Exercise reduces muscle glucose transport protein (GLUT-4) mRNA in type 1 diabetic patients

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Second Department of Medicine, Helsinki University Hospital, 00290 Helsinki, Finland, Section of Applied Physiology, Department of Internal Medicine, Washington University School of Medicine, St. Louis, Missouri 63110; and Department of Metabolism, Hungarian Heart Center, 8231 Balatonfured, Hungary

Koivisto, Veikko A., Raymond E. Bourey, Helena Vuorinen-Markkola, and Laszlo Koranyi. Exercise reduces muscle glucose transport protein (GLUT-4) mRNA in type 1 diabetic patients. J. Appl. Physiol. 74(4): 1755-1760, 1993.—We examined the effect of acute exercise on muscle glucose transporter (GLUT-4) protein and mRNA concentrations in nine male type 1 diabetic patients (age 31 ± 3 yr, body mass index 23.6 ± 0.7 kg/m², insulin dose 44 ± 4 U/day, glycosylated hemoglobin 7.8 ± 0.4%) and in nine healthy control subjects (34 ± 1 yr, 25.3 ± 0.8 kg/m²). Three hours of cycle ergometer exercise was performed after an overnight fast. A needle biopsy (100-150 mg) was taken from the quadriceps femoris 40 min before and immediately after the end of exercise. During exercise, plasma glucose, insulin, cortisol, and growth hormone concentrations were higher in the diabetic patients than in the control subjects. In the basal state, GLUT-4 protein and mRNA concentrations were similar in the two groups. During exercise, GLUT-4 mRNA concentration decreased by 30-45% in the diabetic patients but remained unchanged in the control subjects. GLUT-4 protein content remained unchanged in both groups. These data suggest an abnormal GLUT-4 mRNA production or degradation or both in type 1 diabetic patients during physical exercise.

glucose metabolism; hyperglycemia; hyperinsulinemia

There is a severalfold increase in glucose uptake into exercising muscle, depending on the duration and intensity of exercise (1). Two factors are primarily responsible for the increase in glucose utilization by the exercising muscle: 1) an increase in the delivery of glucose and insulin to the muscle as a result of an increase in muscle blood flow (2) and 2) exercise-activated insulin-independent glucose transport mechanisms in the muscle (19). These events lead to the synergistic interaction between exercise and insulin in the stimulation of muscle glucose disposal (4). The cellular mechanisms of exercise-induced stimulation of glucose transport are still unclear. Recent studies in animals suggested that glucose transport proteins, in particular the insulin-responsive isoform (GLUT-4), could be involved in enhanced glucose transport during acute exercise. An increase in the amount of glucose transport protein in the plasma membrane of skeletal muscle has been observed in the rat after acute exercise (7, 15, 18). The increase in GLUT-4 protein concentration in plasma membrane has occurred simultaneously with a decrease in the intracellular fraction, suggesting a translocation of transporters from intracellular to plasma membrane pool during exercise (8, 12). In other studies, however, an increase in plasma membrane glucose transporters has been observed with no concomitant decrease in cytochalasin binding sites in the intracellular membrane fraction from exercised muscles (7). These latter studies suggest that either increased synthesis or enhanced intrinsic activity of plasma membrane GLUT-4 protein or both contribute to the enhanced muscle glucose uptake during exercise. Alternatively, the intracellular GLUT-4 protein pool is so large that the decrease in not measurable, or the membrane fractionation techniques are too crude to detect possible exercise-induced translocation.

Whereas observations in rats (10, 29, 30) and in humans (20, 23) demonstrate that physical training increases muscle GLUT-4 protein concentration, no data are available in healthy or diabetic humans regarding the influence of acute exercise on the GLUT-4 protein or its mRNA in human skeletal muscle. We recently demonstrated a correlation between muscle GLUT-4 protein content and the rate of insulin-stimulated glucose disposal in healthy humans in the resting state (24). In type 1 diabetic patients, both GLUT-4 mRNA and protein content are normal in the basal state (35). In response to 4 h of insulin infusion and augmented glucose disposal, GLUT-4 mRNA increased and protein content decreased in healthy subjects, whereas both responses were blunted in type 1 diabetic patients (35). Whether the stimulation of glucose disposal by exercise has an effect on glucose transport protein or its mRNA and whether this response is abnormal in diabetes are not known. Consequently, the aim of the present study was to examine muscle GLUT-4 protein and mRNA response to long-term exercise in healthy subjects and type 1 diabetic patients.

SUBJECTS AND METHODS

Subjects

Nine male type 1 diabetic patients (31 ± 3 yr, 23.6 ± 0.7 kg/m²) participated in the study. They were taking insulin (44 ± 4 U/day) with continuous subcutaneous infusion (n = 2) or in three (n = 1) or four (n = 6) injections per day. Their mean duration of diabetes was 10 ± 2 yr, 0161-7567/93 $2.00 Copyright © 1993 the American Physiological Society

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and glycosylated hemoglobin was 7.8 ± 0.4% (reference value 4–6% in our laboratory). None of the patients had any clinically significant micro- or macroangiopathic complications. Nine healthy males (34 ± 1 yr, 25.3 ± 0.8 kg/m²) served as control subjects. All subjects gave their informed consent before participation. The study protocol was approved by the Ethical Committee of the Helsinki University Hospital.

**Design**

The study was started after an overnight fast. On the previous day, the diabetic patients had taken their usual insulin dose. If the fasting blood glucose was >10 mmol/l, diabetic patients (n = 4) were given 4–6 U of intermediate-acting insulin subcutaneously into the arm to prevent a further rise in plasma glucose. The patients with continuous subcutaneous insulin infusion continued the basal insulin infusion; other patients were not given any insulin before or during the exercise. In the diabetic patients the exercise intensity employed was 444 ± 78 kpm/min with a heart rate of 130 ± 4 beats/min, and in the control subjects it was 591 ± 35 kpm/min, raising heart rate to 126 ± 3 beats/min. From a venous catheter in an antecubital vein, the first blood sample (−40 min) was taken before the muscle biopsy and the other blood samples were taken at 30-min intervals during the 180 min of exercise, as indicated in Table 1.

**Muscle Biopsies**

Forty minutes before and immediately after the end of exercise, a percutaneous muscle biopsy (100–150 mg) was performed with a Bergstrom needle under local anesthesia (1% lidocaine). The samples were obtained from vastus lateralis muscle from opposite sites. The specimen was removed from the muscle with suction applied through a syringe attached to the needle, and then it was rapidly injected into liquid nitrogen. This procedure allowed the sample to be frozen within 5 s. The specimens were stored in liquid nitrogen until analysis.

**Quantitation of mRNA and DNA**

Total tissue RNA was extracted using the modified guanidine thiocyanate water-saturated phenol-chloroform extraction method, as previously described (24). Samples were homogenized in 4 M guanidine thiocyanate containing octophenol-ethylenoxide detergent (Nonidet P-40, Sigma Chemical, St. Louis, MO). After determination of the absorbance at 260 (A₂₆₀) and 280 nm (A₂₈₀), aliquots of mRNA were run on MiniGel to verify the integrity of RNA preparations. The A₂₆₀/₂₈₀ and the 28S/18S ratios were 2 for all samples.

**cDNA probes and synthetic mRNA standards.** The GLUT-4 clone from the human intestinal library of K. Tordjman, D. E. James, and M. Mueckler was identical to the published human GLUT-4 sequence (11). A 1.4-kb cDNA fragment was subcloned into the EcoRI site of the plasmid pGEM 3Z (+) (Promega, Madison, WI). Transcription of uniformly labeled [³²P]RNA and synthetic mRNA with T7 or SP6 RNA polymerase was performed according to the protocol provided by the supplier.

**Quantitation of GLUT-4 mRNA was performed using dot-blot analysis.** Aliquots of total RNA (0.5–10 μg) and dilutions of synthetic mRNA (0.5–1,000 pg) as standards were dissolved in 15% formaldehyde-10× saline sodium citrate and blotted onto Nytran (Schleicher and Schuell, Keene, NH) membranes. Membranes were hybridized for 10–18 h at 60°C in 50% formamide, 5× SSPE (0.9 mol/l sodium chloride, 5 mmol/l EDTA, and 50 mmol/l Na₂HPO₄, pH 7.4), 5× Denhardt solution, 0.1% sodium dodecyl sulfate, and 200 μg/ml salmon sperm DNA and then washed at 65°C in 0.1× SSC and 0.1% sodium dodecyl sulfate according to instructions recommended by the vendor. Blots were exposed to Kodak XAR5 film at −80°C by use of intensifying screens (Cronex Lightening Plus, E. I. Dupont de Nemours, Wilmington, DE). The amount of mRNA present in each sample determined in duplicate was measured by densitometric analysis, in which the intensity of the sample dot was compared with that of the standard dots. Autoradiographs were developed for various periods of time so that the intensity of

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**TABLE 1. Effect of 3 h of exercise on plasma glucose, blood lactate, and serum hormone concentrations in type 1 diabetic patients and healthy controls**

<table>
<thead>
<tr>
<th></th>
<th>−40 min</th>
<th>0 min</th>
<th>30 min</th>
<th>60 min</th>
<th>90 min</th>
<th>120 min</th>
<th>150 min</th>
<th>180 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mM</td>
<td>11.3±1.3*</td>
<td>11.3±1.2*</td>
<td>11.1±1.2*</td>
<td>10.7±1.4*</td>
<td>10.2±1.3*</td>
<td>8.4±1.0*</td>
<td>8.1±0.9*</td>
<td>7.6±0.7*</td>
</tr>
<tr>
<td>Control</td>
<td>5.1±0.1</td>
<td>5.0±0.1</td>
<td>4.8±0.1</td>
<td>4.7±0.1</td>
<td>4.6±0.1</td>
<td>4.6±0.2</td>
<td>4.2±0.2</td>
<td>4.2±0.2</td>
</tr>
<tr>
<td>Lactate, mM</td>
<td>0.73±0.11</td>
<td>0.66±0.05</td>
<td>1.33±0.29</td>
<td>1.09±0.16</td>
<td>1.62±0.20</td>
<td>1.49±0.16</td>
<td>1.65±0.29</td>
<td>1.66±0.23</td>
</tr>
<tr>
<td>Diabetic</td>
<td>0.62±0.05</td>
<td>0.64±0.05</td>
<td>0.88±0.11</td>
<td>0.92±0.11</td>
<td>0.75±0.06</td>
<td>0.82±0.07</td>
<td>0.82±0.08</td>
<td>1.18±0.30</td>
</tr>
<tr>
<td>Control</td>
<td>0.64±0.05</td>
<td>0.64±0.05</td>
<td>0.88±0.11</td>
<td>0.92±0.11</td>
<td>0.75±0.06</td>
<td>0.82±0.07</td>
<td>0.82±0.08</td>
<td>1.18±0.30</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.01 vs. control; †P < 0.01 vs. baseline; $P < 0.05 vs. baseline; $P < 0.05 vs. control.
During exercise, plasma glucose concentration decreased whereas blood lactate and serum hormone concentrations were similar to those in healthy subjects (Table 1). Blood lactate, growth hormone, and cortisol concentrations increased in both groups. Blood lactate, growth hormone, and cortisol concentrations were slightly higher in diabetic patients than in control subjects during exercise (Table 1).

**Quantitation of Transporter Protein by Immunoblotting**

Muscle was homogenized (Brinkman, Westbury, NY) on ice at high speed for 45 s in HES buffer (20 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid, 1 mM EDTA, 250 mM sucrose, pH 7.4, 1:20 wt/vol). The protein concentration was determined by the method of Lowry and Passonneau (27) (Sigma Diagnostics, St. Louis, MO), and samples were analyzed by quantitative immunoblotting, as previously described (26). Blots were incubated with phosphate-buffered saline (pH 7.4) containing 50 mg/ml powdered milk (Carnation, Los Angeles, CA) for 60 min at 22°C, with polyclonal antibody P349 raised against COOH-terminal 15-amino acid peptide, and, after they were washed, with 125I-labeled donkey anti-rabbit immunoglobulin A antibody (Amersham, Arlington Heights, IL). Quantitation was performed by excising labeled bands and counting in a gamma counter. Equal areas away from the band of interest were excised and analyzed to establish background.

**Other Determinations**

Plasma glucose was determined using the glucose oxidase method (Glucose Analyzer II, Beckman Instruments, Fullerton, CA). Blood lactate was determined with the lactate dehydrogenase method (16). Serum insulin concentration was determined by radioimmunoassay after precipitation with polyethylene glycol (5). Serum growth hormone (25) and cortisol (32) concentrations were determined radioimmunologically.

**Statistical Analysis**

Analysis of variance, paired t test, and unpaired t tests were used as appropriate. In the analysis of blood values, 0-min concentration was taken as the baseline value. Values are expressed as means ± SE.

**RESULTS**

**Blood Concentrations**

Before exercise, diabetic patients were hyperglycemic, whereas blood lactate and serum hormone concentrations were similar to those in healthy subjects (Table 1). During exercise, plasma glucose concentration decreased in both groups but remained higher in the diabetic patients. Serum insulin concentration decreased in the control subjects but did not change significantly in the diabetic patients. Thus, 30 min before the end of the exercise, diabetic patients were hyperinsulinemic compared with the control subjects. Blood lactate and serum growth hormone and cortisol concentrations increased in both groups. Blood lactate, growth hormone, and cortisol concentrations were slightly higher in diabetic patients than in control subjects during exercise (Table 1).

**GLUT-4 mRNA and Protein Content**

Before exercise, muscle GLUT-4 mRNA and protein content were similar in the diabetic patients and healthy controls when expressed per either total RNA or DNA (Fig. 1). During exercise, GLUT-4 mRNA decreased in the diabetic patients but not in the control subjects. The decrease was observed when the concentration was calculated per total RNA (30% decrease, P < 0.05) or per DNA (44%, P < 0.01, Fig. 1). When calculated per tissue weight, GLUT-4 mRNA concentration fell in the diabetic patients by 38% from 51.8 ± 21.7 to 31.9 ± 18.2 pg/mg tissue (P < 0.02) but remained unchanged in the healthy subjects (53.0 ± 47.0 vs. 49.2 ± 29.0 pg/mg tissue). Total RNA concentration remained unchanged during exercise in the diabetic (0.82 ± 0.12 μg/mg tissue vs. 0.76 ± 0.22 after exercise) and control subjects (0.78 ± 0.21 μg/mg tissue before vs. 0.70 ± 0.14 after exercise). Nor did total DNA concentration change during exercise in the diabetic patients (0.23 ± 0.02 μg/mg tissue before vs. 0.24 ± 0.04 after exercise) or in the control subjects (0.24 ± 0.03 μg/mg tissue before vs. 0.23 ± 0.04 after exercise).

GLUT-4 protein content was similar in the two groups in the basal state and remained unchanged during exercise both in the diabetic patients (706 ± 52 cpm/100 μg total protein before vs. 777 ± 20 after exercise) and the control subjects (743 ± 44 cpm/100 μg total protein vs. 728 ± 48 after exercise).
DISCUSSION

In the streptozocin diabetic, insulin-deficient rat adipose tissue/muscle glucose transport protein (GLUT-4) and its mRNA concentration are decreased in the skeletal muscle (3, 13, 30) and in the adipose tissue (13, 22). This decrease is associated with a catabolic state due to insulin deficiency, because, after insulin treatment, both the protein and the mRNA content are normalized in fat tissue (13). In well-controlled type 1 diabetic patients (35) and in type 2 diabetic patients (9, 14, 17, 28), skeletal muscle GLUT-4 protein concentration is normal in the basal state and GLUT-4 mRNA concentration is even elevated (9, 26). In keeping with our previous observation in type 1 diabetes (35), in the current study both muscle GLUT 4 mRNA and the protein content were normal in diabetic patients in the resting state.

We are not aware of any human studies addressing the effect of acute exercise on glucose transport proteins either in healthy subjects or type 1 diabetic patients. Studies of the rat after acute exercise have reported an increase in the plasma membrane transporters without a concomitant decrease in the intracellular pool (7), suggesting a rise in the transporter concentration. Other studies have shown a translocation of transporters from the intracellular pool to the plasma membrane with no change in the protein concentration (6, 12). In the present study, we employed muscle homogenates. This allows us to determine changes in GLUT-4 protein or its mRNA concentration but not to detect a translocation. With this technique, no change in the muscle glucose transport protein content was observed during exercise in the healthy subjects or diabetic patients. In cross-sectional studies, we (23) and others (20) recently reported elevated levels of muscle GLUT-4 protein concentration in the face of unaltered mRNA concentration in trained healthy subjects. It is thus possible that regular training leads to increased muscle GLUT-4 protein concentration, whereas one exercise is too short for a detectable increase in the protein synthesis, at least when determined immediately after the end of exercise. Moreover, GLUT-4 protein concentration correlates with glucose disposal rate in healthy men (24). Unaltered total concentration does not, however, exclude a possibility for muscle GLUT-4 protein translocation during acute exercise in healthy subjects or diabetic patients.

The GLUT-4 mRNA response was different in the diabetic and control groups; a significant decrease was observed in the diabetic patients with no change in the control subjects. The decrease occurred regardless of whether GLUT-4 mRNA was calculated per total RNA, per DNA, or per tissue weight.

Regarding the reasons for the different response in diabetic and control subjects, several factors should be considered. First, samples from both diabetic patients and control subjects were treated in a similar fashion. Moreover, in previous studies we have used β-actin mRNA determination as control with no change in the face of alterations in GLUT-4 mRNA (35). Thus it is unlikely that a fall in GLUT-4 mRNA in the diabetic patients was artifactual but, rather, a specific decline.

Second, the relative exercise intensity, as determined from the heart rate response, was similar in both groups. Thus the different GLUT-4 mRNA response was probably not due to variations in the exercise intensity.

Third, in diabetic patients, either the degradation or synthesis of GLUT-4 mRNA or both are different from those in the healthy controls. Unfortunately, so far it has not been possible to label the GLUT-4 mRNA with sufficient activity to determine the turnover rate. Moreover, cells in culture express primarily GLUT-1 mRNA (21) and may not be useful in attempts to clarify the turnover rate of muscle GLUT-4 mRNA in vivo. Thus it is unclear whether the kinetics of GLUT-4 mRNA are different in the diabetic and control subjects during exercise.

Fourth, diabetic patients had elevated glucose and insulin concentrations during exercise.

During exercise, glucose utilization rate is higher in diabetic patients with hyperinsulinemia than in control subjects (36). Although we did not measure glucose kinetics, a slightly higher blood lactate concentration suggests more glucose utilization in diabetic patients than in healthy controls. In addition, a greater fall in blood glucose concentration can be, at least in part, due to a higher peripheral glucose disposal in the diabetic patients than in control subjects. Thus, during exercise, the contraction-stimulated glucose disposal may have contributed to the downregulation of GLUT-4 mRNA in diabetic patients.

Finally, growth hormone and cortisol response to exercise was slightly higher in the diabetic patients than in the control subjects. Similarly, the catecholamine response was also higher than normal in hyperglycemic type 1 diabetic patients (35). We recently showed that a 4-h insulin infusion increased GLUT-4 mRNA by 50% in healthy subjects, whereas no change occurred in type 1 (35) or type 2 (26) diabetic patients. We can postulate that because counterregulatory hormones antagonize insulin action, their higher than normal concentration in diabetic patients may have an effect on GLUT-4 mRNA opposite to that of insulin. Thus our current and our previous (26, 35) data indicate that GLUT-4 mRNA response to both insulin and exercise stimulus is abnormal in diabetic patients. Whether the abnormal GLUT-4 mRNA response is related to alterations in glucose metabolism, counterregulatory hormone response, or other factors is still unknown.

The causal relationship between abnormalities in the glucose metabolism and GLUT-4 mRNA response can only be speculated. When whole body glucose flux was normalized by hyperglycemia in the resting state, the GLUT-4 mRNA response was not restored to normal in type 1 diabetic patients (34). Thus, in the resting state, the insulin-stimulated changes in GLUT-4 mRNA are independent of glucose flux. However, we recently demonstrated increased GLUT-4 protein concentration in trained healthy athletes with augmented insulin sensitivity (23). GLUT-4 concentration was elevated in the face of unchanged mRNA level, and it was closely related to increased glucose disposal in these individuals. Thus, with chronic training, there is a posttranscriptional increase in muscle GLUT-4 protein concentration that may contribute to enhanced insulin sensitivity.

Taken together, the current observations in concert
with the abnormalities found during insulin-stimulated glucose disposal in the resting state (26, 34, 35) suggest an abnormal expression of muscle GLUT-4 transporter protein gene in type 1 diabetic patients. The defect is not revealed in the basal state, but only under conditions with stimulated glucose metabolism, where the intracellular trafficking of GLUT-4 protein may have a crucial role, such as during insulin infusion or physical exercise.

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REFERENCES


2. BERGER, B. S., AND J. T. SHEPHERD. Regulation of the circula-


15. KOIVISTO, V. A., P. A. EBBING, A. SOUVINÄKI, AND R. E. BOUREY. Insulin resistant skeletal muscle glucose transporter protein cor-


17. LINNÉSTRÖM, J., V. A. KOIVISTO, I. KORANYI, R. BOUREY, A. M. PERMUTT, AND H. YKI-JÄRVINEN. Level of skeletal muscle glucose transporter protein cor-


