Regulation of hexokinase II activity and expression in human muscle by moderate exercise

J ANICE A. KOVAL, RALPH A. DEFRONZO, ROBERT M. O’DOHERTY, RICHARD PRINTZ, HOSSEIN ARDEHALI, DARYL K. GRANNER, AND LAWRENCE J. MANDARINO

Division of Diabetes, Department of Medicine and Department of Biochemistry, The University of Texas Health Science Center at San Antonio, San Antonio, Texas 78284–7886; and Department of Molecular Physiology and Biophysics, Vanderbilt University School of Medicine, Nashville, Tennessee 37232–0615

Koval, J anice A., Ralph A. DeFronzo, Robert M. O’Doherty, Richard Printz, Hossein Ardehali, Daryl K. Granner, and Lawrence J. Mandarino. Regulation of hexokinase II activity and expression in human muscle by moderate exercise. Am. J. Physiol. 274 (Endocrinol. Metab. 37): E304–E308, 1998.—A single bout of exercise increases the rate of insulin-stimulated glucose uptake and metabolism in skeletal muscle. Exercise also increases insulin-stimulated glucose 6-phosphate in skeletal muscle, suggesting that exercise increases hexokinase activity. Within 3 h, exercise increases hexokinase II (HK II) mRNA and activity in skeletal muscle from rats. It is not known, however, if a single bout of moderate-intensity exercise increases HK II expression in humans. The present study was undertaken to answer this question. Six subjects had percutaneous biopsies of the vastus lateralis muscle before and 3 h after a single 3-h session of moderate-intensity aerobic (60% of maximal oxygen consumption) exercise. Glycogen synthase, HK I, and HK II activities as well as HK I and HK II mRNA content were determined from the muscle biopsy specimens. The fractional velocity of glycogen synthase was increased by 446 ± 84% after exercise (P < 0.005). Hexokinase II activity in the soluble fraction of the homogenates increased from 1.2 ± 0.4 to 4.5 ± 1.6 pmol·min⁻¹·µg⁻¹ (P < 0.05) but was unchanged in the particulate fraction (4.3 ± 1.3 vs. 5.3 ± 1.5). HK I activity in neither the soluble nor particulate fraction changed after exercise. Relative to a 28S rRNA control signal, HK II mRNA increased from 0.091 ± 0.02 to 0.195 ± 0.037 (P < 0.05), whereas HK I mRNA was unchanged (0.414 ± 0.061 vs. 0.498 ± 0.134, P < 0.20). The increase in HK II activity after moderate exercise in healthy subjects could be one factor responsible for the enhanced rate of insulin-stimulated glucose uptake seen after exercise.

A single bout of exercise increases the rate of insulin-stimulated glucose uptake and metabolism in skeletal muscle for 24–48 h (2, 3, 5, 12, 13, 17, 23, 30). This increase has been attributed to an effect of exercise on the translocation of the GLUT-4 glucose transporter (4, 19, 22), hexokinase activity (14, 15, 18), or glycogen synthase activity (2, 3, 9), since electrically induced muscle contraction did not increase insulin-stimulated phosphorylation events involved in insulin signaling (7). Recently Perseghin and colleagues (17) used [³¹P] nuclear magnetic resonance methods combined with hyperglycemic, hyperinsulinemic clamp experiments to show that a single exercise session increased the insulin-stimulated glucose 6-phosphate (G-6-P) concentration in muscle of insulin-resistant subjects and concluded that exercise normalized a defect in glucose transport or glucose phosphorylation (17).

Hexokinase catalyzes the phosphorylation of glucose. Its activity is increased by physical training in humans (18, 19, 26) and by exercise training or chronic contractile activity in experimental animals (22, 26, 28). In humans, 5 days of physical training increases hexokinase activity in skeletal muscle (18). However, the increase in insulin-stimulated glucose uptake that is induced by insulin occurs much earlier, within 24 h (2–4, 12, 13, 17, 23, 30). The recent cloning and characterization (21) of rat skeletal muscle hexokinase II (HK II) have allowed investigators to examine the biochemical and molecular mechanisms by which exercise increases muscle hexokinase activity in greater detail. O’Doherty et al. (15) showed that, within 3 h, moderate exercise had increased HK II mRNA and activity in skeletal muscle from rats before an increase in GLUT-4 expression was observed. The increase in HK II mRNA was due to increased gene transcription (14). It is not known, however, if a single bout of moderate-intensity exercise will increase HK II expression in humans. The present study was undertaken to answer this question.

METHODS

Subjects. Six healthy, sedentary individuals volunteered for this study. There were four women and two men. No subject engaged in regular exercise, and all had normal glucose tolerance.

Study design. The first day of the study consisted of a determination of maximal oxygen consumption (VO₂max) using a cycle ergometer (Ergometrics 800S) and a Sensormedics 2900 Metabolic Measurement System (Sensormedics, Savi Park, CA) in the breath-by-breath mode. Heart rate and rhythm were monitored continuously using a MAX1 Stress System (Marquette Instruments, Milwaukee, WI). The anaerobic threshold was estimated using the V-slope method (1). Subjects exercised to maximum voluntary exhaustion, and all achieved an R of ≥1.10.

On a separate day at least 1 wk later, subjects reported to the General Clinical Research Center (GCRC) of the Audie Murphy Memorial Veterans Hospital at 7:00 AM. After subjects rested for 30–60 min, a percutaneous biopsy of the vastus lateralis muscle was performed under local anesthesia, as described (11). The subjects rested for another 30 min and then exercised on a stationary cycle at a calculated 90% of the anaerobic threshold heart rate (~60% of VO₂max in untrained subjects) for 60 min. Immediately after the exercise, the subjects rested in the GCRC for another 180 min, during which they were not ambulatory. After 180 min, a second biopsy of the vastus lateralis muscle was performed in the
Table 1. Subject characteristics

<table>
<thead>
<tr>
<th>Age, yr</th>
<th>Gender (M/F)</th>
<th>BMI, kg/m²</th>
<th>( V_{O2\text{max}} ), ml·kg⁻¹·min⁻¹</th>
<th>Anaerobic Threshold Heart Rate, beats/min</th>
<th>Exercise Heart Rate, beats/min</th>
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<tr>
<td>27 ± 2</td>
<td>2/4</td>
<td>23.5 ± 0.7</td>
<td>38.5 ± 4.4</td>
<td>143 ± 6</td>
<td>128 ± 5</td>
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Data are given as means ± SE. M, male; F, female; BMI, body mass index; \( V_{O2\text{max}} \), maximal oxygen consumption. Anaerobic threshold heart rate was defined as heart rate at anaerobic threshold chosen by the V-slope method. Exercise heart rate was chosen for each subject to be 90% of anaerobic threshold heart rate.

opposite leg, and the study was concluded. Muscle biopsies were frozen within 15 s in liquid nitrogen and stored in a liquid nitrogen freezer until processing. The study was approved by the Institutional Review Board of The University of Texas Health Science Center at San Antonio, and all subjects gave written consent.

Enzyme activity assays. Glycogen synthase, HK I, and HK II activities were assayed as described (10). A portion of the muscle biopsy specimen was homogenized using a Polytron Homogenizer (Brinkman Instruments) for 20 s at high speed in a buffer consisting of 50 mM potassium phosphate, pH 7.4, 2 mM dithiothreitol, 2 mM EDTA, 20 mM sodium fluoride, 10 mM leupeptin, 10 µg/ml soybean trypsin inhibitor, 20 µg/ml aminobenzamidine, 70 µg/ml Nα-p-tosyl-L-lysine chloromethyl ketone, and 170 mM phenylmethylsulfonyl fluoride. Homogenates were centrifuged at 13,000 g, and the supernatant (soluble fraction) was removed and saved. The pellet (particulate fraction) was resuspended in the extraction buffer plus 0.1% Triton X-100. HK I and HK II activities were determined in soluble and particulate fractions using a temperature sensitivity assay (10), and glycogen synthase was assayed in the soluble fraction using 0.1 and 10 mM G-6-P. Glycogen synthase fractional velocity was calculated as the ratio of the activity determined using 0.1 mM G-6-P to that determined using 10 mM G-6-P (9).

HK I and HK II mRNAs. Hexokinase mRNA content was determined in total RNA isolated from a portion of each muscle biopsy using a ribonuclease (RNAse) protection assay (Ambion, Austin, TX) as described (10). Muscle was extracted using a guanidinium isothiocyanate method (Tel-Test, Friendswood, TX). Content of HK I and HK II mRNA was determined on 4-µg aliquots of total RNA. Riboprobe assays were generated that would yield protected products of 396 nt for HK I and 231 nt for HK II (10, 21). HK I and HK II mRNAs were quantified using a PhosphorImager (Molecular Dynamics, Sunnyvale, CA) and were normalized to a 28S rRNA internal control signal (Ambion). Therefore, values for HK I and HK II mRNA are expressed as dimensionless ratios.

Statistics. Post- and preexercise values were compared using a one- or two-sided paired t-test, as appropriate, with an α of 0.05 considered to be significant.

Table 2. Effect of exercise on glycogen synthase activity

<table>
<thead>
<tr>
<th>Preexercise</th>
<th>Postexercise</th>
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<tr>
<td>G-6-P (mM)</td>
<td>G-6-P (mM)</td>
</tr>
<tr>
<td>0.1</td>
<td>10</td>
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<tr>
<td>0.45 ± 0.20</td>
<td>2.40 ± 0.41</td>
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<td>1.09 ± 0.35*</td>
<td>2.56 ± 0.68</td>
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Data are given as means ± SE in units of nmol·min⁻¹·mg protein⁻¹. G-6-P, glucose 6-phosphate. *P < 0.01 vs. preexercise values (n = 6 subjects).

RESULTS

Exercise. Subjects had an average \( V_{O2\text{max}} \) of 38.5 ml·kg⁻¹·min⁻¹, with a range of 25.2 to 56.3. At anaerobic threshold, subjects had a heart rate of 143 ± 6 beats/min and they performed 1 h of exercise at 90% of this value (128 ± 5 beats/min) on the day they received muscle biopsies (Table 1).

Enzyme activities. Glycogen synthase activity assayed using 0.1 mM G-6-P was increased significantly (P < 0.01) after the bout of exercise (Table 2) and glycogen synthase fractional velocity was increased from 0.16 ± 0.06 basally to 0.46 ± 0.10 after exercise (P < 0.01). There was no increase in total glycogen synthase assayed using 10 mM G-6-P. HK II activity in the soluble fraction of the homogenates increased from 1.2 ± 0.4 to 4.5 ± 1.6 pmol·min⁻¹·µg⁻¹ (P < 0.05) but was unchanged in the particulate fraction. The change in HK II activity in the soluble fraction was inversely correlated with \( V_{O2\text{max}} \) (r = -0.73, P < 0.05). HK I activity in neither the soluble (2.4 ± 0.3 vs. 1.5 ± 0.4) nor particulate (1.2 ± 0.3 vs. 1.1 ± 0.4) fraction changed after exercise (Table 3). Individual values for glycogen synthase fractional velocity and HK II activity in the soluble fraction are shown in Fig. 1.

Hexokinase mRNA levels. Expressed relative to the 28S rRNA internal control signal, HK II mRNA increased approximately twofold, from 0.091 ± 0.02 to 0.195 ± 0.037 (P < 0.05, Fig. 2), but HK I mRNA was unchanged. Exercise had no effect on the 28S rRNA control signal (6.8 ± 0.6 vs. 4.7 ± 0.7 × 10² PhosphorImager units, P = 0.10). The PhosphorImager signal is shown in Fig. 3.

DISCUSSION

The present study was undertaken to determine if 1 h of moderate-intensity, voluntary exercise alters hexokinase expression in skeletal muscle of healthy, seden-
E306 EXERCISE AND HEXOKINASE mRNA AND ACTIVITY

Fig. 1. Individual values for pre- and postexercise glycogen synthase fractional velocity (A) and hexokinase II (HK II) activity in soluble fraction (B). Hexokinase activity units are pmol·min⁻¹·µg protein⁻¹.

Table:<br><br>| HK I mRNA | HK II mRNA | Basal | Exercise | Fold Change |
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Fig. 2. HK I (A) and HK II (B) mRNA, expressed relative to 28S rRNA, before (open bars) and 3 h after (filled bars) 1 h of exercise. Data are given as means ± SE. *P < 0.05 vs. basal values.

Fig. 3. Phosphorimage of RNase protection assay for hexokinase mRNAs. Four micrograms of total RNA were hybridized overnight with 32P-labeled antisense riboprobes that protected products of 396 (HK I), 231 (HK II), and 90–105 nt (28S rRNA). The 28S signal usually is present as a doublet, and both bands were quantified using a PhosphorImager. For quantification, HK I and HK II signals were normalized to their respective 28S rRNA internal control signals. Shown are Phosphor image (A) and HK II mRNA fold stimulation due to exercise (B), where basal signals were normalized to a value of 1.0, for 5 of the 6 subjects. Even-numbered lanes are basal; even-numbered lanes are postexercise.

Fig. 4. Phosphorimage of RNase protection assay for hexokinase mRNAs. Four micrograms of total RNA were hybridized overnight with 32P-labeled antisense riboprobes that protected products of 396 (HK I), 231 (HK II), and 90–105 nt (28S rRNA). The 28S signal usually is present as a doublet, and both bands were quantified using a PhosphorImager. For quantification, HK I and HK II signals were normalized to their respective 28S rRNA internal control signals. Shown are Phosphor image (A) and HK II mRNA fold stimulation due to exercise (B), where basal signals were normalized to a value of 1.0, for 5 of the 6 subjects. Odd-numbered lanes are basal; even-numbered lanes are postexercise.

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Fig. 2. HK I (A) and HK II (B) mRNA, expressed relative to 28S rRNA, before (open bars) and 3 h after (filled bars) 1 h of exercise. Data are given as means ± SE. *P < 0.05 vs. basal values.
In the present study it was found that HK II mRNA and activity were increased. HK II protein was not directly measured in this study. We believe that increased HK II protein is the simplest explanation for the increase in HK II activity. In previous studies in humans, 4 h of insulin infusion increased HK II mRNA, protein, and activity (10). Because the total period of time of the present study is 4 h (1 h of exercise plus 3 h of rest), this is within the time frame for new protein synthesis to occur in this system. It is also possible that changes in a metabolite concentration could affect HK II activity allosterically in vivo. This would not be detected in vitro, however, because of the 100-fold dilution of the muscle homogenate in the activity assay. Moreover, the metabolite most likely to be changed (increased), G-6-P, would decrease HK II activity rather than increase it. Nevertheless, the current results do not rule out the involvement of a mechanism other than new HK II protein synthesis in the increased HK II activity seen after exercise.

Our results may bear on the mechanism underlying the response of skeletal muscle hexokinase activity to chronic training. Other investigators have found that physical training at a moderate intensity of ~60% of VO2max increases muscle hexokinase activity within 5–10 days in healthy subjects (18, 19, 25) and in 2–5 days in rats (22, 26, 28). Our results, together with those of O’Doherty and co-workers (14, 15), suggest that the increases observed by previous investigators (18, 19, 22, 25, 26, 28) were due to increased HK II and not HK I expression. However, further studies are required to establish this.

The effect of exercise on HK II expression and glycolytic synthase activity is similar to the effect of insulin in several ways. First, over the time course studied here, exercise and insulin both increase the fractional velocity of glycogen synthase without increasing its total activity (10). Second, exercise and insulin both increase HK II, but not HK I, mRNA content. Third, exercise and insulin (over several hours) both increase HK II activity in the soluble, but not particulate, fraction of the muscle biopsy. The reason the expression of HK II is limited to increased activity in the soluble fraction of muscle extracts is not clear.

Although the end results are similar, exercise and insulin exert their effects on GLUT-4 translocation and other metabolic steps by different pathways (4, 6, 7, 29). Although the signaling pathways used by exercise to increase HK II expression are unknown, insulin effects on HK II expression are dependent on phosphatidylinositol 3-kinase activation and are independent of the mitogen-activated protein (MAP) kinase pathway (16). It is possible that exercise also uses a different pathway from that of insulin to increase HK II expression, and at least one group has suggested a role for the MAP kinase pathway in the rat (6). However, the signal transduction pathways by which exercise alters enzyme activity or gene expression are relatively undefined, compared with insulin and other hormonal signaling pathways, and deserve more study.

Finally, it is well known that exercise increases insulin sensitivity (2, 3, 5, 12, 17, 23, 30), and it is believed that either glucose transport or hexokinase activities are rate determining under many conditions for glucose uptake into skeletal muscle (8, 24). The exercise-induced increase in hexokinase expression provides a potential explanation for the phenomenon of increased insulin-stimulated glucose uptake after moderate exercise and could, at least partially, explain the increase in insulin-stimulated intramuscular G-6-P concentrations in insulin-resistant subjects who exercise before the performance of a glucose clamp study (2, 3). Because HK II expression and activity are reduced in patients with non-insulin-dependent diabetes mellitus (27), it will be important to determine whether exercise improves insulin-stimulated glucose uptake by increasing HK II expression in these insulin-resistant diabetic individuals.

The excellent technical assistance of Andrea Barrentine and Jean Finlayson and the nursing assistance of Darlene Collins, Patricia Ortiz, and Norma Diaz are gratefully acknowledged.

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