SERIES of signal transduction cascades controlling glucose uptake, glycogen synthesis, gene expression, and protein synthesis (8). In human skeletal muscle, exercise results in increased mitogen-activated protein (MAP) kinase activity and

the activation of downstream targets of MAP kinase (3, 22). It has also been observed that exercise increases c-Jun NH₂-terminal kinase (JNK) activity (2), a signaling molecule responsible for the activation of the early genes *c-jun*, *c-fos*, and *erk1* that, in turn, are involved in the initiation of transcription of various other genes (2, 16). Less attention has been given to the effects of exercise on the expression of genes that encode key regulatory proteins in human skeletal muscle glucose uptake and disposal.

GLUT-4 and hexokinase are two major components of skeletal muscle carbohydrate metabolism; defects in these have been linked to insulin resistance (19). Exercise has been shown to increase GLUT-4 (10, 13, 17) and hexokinase II (14, 15) gene and protein expression in rats. In human skeletal muscle, hexokinase II gene expression was increased by a single bout of exercise (9); however, no data are available on GLUT-4 gene expression.

The major fate of skeletal muscle glucose uptake during the postexercise period is storage as muscle glycogen, the magnitude of which is correlated with skeletal muscle GLUT-4 levels and glycogen synthase activity (11). Glycogen depletion has been associated with enhanced insulin action in the postexercise period (4, 18), and the reversal of glucose transport following exercise is correlated with muscle glycogen levels (5). Glycogenin is a protein primer for glycogen synthesis in muscle and liver that has recently been examined as a potential determinant of maximum glycogen storage capacity (1, 12). Glycogenin is characterized by autocatalytic activity that enables it to add several glucose units from UDP-glucose to its active Tyr-194 site (20) and then to act as a substrate for glycogen synthase, thereby potentially being important for glycogen synthesis (21). No data exist on the effects of exercise on glycogenin gene or protein expression in human skeletal muscle.

The present study was undertaken to describe the effect of a single bout of exercise on GLUT-4, hexokinase II, and glycogenin gene expression in human skeletal muscle.

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METHODS

Subjects. Ten healthy, untrained subjects, (6 women and 4 men, 21.4 ± 1.2 yr, 66.3 ± 5.0 kg, 168 ± 12 cm; peak oxygen consumption of 2.30 ± 0.19 l/min) volunteered to participate in the experiment. Each subject was informed of the risks associated with the procedures and provided written, informed consent before participation. The experiment was approved by the Deakin University Ethics Committee.

Experimental protocol. The subjects reported to the laboratory at 8:00 AM after an overnight fast. They were asked to avoid any vigorous physical activity and to refrain from caffeine and tobacco consumption for at least 24 h before the test. Subjects rested in a supine position, and a muscle sample was obtained from musculus vastus lateralis by using the percutaneous needle biopsy technique with suction. The samples were cleaned of blood and connective tissue and immediately frozen in liquid nitrogen. After the biopsy, subjects commenced cycling at a power output requiring $73 \pm 4\%$ peak oxygen consumption. The exercise bout lasted for 60 min, and subjects were permitted to ingest water ad libitum. When this exercise period ended, a second muscle sample was obtained, and the subjects rested for 3 h before a third and final muscle sample was obtained. Each muscle sample was obtained from a separate incision at least 2 cm apart.

Extraction of total RNA and RT-PCR quantitation. Total RNA was extracted from 50 mg of muscle tissue by using the single-step, acid guanidium thiocyanate, phenol-chloroform extraction as described by Chomczynski and Sacchi (6). First-strand cDNA was generated from 1 μ g of RNA in a 30- μ l volume by using oligo(dT) primer in the first-strand synthesis kit (Promega, Madison, WI). One microliter of the reverse transcription reaction mix was amplified with oligonucleotides specific for human GLUT-4 (GenBank M20747), hexokinase II (GenBank Z46376), and glycogenin (GenBank U44131) in a total volume of 20 μ l. The samples were amplified in the linear phase, optimized for each gene (GLUT-4: 32 cycles; hexokinase II: 40 cycles; glycogenin: 37 cycles) by using a three-step PCR that involved denaturation at 94°C for 30 s, oligonucleotide annealing at 55°C (except glycogenin at 58°C) for 30 s, and elongation at 72°C for 1 min. To correct for any nonspecific changes in skeletal muscle gene expression, the ribosomal protein 29S (RS 29S, GenBank U14973) was used as a control for all quantitation and was amplified in the linear phase for 24 cycles at 94°C for 30 s, 55°C for 30 s, and 72°C for 1 min. All PCR products were electrophoresed on a 6% polyacrylamide gel (40% acrylamide 19:1 cross-linker) at 220 V for 25 min, visualized using ethidium bromide, and quantitated by computer integrated densitometry (Kodak, New Haven, CT). Levels of mRNA were expressed as the ratio of signal intensity for the genes of interest to that of RS 29S.

Statistical analysis. Data were analyzed by using one-way ANOVA for repeated measures. All results are expressed as means \pm SE, and *P* values of 0.05 or less were considered significant.

RESULTS

The effects of exercise on skeletal muscle gene expression are summarized in Fig. 1. GLUT-4 gene expression increased immediately after exercise (1.7 ± 0.4 vs. 0.9 ± 0.3 arbitrary units; *P* < 0.05) and remained significantly higher than baseline 3 h after the end of exercise (2.2 ± 0.4 vs. 0.9 ± 0.3 arbitrary units; *P* < 0.05). Hexokinase II gene expression was not significantly different after exercise but was significantly higher than resting values 3 h after the end of exercise

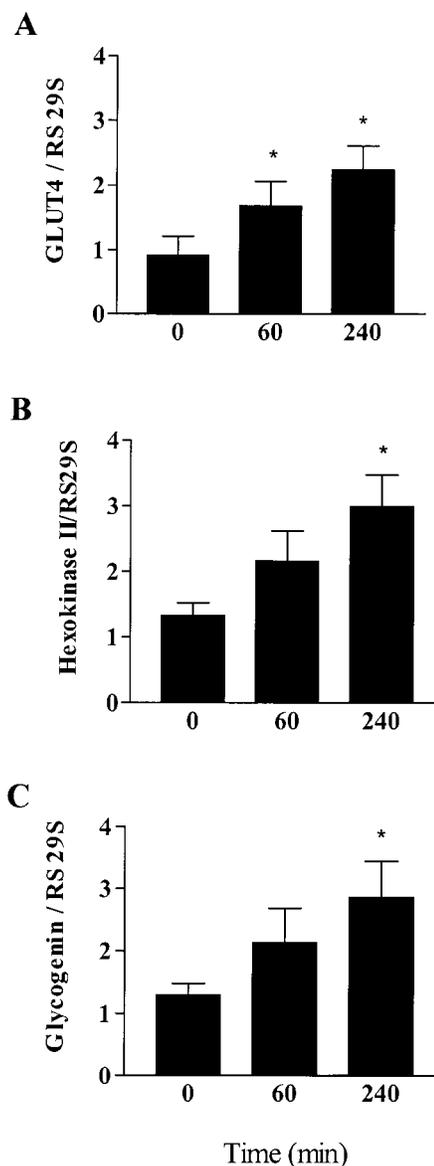


Fig. 1. GLUT-4 (A), hexokinase II (B), and glycogenin (C) gene expression before and after 60 min of moderate-intensity exercise. Values are means \pm SE (*n* = 10). *Significant difference from 0 value (*P* < 0.05).

(2.9 ± 0.4 vs. 1.3 ± 0.3 arbitrary units; *P* < 0.05). A similar pattern was observed for glycogenin gene expression, with a significantly higher value 3 h after the end of exercise compared with baseline (2.8 ± 0.6 vs. 1.2 ± 0.2 arbitrary units; *P* < 0.05).

DISCUSSION

Our results have demonstrated, for the first time, that a single bout of exercise increased GLUT-4 and glycogenin gene expression in human skeletal muscle and confirm recent observations of increased hexokinase II gene expression following exercise (9).

Previous studies on rat skeletal muscle GLUT-4 have observed an increased rate of transcription (13) and protein content (17) after a single exercise bout. In the latter study, the increased GLUT-4 was associated with

enhanced insulin-stimulated muscle glucose transport and glycogen storage. Another recent investigation has provided evidence that GLUT-4 gene and protein expression were upregulated very early after exercise and remained elevated for several hours during recovery (10). It was suggested that the increased GLUT-4 facilitated postexercise glycogen synthesis (10). Our results indicate that exercise also increases GLUT-4 gene expression in human skeletal muscle.

In humans, 1 h of moderate-intensity exercise increases hexokinase II transcription and mRNA and protein levels 3 h after the end of exercise (9), and similar observations have been made in rat skeletal muscle (14, 15). Our results on hexokinase II gene expression are in agreement with these studies.

Conversion to muscle glycogen is the major metabolic fate of skeletal muscle glucose uptake during recovery from exercise and in response to insulin stimulation. Recently, it has been argued that glycosylated glycogenin is the primary substrate for glycogen synthase (1), and it is possible that the glycogenin level may influence the maximum glycogen storage capacity of skeletal muscle. Overexpression of glycogenin in fibroblasts (12) and L6 muscle cells (7) results in increased glycogen storage. The increase in glycogenin gene expression we have observed, together with the associated increases in GLUT-4 and hexokinase II gene expression, could contribute to enhanced protein expression, thereby facilitating muscle glycogen resynthesis during recovery from exercise.

It is worth noting that the gene expressions of proteins involved in muscle glucose transport, phosphorylation, and conversion to glycogen were all increased in a similar manner following exercise. Whether this represents coordinated regulation of postexercise glucose metabolism requires further investigation.

In summary, we have observed increased GLUT-4, hexokinase II, and glycogenin gene expression in human skeletal muscle following a single bout of exercise. Whether such changes lead to increased expression of these key proteins in skeletal muscle and contribute to the enhanced skeletal muscle glucose uptake, glycogen synthesis, and insulin action observed following exercise remains to be determined.

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