Differential Regulation of Glucose Transporter Activity and Expression in Red and White Skeletal Muscle*

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Jeanne M. Richardson‡, Thomas W. Balon§, Judith L. Treadway¶, and Jeffrey E. Pessin†

From the ‡Department of Physiology and Biophysics, The University of Iowa College of Medicine and the ¶Department of Exercise Science, The University of Iowa, Iowa City, Iowa 52242

Insulin-stimulated glucose transport activity and GLUT4 glucose transporter protein expression in rat soleus, red-enriched, and white-enriched skeletal muscle were examined in streptozotocin (STZ)-induced insulin-deficient diabetes. Six days of STZ-diabetes resulted in a nearly complete inhibition of insulin-stimulated glucose transport activity in perfused soleus, red, and white muscle which recovered following insulin therapy. A specific decrease in the GLUT4 glucose transporter protein was observed in soleus (3-fold) and red (2-fold) muscle which also recovered to control values with insulin therapy. Similarly, cardiac muscle displayed a marked STZ-induced decrease in GLUT4 protein that was normalized by insulin therapy. White muscle displayed a small but statistically significant decrease in GLUT4 protein (23%), but this could not account for the marked inhibition of insulin-stimulated glucose transporter activity observed in this tissue. In addition, GLUT4 mRNA was found to decrease in red muscle (2-fold) with no significant alteration in white muscle.

The effect of STZ-induced diabetes was time-dependent with maximal inhibition of insulin-stimulated glucose transport activity at 24 h in both red and white skeletal muscle and half-maximal inhibition at approximately 8 h. In contrast, GLUT4 protein in red and white muscle remained unchanged until 4 and 7 days following STZ treatment, respectively. These data demonstrate that red skeletal muscle displays a more rapid hormonal/metabolic-dependent regulation of GLUT4 glucose transporter protein and mRNA expression than white skeletal muscle. In addition, the inhibition of insulin-stimulated glucose transport activity in both red and white muscle preceded the decrease in GLUT4 protein and mRNA levels. Thus, STZ treatment initially results in a rapid uncoupling of the insulin-mediated signaling of glucose transport activity which is independent of GLUT4 protein and mRNA levels.

The mammalian facilitative glucose transporters are members of a multigene family with distinct but overlapping tissue distributions (1, 2). Initial studies examining the regulation of glucose transport by insulin have been conducted with isolated rat adipocytes due to their high degree of sensitivity and responsiveness. These cells express the GLUT4 glucose transporter protein as well as a substantially lower amount (approximately 10-20-fold) of the GLUT1 isoform (3-11). It has been well established that acute insulin stimulation of adipocyte glucose transport activity occurs primarily by the recruitment of the GLUT4 protein from an intracellular pool to the cell surface with a significantly smaller effect on the subcellular distribution of the GLUT1 protein (12-13). Recently, insulin resistance induced by streptozotocin (STZ) diabetes and fasting has been observed to markedly reduce (~10 fold) the level of GLUT4 protein and mRNA in rat adipose tissue with no significant alteration in the expression of the GLUT1 glucose transporter (14-17). The decrease in adipose GLUT4 protein expression has been generally thought to account for the inhibition of insulin-stimulated glucose transport activity that occurs under these altered metabolic conditions.

Quantitatively, however, the most important tissue with regard to insulin-dependent glucose transport is skeletal muscle, which is primarily responsible for postprandial glucose uptake (18) and is the major site of peripheral tissue resistance to insulin in diabetes (19). As in adipose tissue, the GLUT4 glucose transporter is expressed in skeletal muscle (5-8) and recently has been demonstrated to undergo insulin-stimulated recruitment (20, 21). Intact skeletal muscles are composed of varying proportions of muscle fibers, which differ in contractile and metabolic properties, including sensitivity and responsiveness to insulin (22-24).

Since insulin action is greater in the red Type I (slow twitch, oxidative) and Type IIA (fast twitch, oxidative/glycolytic) compared with the white Type IIB (fast twitch, glycolytic) fiber type, we have examined the regulation of glucose transport activity and expression in white- and red-enriched skeletal muscle from control, diabetic, and insulin-treated diabetic rats. The data presented in this study demonstrate a skeletal muscle fiber type-specific regulation of GLUT4 expression as well as a dissociation between GLUT4 protein levels and insulin-stimulated glucose transport activity.

EXPERIMENTAL PROCEDURES

Animals—Male Sprague-Dawley rats (160-180 g) were either given a single intraperitoneal injection of 125 mg/kg STZ in citrate buffer (The Upjohn Co.) to induce a diabetic state or injected with vehicle alone. This large dose of STZ has been previously demonstrated to...
induce a rapid and uniform decrease in insulin levels such that variation in the time dependence of insulin deficiency is minimized (25). Briefly, the animals were maintained for up to 7 days and blood glucose, insulin, and body weights were monitored. Rats were considered diabetic only if tail blood glucose levels exceeded 300 mg/dl following STZ treatment. Under these conditions, 88% of the STZ-diabetic animals survived the 7-day protocol, and the same animals were randomly selected for subsequent treatment with daily injections of 2 units of human regular insulin (Humulin R) and 3 units of intermediate acting human NPH insulin (Humulin N) for up to 18 days. All animal care was performed in accordance with institutional guidelines.

**Glucose Transport**—Following the above treatments, rats were anesthetized with an intraperitoneal injection of pentobarbital (5 mg/100 g) and surgically prepared for perfusion by the method of Ruderman et al. as modified by Goodman et al. (26, 27). The initial perfusion medium consisted of Krebs-Henseleit solution, bovine erythrocytes (30% hematocrit), 4% bovine serum albumin (Cohn fraction V; Pentex, Kankakee, IL), and 0.15 mM pyruvate. Insulin (crystalline pork insulin; Lilly) was added to the perfusate to a concentration of 140 nM. The hindquarter muscles were then perfused for 30 min to allow for equilibration prior to experimental measurements. For measurement of skeletal muscle glucose transport, 3-O-[U-14C]methyl-D-glucose (3 mM, 20 mCi) plus 20 mCi of [3H]mannitol (Amersham Corp.) was added to the perfusate for 7 min. Transport of 3-O-methylglucose was determined by dissection of the individual muscles and extraction in HClO4. Nonspecific trapping in the extracellular space was corrected for by the amount of recovered [3H]mannitol. Microscopic examination of representative sections of the dissected muscle groups demonstrated the absence of any detectable adipose cells.

**Western Blotting**—Skeletal muscle tissues were dissected, frozen under liquid nitrogen, and pulverized with a mortar and pestle. The tissue was then homogenized with a Brinkmann polytron in 10 volumes of ice-cold buffer containing 250 mM sucrose, 10 mM Tris, and 2 mM EDTA, pH 7.4. The homogenates were centrifuged at 3700 g for 25 min at 4 °C. The supernatant was adjusted to 0.80 M KCl, mixed end-over-end at 4 °C for 30 min, and then centrifuged at 150,000 × g for 60 min at 4 °C to obtain total post-nuclear membranes. The membranes were resuspended in homogenization buffer by repeated passage through a 25-gauge needle. Total muscle protein extracts were prepared by the direct solubilization of the muscle tissues in RIPA buffer (50 mM Tris, pH 7.4, 150 mM NaCl, 1% Triton X-100, 0.1% SDS, and 1% sodium deoxycholate) followed by centrifugation at 100,000 × g at 4 °C for 60 min. The samples (25–50 μg) were then subjected to SDS-polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes. For the determination of relative GLUT4 protein levels, the nitrocellulose membranes were incubated overnight at 4 °C with 4 μCi/ml of the anti-GLUT4 protein monoclonal antibody 1F8 (28) followed by addition of 1 μCi of [35S]-labeled sheep anti-mouse antibody (Amersham Corp.) and autoradiography. In some experiments, a rabbit GLUT4 polyclonal antibody (IRGT, East Acres) was used at a 1:1000 dilution with the addition of 1 μCi of [35S]-labeled protein A and autoradiography. Control experiments demonstrated that under these conditions the Western blot signal of the GLUT4 protein was linear from 10–100 μg of loaded membrane protein.

**Northern Blotting**—Total RNA was isolated according to the method of Chirgwin (29) from the individual hindquarter skeletal muscles. The RNA (20 μg) was denatured, size-fractionated on a 1% agarose gel, and transferred to nitrocellulose. The samples were then hybridized at 60 °C in a solution containing 50% formamide, 5 × SSPE, 5 × Denhardt's, 100 μg/ml salmon sperm DNA, and 1% SDS containing 2 × 106 cpm/ml of an anti-sense riboprobe generated from pSM1-1, which encodes the rat GLUT4 protein (7). The membranes were washed three times in 1 × SSPE, 0.5% SDS at 65 °C for 15 min, followed by incubation with 2 × SSPE containing 1 μg/ml RNase A (Sigma) at 70 °C for 15 min. The membranes were then washed in 2 × SSPE plus 0.1% SDS at room temperature and subjected to autoradiography. Under these conditions, the relative detected levels of GLUT4 mRNA were linear from 5–40 μg of loaded RNA.

**Statistical Analysis**—Statistical analyses were performed by one-way analysis of variance. A Sheffe post hoc comparison test was applied to this analysis. A P value was considered significant (p < 0.05). Results are represented as the mean ± S.D. from 3–10 independent determinations as indicated in the figure legends.

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**RESULTS**

Previous studies have demonstrated that STZ-induced diabetes decreases insulin-stimulated glucose uptake in total rat hindquarter muscle without any major effect on basal glucose uptake (25, 30). Consistent with these studies, basal 3-O-methylglucose glucose transport activity in pooled white superficial quadriceps/gastrocnemius muscles (WQ/G), which are enriched for type IIB fibers, pooled deep red quadriceps/gastrocnemius muscles (RQ/G), which are enriched for type I and IIA fibers, and soleus, which is primarily composed of type I fibers, was not affected by STZ-induced diabetes to any great extent compared with control animals (Fig. 1). In contrast, 6 days of STZ-induced diabetes resulted in essentially complete inhibition of insulin-stimulated glucose transport activity in all three skeletal muscle types. The inhibition of insulin-stimulated glucose transport activity was observed to gradually recover with daily insulin therapy (data not shown) and was fully restored to control values by 18 days (Fig. 1).

To correlate the changes in glucose transport activity with the levels of the muscle-specific glucose transporter protein, Western blot analysis was performed on total post-nuclear muscle membrane from white, red, and soleus skeletal muscle (Fig. 2). In STZ-diabetic rats, the GLUT4-specific monoclonal antibody 1F8 demonstrated a 2- and 3-fold decrease in this specific isoform in red and soleus skeletal muscles, respectively (Fig. 2, lanes 5 and 8), compared with the control animals (Fig. 2, lanes 4 and 7). Insulin therapy resulted in a complete recovery of the red and soleus skeletal muscle GLUT4 isoform to control levels (Fig. 2, lanes 6 and 9). The decrease in GLUT4 protein by STZ treatment and subsequent recovery by insulin therapy was apparently consistent with the alterations in insulin-stimulated glucose transport activity in the red and soleus skeletal muscle (Fig. 1). In addition, cardiac muscle also displayed an approximate 2-fold specific decrease in GLUT4 protein, which fully recovered after the initiation of insulin therapy (Fig. 2, lanes 10–12). Surprisingly,
Western blot analysis of the GLUT4 glucose transporter protein in muscle membranes from control, STZ-diabetic, and insulin-treated STZ-diabetic rats. Total post-nuclear muscle membranes from WQ/G, RQ/G, soleus, and heart were isolated from groups of control (C), 7-day STZ-diabetic (D), and 15-day insulin-treated STZ-diabetic (I) rats. WQ/G (50 µg), RQ/G (50 µg), soleus (25 µg), and heart (50 µg) muscle membrane proteins were fractionated by SDS-PAGE and transferred to nitrocellulose membranes for immunoblotting with GLUT1, GLUT4, and IRGT antisera (see Experimental Procedures).

Data from multiple Western blots of muscle GLUT4 protein, as described in Fig. 2, were independently performed nine times for the white skeletal muscle groups and four times for the red skeletal muscle groups. A quantitative summary of multiple Western blots for white and red skeletal muscle is presented in Fig. 3. Therefore, the relative expression of the GLUT1 isoform in white skeletal muscle in STZ-induced diabetic soleus, red, white, and cardiac muscles (data not shown) was subjected to obtain samples from 3–10 independent observations. * , p < 0.05; ** , p < 0.01 compared with the GLUT4 levels in muscle from control animals.

To further investigate the discordance between GLUT4 protein and mRNA expression with insulin-stimulated glucose transport activity, we examined the time-dependent alterations of GLUT4 function and expression in both white and red hindquarter skeletal muscle (Fig. 6). Insulin deficiency resulted in a rapid decrease of insulin-stimulated glu-
cose transport activity in both white (Fig. 6A) and red (Fig. 6B) muscle with half-maximal inhibition at approximately 8 h and maximal inhibition by 24 h. In contrast, white muscle GLUT4 protein levels were unaltered following the first 4 days of insulin deficiency but were marginally decreased by 23% at day 7 (Fig. 6A). The small decrease in white muscle GLUT4 protein was not reflected in GLUT4 mRNA levels, which remained unchanged under these conditions. Although red muscle GLUT4 protein and mRNA decreased to a greater extent and at a faster rate than white muscle, no significant change was detected within the first 24 h (Fig. 6B). However, 4 days of insulin deficiency resulted in a statistically significant 19% and 12% decrease in GLUT4 protein and mRNA in red muscle, which continued to decline to 51 and 44%, respectively, by day 7. Analysis of soleus muscle yielded results essentially identical to that observed for the red hindquarter muscle (data not shown). Even though at the 6–7-day time points there is a reasonable correlation between GLUT4 protein expression and insulin-stimulated glucose transport activity in red muscle, these data clearly demonstrate a temporal separation of these two events in both white and red skeletal muscles at earlier times.

**DISCUSSION**

Initial studies examining the regulation of glucose transport activity have utilized rat adipocytes as a model system due to their relative ease of isolation and high degree of sensitivity and responsiveness to insulin. However, the major site of insulin-mediated peripheral glucose disposal in mammals is skeletal muscle, in which glucose transport is the rate-limiting step for glucose utilization (31). To date, studies which have examined the tissue distribution and insulin regulation of the various glucose transporter gene family members have indicated that the predominant insulin-responsive glucose transporter in both adipose and skeletal muscle is GLUT4 (6, 10).

Several studies have documented that STZ-induced diabetes results in a dramatic decrease in adipose GLUT4 protein and mRNA, which apparently accounts for the loss of insulin-stimulated glucose transport activity under these conditions (14–17). In contrast to the marked decrease in GLUT4 protein and mRNA levels in adipocytes of STZ-diabetic rats, previous studies have observed a modest decrease (~30%) in GLUT4 levels measured by cytochalasin B binding and immunoblotting in pooled hindquarter skeletal muscle (16, 30, 32). Nevertheless, skeletal muscle insulin-stimulated glucose transport activity was completely inhibited in the STZ-diabetic rat (Refs. 25, 30 and Fig. 1). However, such studies in skeletal muscle tissue are complicated by the heterogeneous composition of muscle with fiber type differences in insulin sensitivity and responsiveness (22–24). In addition, it has recently been observed that white fast twitch glycolytic fibers are significantly less insulin-responsive and express lower levels of GLUT4 protein and mRNA than red slow twitch oxidative muscle fibers (33).

In order to address the possible muscle fiber type-specific regulation of glucose transporter function and expression in an experimental model of type I diabetes, we examined the relationship between glucose transport activity, GLUT4 protein and mRNA levels in control, STZ-diabetic, and insulin-treated STZ-diabetic rats. The data presented in this manuscript demonstrate that the GLUT4 glucose transporter isoform in red, soleus, and cardiac muscles is regulated by STZ-induced diabetes and insulin treatment in a manner qualitatively similar to that observed in adipose tissue. In contrast, white skeletal muscle apparently down-regulates GLUT4 protein and mRNA at a significantly lower rate and extent than the red and cardiac muscles.

The physiological consequences of this very surprising muscle-specific regulation of GLUT4 expression may reflect the distinct metabolic properties of these different muscle fibers. Red muscle fibers are enriched in mitochondria and have a correspondingly greater oxidative capacity compared with white muscle fibers (34). The disparity in the regulation of GLUT4 expression in STZ-induced diabetes between red- and white-enriched skeletal muscle suggests that the hormonal control of substrate utilization for energy production may be related to a fiber type-specific gene regulatory mechanism. Recently, several studies have reported variable effects of STZ-induced diabetes on GLUT4 protein and mRNA levels in hindquarter skeletal muscle of diabetic rats (16, 35). In addition, several laboratories examining the levels of muscle GLUT4 expression in obese and non-insulin-dependent diabetes mellitus patients have either observed a decrease in GLUT4 expression (36) or no change (37). The skeletal muscle fiber type specificity for GLUT4 expression observed in this study may account for these discrepant findings by other laboratories.

The data presented in Fig. 6 also demonstrate that STZ-diabetes results in a rapid inhibition of insulin-stimulated glucose transport activity in red as well as in white skeletal muscle. This occurs prior to any alteration in the expression of GLUT4 protein and mRNA. Although STZ-induced diabetes has been reported to result in a partial loss of skeletal muscle insulin receptor binding and tyrosine-specific protein kinase activity (38, 39), these receptor-related defects are unlikely to account for the complete lack of insulin responsiveness observed in this study. Thus, these data suggest that STZ-induced diabetes induces a rapid uncoupling of the insulin-stimulated glucose transport system prior to the down-regulation of GLUT4 protein and mRNA. This occurs at a step distal to the insulin receptor but prior to the GLUT4 glucose transporter protein itself.

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**REFERENCES**


![Fig. 6. Time-dependent effect of STZ-induced insulin deficiency on insulin-stimulated glucose transport activity, GLUT4 protein, and mRNA expression in white and red hindquarter skeletal muscles.](image-url)