Exercise Increases Hexokinase II mRNA, But Not Activity in Obesity and Type 2 Diabetes

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Glucose phosphorylation, catalyzed by hexokinase, is the first committed step in glucose uptake in skeletal muscle. Hexokinase II (HKII) is the isoform that is present in muscle and is regulated by insulin and muscle contraction. Glucose phosphorylation and HKII expression are both reduced in obese and type 2 diabetic subjects. A single bout of exercise increases HKII mRNA and activity in muscle from healthy subjects. The present study was performed to determine if a moderate exercise increases HKII mRNA expression and activity in patients with type 2 diabetes. Muscle biopsies were performed before and 3 hours after a single bout of cycle ergometer exercise in obese and type 2 diabetic patients. HKII mRNA and activity and glycogen synthase activity were determined in the muscle biopsies. Exercise increased HKII mRNA in obese and diabetic subjects by 1.67 ± 0.34 and 1.87 ± 0.26-fold, respectively ($P < .05$ for both). Exercise did not significantly increase HKI mRNA. When HKII mRNA increases were compared with the 2.26 ± 0.36-fold increase in HKII mRNA previously reported for healthy lean subjects, no statistically significant differences were found. In contrast to the increase in HKII activity observed after exercise by lean healthy controls, exercise did not increase HKII activity in obese nondiabetic or diabetic subjects. Exercise increased glycogen synthase activity ($GS_{0.1}$ and GS_{FV}) significantly in both obese nondiabetic and type 2 diabetic patients. The present results indicate that there is a posttranscriptional defect in the response of HKII expression to exercise in obese and type 2 diabetic subjects. This defect may contribute to reduced HKII activity and glucose uptake in these patients.

THE PHOSPHORYLATION OF glucose is the first committed step in its metabolism in skeletal muscle. Recent evidence from nuclear magnetic resonance (NMR) spectroscopy studies[1] and isotopic tracer and modeling studies[2] in vivo provide evidence that glucose transport and phosphorylation both contribute to determining the rate of glucose uptake. Furthermore, investigators have used these techniques to show that insulin stimulation of glucose transport/phosphorylation is reduced in type 2 diabetes mellitus.[2] Results from studies using positron-emission tomography (PET) scanning have led to similar conclusions.[3] These findings have generated interest in determining the mechanism of the defect in glucose phosphorylation in skeletal muscle in type 2 diabetes.

Hexokinase catalyzes the conversion of glucose to glucose 6-phosphate. Skeletal muscle expresses 2 isoforms of hexokinase, HKI and HKII, and these proteins have been cloned and characterized in rats[4] and humans.[5] Insulin and muscle contraction increase the expression of HKII in skeletal muscle.[4,6-9] Insulin increases HKII transcription in cultured L6 rat skeletal muscle cells by a wortmannin-sensitive pathway.[10] Insulin administration increases HKII expression in vivo in rats[11] and humans,[7,8] while HKI expression is unaffected. Muscle contraction increases HKII transcription in rats[6] and mRNA levels and activity in muscle from lean, healthy human volunteers.[9] HKII overexpression in skeletal muscle of transgenic mice increases muscle glucose uptake in response to either insulin or exercise.[12] Finally, insulin fails to increase HKII expression or activity in muscle of type 2 diabetic patients, and HKII activity is reduced in diabetic patients compared with healthy control subjects.[7,8] This reduction in HKII expression in insulin-resistant patients may contribute to the decrease in insulin-stimulated glucose uptake into muscle that characterizes these subjects.

Insulin and muscle contraction produce similar effects on glucose transport,[13,14] HKII expression (see above), and glycogen synthase activity[15,16] in muscle of rodents and humans, although the signaling pathways appear to be different.[14,17] A single bout of exercise can increase insulin-stimulated glucose uptake in patients with type 2 diabetes,[16] who are characteristically resistant to the effects of insulin on glucose metabolism.[18] The present study was performed to determine if a single bout of moderate exercise increases HKII mRNA expression and activity in patients with type 2 diabetes. If so, this could, in part, explain the effect of an acute bout of exercise on glucose uptake in insulin-resistant patients.

MATERIALS AND METHODS

Subjects

Eight patients with type 2 diabetes and 6 obese nondiabetic subjects took part in this study. The characteristics of the subjects are given in Table 1. No subject engaged in regular exercise. All diabetic patients were treated with oral hypoglycemic agents that were discontinued for 48 hours before they were studied. Data from 6 lean, healthy subjects that was reported previously was used for comparative purposes.[9]

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Table 1. Subject Characteristics

	No.	Gender (M/F)	Ethnicity (M/A)	Age (yr)	BMI (kg/m^2)	VO_2max (mL/min · kg)	Plasma Glucose (mg/dL)	HbA_{1c} (%)
Diabetic	8	7/1	7/1	47 ± 3	30.7 ± 1.1	21.6 ± 1.4	168 ± 15*	8.6 ± 0.6
Obese	6	2/4	5/1	42 ± 2	31.3 ± 1.2	26.5 ± 2.3	93 ± 2	5.6 ± 0.3

NOTE. Data are means ± SEM.
Abbreviations: M, Mexican American; A, Anglo.
* $P < .05$ *v* obese nondiabetics.

Study Design

The design of the study was identical to that described for lean control subjects.[9] The first day of the study consisted of a determination of VO_2max 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.[19] Subjects exercised to maximum voluntary exhaustion.

On a separate day at least 1 week later, subjects reported to the General Clinical Research Center (GCRC) of the Audie Murphy Memorial Veterans Hospital at 7:00 AM. After resting for 30 to 60 minutes, a percutaneous biopsy of the vastus lateralis muscle was performed under local anesthesia, as described.[20] The subjects rested for another 30 minutes and then exercised on a stationary cycle at a calculated 90% of anaerobic threshold heart rate (approximately 60% of VO_2max in untrained subjects) for 60 minutes. Immediately after the exercise, the subjects rested in the GCRC for another 180 minutes, during which they were not ambulatory. After 180 minutes, a second biopsy of the vastus lateralis muscle was performed in the opposite leg, and the study was concluded. Muscle biopsies were frozen within 15 seconds 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, HKI, and HKII activities were assayed as described.[21] A portion of the muscle biopsy specimen was homogenized using a Polytron Homogenizer (Brinkman Instruments) for 20 seconds at high speed in a buffer consisting of 50 mmol/L potassium phosphate, pH 7.4, 2 mmol/L dithiothreitol, 2 mmol/L EDTA, 20 mmol/L sodium fluoride, 10 μg/mL leupeptin, 10 μg/mL soybean trypsin inhibitor, 20 μg/mL p-aminobenzamidine, 70 μg/mL N-p-tosyl-L-lysine chloromethyl ketone, and 170 μg/mL 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 X100. HKI and HKII activities were determined in soluble and particulate fractions using a temperature sensitivity assay,[21] and glycogen synthase was assayed in the soluble fraction using 0.1 and 10 mmol/L glucose 6-phosphate (G6P). Glycogen synthase fractional velocity was calculated as the ratio of the activity determined using 0.1 mmol/L G6P ($GS_{0.1}$) to that determined using 10 mmol/L G6P (GS_{10}), as described.[15]

Hexokinase I and II mRNAs

Hexokinase mRNA content was determined in total RNA isolated from a portion of each muscle biopsy using an RNase protection assay (Ambion, Austin, TX) as described.[21] Muscle was extracted using a guanidinium isothiocyanate method (Tel-Test, Friendswood, TX). Content of HKI and HKII mRNA was determined on 4-μg aliquots of total RNA. Riboprobes were generated that would yield protected products of 396 nt for HKI and 231 nt for HKII.[4,5,21] HKI and HKII RNAs were quantified using a PhosphorImager (Molecular Dynamics, Sunnyvale, CA) and were normalized to a 28S rRNA internal control signal (Ambion). Therefore, values for HKI and HKII mRNA are expressed as dimension-less ratios.

Statistics

Post- and pre-exercise values were compared using a 1- or 2-sided paired *t* test, as appropriate, with an α of 0.05 considered to be significant. Differences between groups were compared by analysis of variance or unpaired *t* test.

RESULTS

Subject Characteristics

The subjects were primarily Mexican American, and both groups were moderately obese. The diabetic patients were moderately hyperglycemic, with an average fasting plasma glucose concentration of 168 ± 15 mg/dL. Subjects underwent a cycle ergometer determination of VO_2max, which was used to determine the exercise intensity to be used to assess how exercise affects HKII expression. Patients with diabetes had a VO_2max of 21.6 mL/kg · min (1.84 L/min), and obese nondiabetic subjects had a VO_2max of 26.5 mL/kg · min (2.18 L/min). The values for the diabetic and obese subjects did not differ significantly. Both groups had a VO_2max that was less than that predicted for healthy control subjects.

Hexokinase mRNA

To determine the extent to which exercise increases HKII mRNA, subjects had a biopsy of the vastus lateralis muscle and performed 1 hour of cycle ergometer exercise at 65% of their VO_2max. The subjects rested for 3 hours and then had a second biopsy. HKI and HKII mRNA were determined using an RNase protection assay. The results are shown in Fig 1. Exercise increased HKII mRNA in obese and diabetic subjects by 1.67 ± 0.34 and 1.87 ± 0.26-fold, respectively ($P < .05$ for both). Exercise did not significantly increase HKI mRNA. When HKII mRNA increases were compared by analysis of variance with the 2.26 ± 0.36-fold increase in HKII mRNA previously reported for healthy lean subjects,[9] no statistically significant differences were found.

Enzyme Activities

Glycogen synthase and HKI and HKII enzyme activities were determined in separate portions of the same muscle biopsies used to determine hexokinase mRNA levels. Exercise

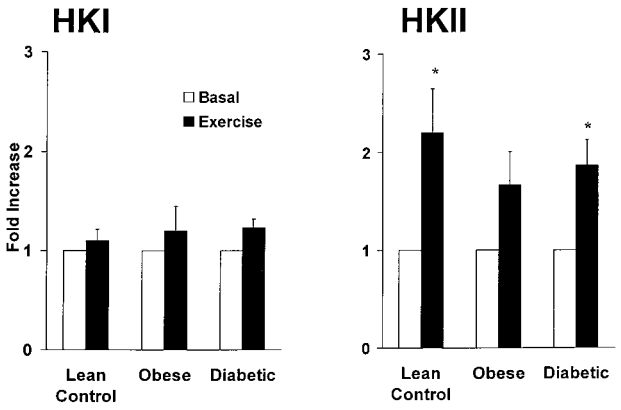

Fig 1. Effects of exercise on hexokinase mRNA. Muscle biopsies were performed before and after 3 hours of 60 minutes of cycle exercise at 65% of VO_2max. HKI and HKII mRNA were determined by RNase protection assay as described in the text. Data are shown as means ± SEM and expressed as fold-increase over basal values. Basal values are shown as open bars and postexercise values as black bars. Data from lean control subjects is taken from Koval et al.[9] *$P < .05$ *v* basal values.

increased glycogen synthase activity assayed using 0.1 mmol/L G6P ($GS_{0.1}$) and glycogen synthase fractional velocity (GS_{FV}) significantly in both obese nondiabetic and type 2 diabetic patients (Fig 2). Compared with the effect of exercise on glycogen synthase in lean controls reported previously,[9] the obese and diabetic subjects had reduced $GS_{0.1}$ and GS_{FV} under basal conditions, but the increment in glycogen synthase activity ($GS_{0.1}$) was not different from control subjects (0.618 ± 0.208, 0.638 ± 0.128, and 0.742 ± 0.183 nmol/min · mg in lean control, obese nondiabetic, and type 2 diabetic subjects, respectively, P = not significant [NS]). The effect of exercise on hexokinase activities is given in Table 2. Exercise did not increase HKII activity in either soluble or particulate fractions of skeletal muscle in the obese or diabetic subjects. This was in contrast to healthy control subjects reported previously using an identical protocol,[9] in whom exercise induced an increase in HKII activity in the soluble fraction of the muscle homogenate from 1.2 ± 0.4 pmol/min · μg protein basally to 4.5 pmol/min · μg 3 hours after exercise. Exercise also did not increase HKI activity in either fraction of muscle homogenates.

DISCUSSION

The results of previous studies show that muscle contraction or voluntary exercise increase HKII transcription, mRNA level, and activity in skeletal muscle of normal rodents and healthy humans.[6,9] Insulin also increases HKII transcription and activity in skeletal muscle,[4,7,8,11] but the effects of exercise and insulin are likely to be produced through different signaling pathways.[17] Because basal and insulin-stimulated HKII activity are decreased in insulin-resistant patients with obesity or type 2 diabetes mellitus, the present study was undertaken to determine if exercise increases HKII mRNA expression and activity in such patients, or alternatively, whether these insulin-resistant subjects were also resistant to the effects of exercise. Because even a single bout of exercise can increase insulin-stimulated glucose uptake in patients with type 2 diabetes,[16] an increase in HKII activity could be responsible, in part, for this effect.

The results of the present study show that exercise resulted in a nearly 2-fold increase in HKII, but not HKI, mRNA in both the obese and diabetic subjects. This increase was not different from the 2.2-fold increase in HKII mRNA that was reported previously in lean, healthy subjects.[9] Exercise increases HKII mRNA by increasing the rate of transcription of the HKII gene.[10] Therefore, it is likely that the present results imply that exercise has a normal effect on the rate of transcription of the HKII gene in insulin-resistant subjects. In lean subjects, the increase in HKII mRNA led to an increase in HKII activity in muscle.[9] In contrast, the present results show that the normal increase in HKII mRNA in the obese and diabetics groups was not followed by an increase in HKII activity, at least within the time frame used in this study (3 hours after exercise). This suggests that there may be a posttranscriptional defect in the obese and diabetic subjects. Recently, the assumption of stability of the activity of HKI at 45°C has been questioned.[22] If this is correct and some HKI activity (as well as all HKII activity) is lost when samples are heated to 45°C, HKI activity would have been underestimated and HKII overestimated. However, the total HKI plus HKII activity assayed without heating, was 5.51 ± 0.45 basally versus 4.43 ± 0.62 after exercise in the diabetics and 3.24 ± 0.34 basally versus 3.59 ± 0.70 after exercise in the obese subjects (P = NS for both, basal *v* exercise). Therefore, the overall conclusion that HKII mRNA was increased without an increase in hexokinase activity is unaffected.

Obese and type 2 diabetic patients are resistant to the ability of insulin to increase HKII expression and activity and have reduced basal HKII activity compared with lean control subjects.[7,8] The present findings indicate that insulin-resistant subjects are also resistant to the effects of exercise. However, this

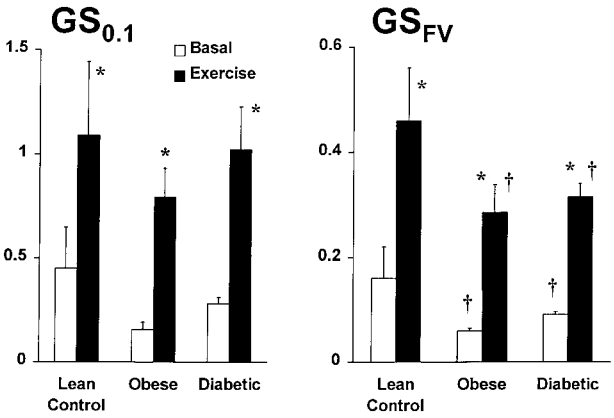

Fig 2. Effects of exercise on $GS_{0.1}$ activity and GS_{FV}. Muscle biopsies were performed before and after 3 hours of 60 minutes of cycle exercise at 65% of VO_2max. Glycogen synthase activity was determined using 0.1 and 10 mmol/L G6P, as described in the text. GS_{FV} is defined as $GS_{0.1}/GS_{10}$. Data are shown as means ± SEM in units of nmol/min · mg for $GS_{0.1}$. Values for GS_{FV} are unit-less. Basal values are shown as open bars and postexercise values as black bars. Data from lean control subjects are taken from Koval et al.[9] *$P < .05$ *v* basal values, †$P < .05$ *v* lean controls.

Table 2. Effect of Exercise on Hexokinase Activity

	HKI				HKII			
	Soluble		Particulate		Soluble		Particulate	
	Basal	Exercise	Basal	Exercise	Basal	Exercise	Basal	Exercise
Diabetic	1.26 ± 0.24	1.11 ± 0.34	1.03 ± 0.29	0.62 ± 0.26	1.39 ± 0.37	1.32 ± 0.38	1.82 ± 0.61	1.37 ± 0.38
Obese	0.97 ± 0.25	0.52 ± 0.09	0.61 ± 0.23	0.65 ± 0.23	0.68 ± 0.21	1.09 ± 0.23	0.99 ± 0.14	1.32 ± 0.47

NOTE. Data are given as means ± SEM in units of pmol/min · μg protein.
Abbreviations: HKI and HKII, hexokinase I and II, respectively.

"exercise resistance" is manifested in a different way than insulin resistance. Insulin resistance apparently decreases the ability of insulin to increase transcription of the HKII gene, while exercise resistance decreases the ability of exercise either to increase translation of HKII mRNA or alters the function of newly translated HKII protein. The data in the present study do not speak to whether there is an abnormality that is common to insulin resistance and exercise resistance.

Regardless of the mechanism by which obese and type 2 diabetic patients are resistant to the ability of insulin to increase HKII activity in skeletal muscle, this abnormality could be responsible, in part, for decreased basal HKII activity in obese and diabetic patients described previously.[7,8] This decrease in basal HKII activity could influence subsequent insulin stimulation of glucose phosphorylation. One way that insulin could increase glucose phosphorylation is to induce a translocation of HKII protein to mitochondria. There is evidence that this occurs in vivo in human muscle.[23] A decrease in basal HKII activity could reduce the amount of HKII available for translocation to the mitochondria and might reduce the ability of insulin to increase glucose phosphorylation. This may have important ramifications with regard to glucose metabolism, as there is a defect in insulin-stimulated glucose phosphorylation in human muscle in vivo.[2] In this regard, it may be significant that training improves glucose transport/phosphorylation in muscle.[24] This suggests that repeated bouts of exercise may increase HKII expression, whereas the single bout of exercise in the present study did not.

The obese and diabetic subjects who took part in the present study had a lower average VO_2max compared with the lean control subjects reported previously.[9] Because all of these subjects exercised at 65% of VO_2max, this means that the lean control subjects exercise at a higher absolute workload. It is possible that this higher absolute workload could have been responsible for the differences in response among these groups. Arguing against this possibility, however, is the finding that glycogen synthase responded to these different levels of absolute exercise in a similar manner. This indicates that even though different absolute workloads were used in the 2 studies, there were similar effects on the muscle produced by the same relative workloads. This is true to the extent that activation of glycogen synthase reflects other contraction-mediated events in muscle.

In summary, these results show that a single bout of exercise increases HKII mRNA levels, but not HKII activity, in muscle from obese and type 2 diabetic patients. This abnormality may be responsible, in part, for decreased basal hexokinase activity and decreased glucose phosphorylation in muscle of these patients.

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