Defect in Insulin Action on Expression of the Muscle/Adipose Tissue Glucose Transporter Gene in Skeletal Muscle of Type 1 Diabetic Patients*

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ABSTRACT

Recently several members of the glucose transporter family have been identified by molecular cloning techniques. We determined the effect of a 4-h insulin infusion on the expression of the muscle/adipose tissue (GLUT-4) glucose transporter mRNAs and protein in 14 insulin-treated type 1 diabetic patients and 15 matched nondiabetic subjects. GLUT-4 mRNAs and protein concentrations were determined in muscle biopsies taken before and at the end of the insulin infusion during maintenance of normoglycemia. In response to insulin, muscle GLUT-4 mRNA increased in the nondiabetic subjects from 24 ± 3 to 36 ± 4 pg/μg RNA (P < 0.001) but remained unchanged in the insulin-resistant diabetic patients (24 ± 2 vs. 26 ± 2 pg/μg RNA, before vs. after insulin). The glucose transporter protein concentrations were similar in the basal state and decreased by 21 ± 7% (P < 0.02) in the normal subjects but remained unchanged in the diabetic patients. The increase of the GLUT-4 mRNA and the decrease in the GLUT-4 protein correlated with the rate of glucose uptake [correlation coefficient (r) = -0.55, P < 0.01, and r = -0.44, P < 0.05, respectively]. We conclude that the insulin response of both the GLUT-4 glucose transporter mRNA and protein are absent in skeletal muscle of insulin-resistant type 1 diabetic patients. Thus, impaired insulin regulation of glucose transporter gene expression can be one of the underlying mechanisms of insulin resistance in type 1 diabetes. (J Clin Endocrinol Metab 75: 795-799, 1992)

THE MAJORITY of type 1 diabetic patients treated with conventional insulin injection regimens are insulin resistant (1). This resistance to insulin is characterized by a decrease in the ability of body tissues, especially skeletal muscle, to use glucose through both oxidative and glycogen synthetic pathways (2). At the cellular level, glucose transport has recently been shown to be the rate-limiting step for glucose metabolism in these patients (2). Thus, the abnormalities in intracellular glucose metabolism could be secondary to reduced glucose transport in insulin-sensitive tissues.

In recent years, major advances have been made in our understanding of the molecules transporting glucose across the cell membrane in various tissues. At least four members of the glucose transporter gene family have been identified. In skeletal muscle, glucose transport occurs through a facilitated diffusion system that involves two glucose transporter isoforms, the HepG2/rat brain (GLUT-1) and the muscle/adipose tissue (GLUT-4) glucose transporter (3). Whereas the GLUT-1 is widely distributed and is found, e.g. in neural tissue, connective tissue, and blood cells, GLUT-4 is expressed exclusively in insulin-sensitive tissues, especially skeletal muscle, fat, and heart (3).

In muscle of severely insulin-deficient streptozotocin diabetic rats, the rate of glucose transport is reduced, and the GLUT-4 mRNA and protein are both depleted (4, 5). Insulin treatment normalizes GLUT-4 messenger RNA (mRNA) and protein levels but does not change GLUT-1 mRNA or protein (4, 5). In patients with noninsulin dependent diabetes mellitus, muscle GLUT-4 mRNA and protein levels are normal when measured in the basal state after an overnight fast (6–8). However, because GLUT-4 is only expressed in insulin-sensitive tissues and could be responsible for glucose transport during insulin stimulation, it seems of interest to compare GLUT-4 mRNA and protein levels in the insulin-stimulated state in diabetic and nondiabetic subjects. In the present study we determined whether and how the putative insulin-sensitive glucose transporter (GLUT-4) responds to hyperinsulinemia induced by a 4-h insulin infusion in insulin-resistant type 1 diabetic patients.

Subjects and Methods

Subjects and study design

Fourteen type 1 diabetic patients and 15 nondiabetic males participated in the studies. Clinical characteristics of the study groups are given in Table 1. Except for diabetes, the patients were healthy, and they did not use any medication in addition to insulin. None of the patients had...
TABLE 1. Clinical characteristics of the study groups

<table>
<thead>
<tr>
<th></th>
<th>Type 1 diabetic patients</th>
<th>Normal subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>14</td>
<td>15</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>29 ± 2</td>
<td>25 ± 1</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23 ± 1</td>
<td>22 ± 1</td>
</tr>
<tr>
<td>Fasting plasma glucose (mmol/L)</td>
<td>7.2 ± 0.4</td>
<td>5.4 ± 0.1</td>
</tr>
<tr>
<td>HbA₁c (%)</td>
<td>8.2 ± 0.5</td>
<td>4.9 ± 0.1</td>
</tr>
<tr>
<td>Fasting serum (free insulin) (pmol/mL)</td>
<td>42 ± 7</td>
<td>29 ± 7</td>
</tr>
<tr>
<td>Duration of diabetes (yr)</td>
<td>10 ± 2</td>
<td></td>
</tr>
<tr>
<td>Fasting serum C-peptide (pmol/mL)</td>
<td>0.1 ± 0.03</td>
<td>0.5 ± 0.03</td>
</tr>
<tr>
<td>Mean insulin dose (IU/day)</td>
<td>43 ± 4</td>
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</table>

*Reference range 4-6%. *Median, C-peptide undetectable in 11/14 of the patients.

clinical signs of neuropathy, nephropathy, or macrovascular complications. Four patients had background retinopathy but none had proliferative retinopathy. Three patients were treated with continuous SC insulin infusion, whereas the others used one (n = 1), two (n = 1), three (n = 3), or four (n = 6) injections of short- or intermediate-acting insulin per day. The nondiabetic subjects were healthy, as judged by history, physical examination, and routine laboratory tests. None of the normal subjects took any medications or had a family history of diabetes. The subjects consumed a weight-maintaining diet containing at least 200 g carbohydrate/day for 3 days before the study, which was performed at the metabolic ward of the Second Department of Medicine of Helsinki University.

After an overnight fast, the rate of whole-body glucose uptake was determined under normoglycemic hyperinsulinemic conditions in both groups, as detailed below. Muscle biopsies for determination of GLUT-4 mRNA and protein concentrations were taken before and after the insulin infusion.

Whole-body glucose uptake measurements

After an overnight fast, two catheters were inserted, one in an antecubital vein for infusions of glucose and insulin and another retrogradely in an ipsilateral heated (70 °C) hand vein for sampling of arterialized venous blood (2). At 0 min, serum insulin was increased to approximately 200 pmol/L by a bolus of 100 IU of insulin (2). The rate of the continuous insulin infusion was 11 pmol/kg/min. Plasma glucose was maintained at approximately 5 mM by a continuous intravenous infusion of 20% glucose. Adjustments in the glucose infusion rate were made based on plasma glucose measurements performed every 5 min in blood samples taken from arterialized venous blood. Normoglycemia was achieved in all patients within 30 min. The rate of glucose uptake was calculated as the mean between 30 and 240 min. Because previous studies have repeatedly demonstrated complete suppression of endogenous glucose production in both type 1 diabetic patients and nondiabetic subjects at insulin concentrations similar to or lower than those achieved in the present study (1, 2), the rate of glucose uptake was calculated from the glucose infusion rate.

Muscle biopsies

Muscle biopsies were taken from vastus lateralis muscle before (~10 min) and at the end of the insulin infusion (240 min) under local anesthesia using a Bergstrom needle. The two biopsies were taken from opposite sites, and rapidly (<5 s) frozen in liquid nitrogen. The samples were kept in liquid nitrogen until analysis. Enough muscle was obtained to determine the GLUT-4 protein in all except one normal subject and the GLUT-4 mRNA was determined in 13 of the normal subjects and in 12 of the diabetic patients.

Complementary DNA (cDNA) probes, synthetic mRNA standards

Human GLUT-4 cDNA was isolated from a jejunal cDNA library (9) using rat GLUT-4 as the probe (10). Sequence analysis indicated that the 1.7-kb human cDNA insert was identical to the hJHT-3 clone previously described by Fukumoto et al. (11). This clone contains a 260-base pair intron at its 3'-end. The cDNA was subcloned into a Bluescript SK+ plasmid (Stratagene, La Jolla, CA). Transcription of 32P-labeled cRNA with T7 RNA polymerase was performed according to the manufacturer's recommendations.

Quantitation of mRNAs and DNA

Total tissue RNA was extracted using a modified guanidine thiocyanate-water-saturated phenol/chloroform extraction method (12). Samples were homogenized in 4 mol/L GuSCN containing o�cylphenyl-ethenylamine detergent (Nonident P-40, Sigma Chemical Co., St. Louis, MO). The quantity and quality of RNA were determined by absorbance at 260 and 280 nm; the values for the absorbance 260/280 ratio were over 2.0 in each sample. Quantitation of GLUT-4 mRNA was performed using dot blot analysis. Aliquots of total RNA (0.5–10 μg) and dilutions of synthetic mRNA (0.5–1000 pg) as standards were dissolved in 15% formaldehyde/10 × SSC (SSC = 0.15 mol/L sodium chloride, 0.015 mol/L sodium citrate, pH 7.0) and blotted onto Nytran (Schleicher & Schuell, Inc., Keene, NH) membranes. Membranes were hybridized for 16–18 h at 60 °C in 50% formamide, 5 × SSPE (SSPE = 0.15 mol/L sodium chloride, 5 mmol/L EDTA, and 50 mmol/L NaH₂PO₄, pH 7.4), and then washed at 65 °C in 0.1 SSC, 0.1% sodium dodecyl sulfate, according to the instructions recommended by the vendor. Filters were exposed to Kodak XAR5 film at 70 °C using intensifying screens (Cronex Lightening Plus, E. I. Dupont de Nemours Co., Wilmington, DE). The amount of mRNA present in each sample, determined in duplicate, was measured by densitometric analysis, comparing the intensity of the sample dot with synthetic mRNA standard dots. Autoradiographs were developed for various periods of time so that the intensity of the unknown samples was within the linear range of standards. In 75% of the determinations, the sample size allowed the determination of actin mRNA as a control. There were no differences in the levels of actin mRNA in the muscle samples taken before and after insulin clamp (data not shown).

Immunoblotting

Muscle was homogenized (Polytron, Brinkman, Westbury, NY) on ice at high speed for 45 s in HES buffer (20 mmol/L HEPES, 1 mmol/L EDTA, 250 mmol/L sucrose, pH 7.4, 1:20, wt/vol). No attempt was made to fractionate muscle into subfractions. Thus, the data reflect changes in total muscle GLUT-4 content. The protein concentration was determined by a modification of the method of Lowry (13), and samples were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Protein was electrophoretically transferred (Polyblot, ABN, Hayward, CA) to nitrocellulose paper. Nitrocellulose blots were incubated with phosphate-buffered saline (pH 7.4) containing 50 mg/mL powdered milk (Carnation Co., Los Angeles) for 60 min at 22 °C and thereafter with a polyclonal antibody specific for the GLUT-4 carboxy-terminus (RB20, Ref. 5, or F349), and after washing with 3% labeled protein A (Amersham Corp., Arlington Heights, IL). Quantitation was performed by excising labeled bands and counting in a γ-counter (Packard Instrument Co., Downers Grove, IL). The samples taken before and after the insulin infusion were always run in adjacent lanes on the same gel. Since all samples could not be simultaneously run on the same gel, only the percentage change [GLUT-4 (dpm/μg protein) change by insulin × 100] was calculated (but not the absolute amount of GLUT-4 protein (dpm/μg) can be compared between the groups and correlated to whole-body glucose metabolism. To quantitate the basal GLUT-4 protein concentration, muscle biopsies were taken after an overnight fast from 11 additional C-peptide negative type 1 diabetic men (age 43 ± 2 yr, body mass index (BMI) 23.4 ± 0.8 kg/m², duration of diabetes 16 ± 3 yr, insulin dose 42 ± 3 IU/day, fasting plasma glucose 9.9 ± 0.7 mmol/L, fasting free insulin 54 ± 7 pmol/L, HbA₁c 8.3 ± 0.5%) and 12 matched nondiabetic men (age 44 ± 2 yrs, BMI 24.8 ± 0.5 kg/m², fasting plasma glucose 4.8 ± 0.1 mmol/L, fasting free insulin 42 ± 7 pmol/L). Equal areas away from the band of interest were excised and analyzed to establish background. The mean counts per minute above background was 538 ± 38 cpm (mean background 97 ± 5 cpm).
Glucose and insulin determinations

Plasma glucose was measured with the glucose oxidase method (14) using Beckman Glucose Analyzer II (Beckman Instruments, Fullerton, CA). Blood for measurement of serum free insulin was drawn every 30 min during the insulin infusion. Serum free insulin was measured with RIA using the Phadeseph Insulin RIA kit (Pharmacia, Uppsala, Sweden) (15).

Data analysis

Comparison of measurements before and after the 4-h insulin infusion was done with the paired t test, and correlation analyses with Spearman’s rank correlation coefficient (r), using the Statsgraphics statistical package (STSC and Statistical Graphics, Rockville, MD). All data are expressed as mean ± SEM.

Results

Glucose uptake in vivo

The 4-h insulin infusion increased plasma free-insulin concentrations similarly in the normal subjects (from 29 ± 7 to 746 ± 29 pmol/L) and in the diabetic patients (from 42 ± 7 to 725 ± 43 pmol/L). During the insulin infusion, the mean (0–240 min) plasma glucose averaged 5.3 ± 0.1 mmol/L in the normal subjects and 5.6 ± 0.1 mmol/L in the diabetic patients (NS). The rate of glucose uptake was lower in the diabetic patients (33.4 ± 1.7 mmol/kg/min) than in the normal subjects (42.3 ± 2.2 mmol/kg/min, P < 0.01).

Expression of GLUT-4 mRNA in skeletal muscle

In the normal subjects, the GLUT-4 mRNA increased during the 4-h period of hyperinsulinemia from 24 ± 3 to 36 ± 4 pg/μg RNA (P < 0.001), or when expressed per DNA, from 24 ± 5 to 41 ± 5 pg/μg DNA (P < 0.001). No change was observed in the diabetic patients (24 ± 2 vs. 26 ± 2 pg/μg RNA, or 39 ± 7 vs. 32 ± 3 pg/μg DNA, before vs. after insulin, Fig. 1). The absolute (P < 0.01) and percentage (P < 0.02) increments in the GLUT-4 mRNA were significantly higher in the normal subjects than in the diabetic patients. The absolute (r = 0.54, P < 0.01) and percentage (r = 0.55, P < 0.01, Fig. 2) change in the GLUT-4 mRNA correlated with whole-body glucose uptake.

Effect of insulin on the GLUT-4 protein in skeletal muscle

The basal GLUT-4 protein concentrations were virtually identical in the type 1 diabetic patients (523 ± 59 dpm/100 μg protein) and the nondiabetic subjects (523 ± 56 dpm/100 μg protein, Fig. 3). The muscle GLUT-4 protein concentration decreased by 21 ± 7% (P < 0.02) in the nondiabetic subjects. In the diabetic patients, the GLUT-4 protein remained unchanged (mean decrease 5 ± 4%, NS). The percentage change of the GLUT-4 protein correlated inversely with the rate of whole-body glucose uptake (r = −0.44, P < 0.05), and the percentage change of the GLUT-4 mRNA (r = −0.51, P < 0.02, Fig. 4).

Discussion

Three new observations were made in the present study. First, in the basal state GLUT-4 mRNA and protein concen-
tations were similar in type 1 diabetic patients and normal subjects, as was also recently found in patients with non-insulin dependent diabetes mellitus (6-8). Second, GLUT-4 mRNA and the total protein concentration were shown to respond to insulin in normal human skeletal muscle. Third, these responses were lacking in insulin resistant-type 1 diabetic patients.

There are presently no data available on the time course of insulin-induced changes in GLUT-4 mRNA in normal muscle. In adipose tissue from streptozotocin diabetic rats, insulin treatment restores the GLUT-4 mRNA concentration within 3-6 h but does not change the GLUT-1 mRNA (16). In L6 muscle cells (17), cultured human fibroblasts (18), and 3T3-L1 adipocytes (19), a maximal increase of GLUT-1 mRNA is observed after 4-6 h of insulin addition. In these cells, insulin does not change the GLUT-4 mRNA (3, 16). The reason for the opposite responses of GLUT-4 and GLUT-1 mRNA to insulin in differentiated muscle vs. cultured cells is unknown, but the time within which the glucose transporter mRNA increased in the present study is comparable to that previously observed in cell cultures and rat adipocytes.

Unexpectedly, the 4-h insulin infusion decreased the total muscle GLUT-4 protein content by 21% in the normal subjects. This finding was also recently reported in preliminary studies by Handberg et al. (20), who observed an 18% decrease in GLUT-4 protein during a 3-h insulin infusion. The decrease in GLUT-4 protein concentration probably indicates a specific decrease of this protein rather than a dilution, because even during combined insulin and amino acid infusions, the calculated leg muscle fractional protein synthetic rate is approximately 0.14%/h (21). Although some proteins may be synthesized rapidly, rates of protein synthesis are usually measured in days rather than hours (22, 23). Whether the decrease in GLUT-4 represents a transient imbalance between synthesis and degradation of GLUT-4 remains to be established. One interpretation is that the decrease in GLUT-4 protein is a signal that stimulates enhanced gene transcription. The inverse relationship between the decrease in protein and the increase in mRNA (Fig. 4) might amply support such a hypothesis. On the other hand, as long as the effect of insulin on the intracellular location of GLUT-4 protein in muscle remains unclear, the physiological significance of the decrease in GLUT-4 is uncertain.

Regarding the cause(s) of insulin resistance in type 1 diabetic patients, the average diurnal insulin concentration in patients with moderate glycemic control is similar to that in normal subjects (24). Under such circumstances, hyperglycemia results from hepatic overproduction of glucose (25). Complete normalization of glycemic control, e.g., with the artificial pancreas, inhibits hepatic glucose production at the expense of peripheral hyperinsulinemia (26). Thus, peripheral insulin deficiency cannot be the cause of insulin resistance in skeletal muscle in these patients.

We have previously demonstrated that hyperglycemia per se can decrease glucose uptake in type 1 diabetic patients (27), and suggested that chronic hyperglycemia is the major cause of insulin resistance in these patients. In support of this proposal, insulin sensitivity is normal in type 1 diabetic patients with excellent glycemic control and during remission (1), and can be ameliorated by improving glycemic control with continuous subcutaneous insulin infusion therapy, even in the face of reduced (25) insulin requirements. We have therefore postulated that insulin resistance develops in peripheral tissues as a consequence of chronic hyperglycemia (27). Normalization of glycemic control could therefore possibly correct the defect in GLUT-4 gene expression characterizing type 1 diabetic patients.

In conclusion, the present data demonstrate that the mRNA for the GLUT-4 glucose transporter increases in response to insulin in human skeletal muscle. The data provide evidence for abnormal insulin regulation of glucose transporter gene expression in insulin resistant type 1 diabetic patients. Because insulin resistance in type 1 diabetic patients is acquired rather than inherited (1), these data imply that secondary metabolic alterations associated with the hyperglycemic state in type 1 diabetes, or chronic hyperglycemia per se, may induce pretranslational defects in insulin-induced glucose transport.

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