Transgenic mice expressing the human GLUT4/muscle–fat facilitative glucose transporter protein exhibit efficient glycemic control

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ABSTRACT To examine the physiological role of the GLUT4/muscle-fat specific facilitative glucose transporter in regulating glucose homeostasis, we have generated transgenic mice expressing high levels of this protein in an appropriate tissue-specific manner. Examination of two independent founder lines demonstrated that high-level expression of GLUT4 protein resulted in a marked reduction of fasting glucose levels (~70 mg/dl) compared to wild-type mice (~130 mg/dl). Surprisingly, 30 min following an oral glucose challenge the GLUT4 transgenic mice had only a slight elevation in plasma glucose levels (~90 mg/dl), whereas wild-type mice displayed a typical 2- to 3-fold increase (~250–300 mg/dl). In parallel to the changes in plasma glucose, insulin levels were ~2-fold lower in the transgenic mice compared to the wild-type mice. Furthermore, isolated adipocytes from the GLUT4 transgenic mice had increased basal glucose uptake and subcellular fractionation indicated elevated levels of cell surface-associated GLUT4 protein. Consistent with these results, in situ immunocytochemical localization of GLUT4 protein in adipocytes and cardiac myocytes indicated a marked increase in plasma membrane-associated GLUT4 protein in the basal state. Taken together these data demonstrate that increased expression of the human GLUT4 gene in vivo results in a constitutively high level of cell surface GLUT4 protein expression and more efficient metabolic control over fluctuations in plasma glucose concentrations.

The GLUT4/muscle–fat glucose transporter is one member of the facilitative glucose transporter super-gene family that is specifically expressed in muscle and adipose tissues (1, 2). In contrast to the other glucose transporter isoforms, GLUT4 contains specific amino acid targeting sequences (3–5) responsible for its localization to unique intracellular vesicular compartments found in adipocytes and muscle cells (6–11). In response to acute insulin stimulation, these preformed GLUT4-containing vesicles rapidly translocate to the plasma membrane in a GTP-dependent process, resulting in a large increase in plasma membrane-associated GLUT4 protein (9–17).

In contrast to this acute pathway of insulin action, catabolic states such as fasting and non-insulin-dependent diabetes are directly associated with a marked resistance of adipose and muscle tissue to insulin-stimulated glucose uptake (18–20). Recently, several studies have suggested that a decrease in GLUT4 expression may be the initial cause of insulin resistance in adipose tissue, which contributes to the maintenance of insulin resistance in muscle (21–29). Since the pathophysiological mechanisms responsible for insulin resistance are poorly understood, we have recently generated transgenic mice expressing high levels of the human GLUT4 protein in an appropriate tissue-specific pattern (30). In this study we demonstrate that expression of the human GLUT4 protein in transgenic mice results in a constitutively high level of basal glucose uptake that is directly associated with marked hypoglycemia and insulinopenia relative to wild-type animals. Furthermore, circulating glucose levels remain refractory to an oral glucose challenge, indicating that the transgenic animals display a substantially greater degree of glycemic control than wild-type mice.

EXPERIMENTAL PROCEDURES

Preparation of Transgenic Mice. Transgenic mice carrying 11.5 kb (Kpn I/EcoRI fragment) of the human GLUT4 glucose transporter genomic DNA (hGLUT4-11.5) were produced as described (30). In this study we utilized two hemizygotic transgenic lines (hGLUT4-11.5B and hGLUT4-11.5C) that displayed the correct pattern of tissue-specific expression and with ~10-fold higher protein levels compared to the endogenous mouse GLUT4 protein.

Oral Glucose Tolerance Test and Metabolite Measurements. GLUT4 transgenic mice (7–9 weeks) and age-matched nontransgenic littermates were fasted overnight and bled via the orbital sinus (0.025 ml) immediately prior to administration of an oral glucose load (1 g of glucose per kg of body weight). Mice were subsequently bled after 30, 75, and 120 min. Plasma glucose and triglyceride levels were measured using the VP Super System autoanalyzer (Abbott). Plasma insulin was determined using a radioimmunoassay (Binax) with human insulin as standard. Plasma β-hydroxybutyrate and nonesterified free fatty acids were determined spectrophotometrically using kits from Sigma and Amano International Enzyme (Troy, VA), respectively. Cardiac glycogen levels were determined by the method of Hassid and Abraham (31).

Glucose Transport and Adipocyte Cell Size. 2-Deoxy-d-glucose uptake was determined in isolated primary parametrial adipocytes (32, 33) as described by Traxinger and Marshall (34). Adipocyte cell size was determined by the photomicrographic technique of Lavau et al. (35).

Immunofluorescence. Wild-type or GLUT4 transgenic mice were either fasted overnight and left untreated or allowed to eat ad lib and then given an intraperitoneal injection of glucose (1 g/kg) and insulin (8 units/kg) as described by Slot et al. (9). Thirty minutes later the mice were anesthetized and perfused through the left ventricle with 25 ml of 4% formaldehyde plus 0.2% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) at 23°C. Following fixation in situ, perirenal adipose tissue and cardiac muscle were removed, fixed by immersion for 1 hr, and processed for frozen sectioning as described (36) with 4.8 μg of rabbit anti-human GLUT4 IgG per ml (37) for 1 hr followed by incubation with 5 μg of donkey anti-rabbit IgG per ml conjugated to Texas Red (The Jackson Laboratory). Expo-

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Table 1. Comparison of several physiological characteristics between wild-type and GLUT4 transgenic mice

<table>
<thead>
<tr>
<th>Physiological characteristic</th>
<th>Male</th>
<th></th>
<th>Female</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>WT</td>
<td>GLUT4-B</td>
<td>GLUT4-C</td>
<td></td>
</tr>
<tr>
<td></td>
<td>26.0 ± 0.8</td>
<td>25.8 ± 0.6</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(4)</td>
<td>(6)</td>
<td>(3)</td>
<td></td>
</tr>
<tr>
<td>Adipose tissue weight, mg</td>
<td>330 ± 55</td>
<td>340 ± 25</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(4)</td>
<td>(6)</td>
<td>(3)</td>
<td></td>
</tr>
<tr>
<td>Adipocyte cell size, μm</td>
<td>63.4 ± 1.3</td>
<td>63.4 ± 1.2</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(4)</td>
<td>(6)</td>
<td>(3)</td>
<td></td>
</tr>
<tr>
<td>Plasma glucose, mg/dl</td>
<td>143 ± 8</td>
<td>65 ± 7*</td>
<td>55 ± 6*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(10)</td>
<td>(5)</td>
<td>(3)</td>
<td></td>
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These data (mean ± SEM) were obtained from the analysis of wild-type, hGLUT-11.5B, and hGLUT-11.5C transgenic male and female mice at 9 weeks of age. The value (n) in parentheses indicates the number of animals used for each analysis. Significant differences from age- and sex-matched wild-type littermates are indicated (*, P < 0.01). WT, wild-type mice; GLUT4-B, hGLUT-11.5B transgenic mice; GLUT4-C, hGLUT-11.5C transgenic mice; ND, not determined.

sure-matched fluorescent micrographs were taken on a Nikon FXA microscope.

Subcellular Membrane Fractionation. Plasma membranes and low density microsome fractions were obtained from isolated primary parametrial adipocytes as described by Mckee and Jaret (38) and modified by Simpson et al. (39). Membrane protein (25 μg of protein) was electrophoresed on 10% reducing SDS/polyacrylamide gels and subjected to Western blot analysis using a rabbit GLUT4 polyclonal antibody (IRGT, East Acres, Southbridge, MA).

RESULTS

To investigate the functional role of the GLUT4 glucose transporter in a physiologically appropriate context, we have generated transgenic mice expressing high levels of the human GLUT4 protein in a tissue-specific manner (30). Two independent founder lines (hGLUT4-11.5B and hGLUT4-11.5C) were found to direct the expression of the GLUT4 protein ≈10-fold greater than the endogenous mouse GLUT4 protein in muscle tissues as well as in brown (intercapular) and white (parametrial) adipose tissues. Characterization of these animals demonstrated normal rates of growth and no difference in total body weight compared to nontransgenic littermates (Table 1). In contrast, examination of parametrial white adipose tissue in female transgenic mice of both lines demonstrated an approximate 30–50% increase in adipose tissue wet weight as well as in adipocyte cell size. However, the epididymal white adipose tissue of the transgenic male mice was not significantly different from the epididymal adipose tissue of the control male mice. This gender-specific difference in adipose tissue characteristics is consistent with the relatively poor expression of the human GLUT4 mRNA and protein in male epididymal adipose tissue compared to female parametrial adipose tissue in these transgenic animals (30). Nevertheless, both male and female GLUT4 transgenic mice were found to have marked fasting hypoglycemia (≈70 mg/dl) compared to nontransgenic wild-type littermates (≈130 mg/ml).

To determine the functional consequences of this 10-fold elevated expression of human GLUT 4 in vivo, we assessed the physiological responses of these animals to an oral glucose challenge (Fig. 1). As typically observed, 30 min following an oral glucose load of 1 g/kg a substantial increase in plasma glucose levels (2–10-fold) occurred in wild-type male and female mice (Fig. 1). This increase in circulating glucose levels was transient and gradually declined toward basal levels by 120 min. In contrast, male and female GLUT4 transgenic mice from both lines displayed only minimal excursion of plasma glucose levels in response to the same oral glucose load (Fig. 1). This increased glucose disposal most likely resulted from increased glucose uptake/metabolism in muscle tissue since the male transgenic mice do not overexpress the transgene in

![Fig. 1](image_url)
white adipose tissue (30). Further, the functional expression of the GLUT4 transgene in cardiac muscle was confirmed by the 3- to 5-fold increase in cardiac muscle glycogen content (Table 2).

Similarly, the plasma insulin response following the oral glucose challenge was also markedly different between the wild-type and transgenic animals (Table 2). Although wild-type animals displayed an increase in plasma insulin levels in direct proportion to the changes in plasma glucose, circulating insulin levels in both human GLUT4 transgenic mouse lines were ≈2-fold lower. This apparent insulinopenia was also reflected by elevated levels of plasma triglycerides, free fatty acids, and β-hydroxybutyrate.

We next determined basal and insulin-stimulated glucose transport activity in isolated adipocytes (Fig. 2). Adipocytes isolated from white parametrial adipose tissue of wild-type mice demonstrated an approximate 4- to 5-fold insulin stimulation of 2-deoxyglucose uptake. In comparison to the wild-type animals, adipocytes from the human GLUT4 transgenic animals displayed an approximate 2- to 3-fold elevation in basal 2-deoxyglucose uptake. Although the maximal extent of 2-deoxyglucose uptake in the presence of insulin was similar between the wild-type and GLUT4 transgenic adipocytes, the insulin stimulation was only 1.6-fold in the adipocytes from the transgenic animals due to the high level of basal transport.

This apparent increase in basal glucose transport by adipocytes from the GLUT4 transgenic mice was found to correlate with an increased localization of the human GLUT4 transporter protein at the adipocyte cell surface as assessed by subcellular fractionation and immunoblotting (Fig. 3). In the absence of insulin, the basal level of GLUT4 protein was ≈14-fold greater in the isolated plasma membrane fraction of the transgenic adipocytes compared to the wild-type adipocytes.

Fig. 2. Basal and insulin-stimulated glucose transport in isolated primary adipocytes from wild-type and hGLUT4-11.5 transgenic mice. 2-Deoxyglucose (2-DOG) uptake was determined in the absence (●) or presence (○) of 20 nM insulin in female parametrial adipocytes isolated from wild-type (WT), hGLUT4-11.5B (TG-B), and hGLUT4-11.5C (TG-C) transgenic mice. These results (mean ± SD) were obtained from the average of n = 5, n = 3, and n = 2 independent experiments for the wild-type, hGLUT4-11.5B, and hGLUT4-11.5C animals, respectively. Each experiment was performed in triplicate using pooled adipocytes from eight mice.

Fig. 3. Subcellular distribution of GLUT4 protein from control and insulin-treated adipocytes. Adipocyte plasma membranes (PM) and low density microsome membranes (LDM) were prepared from wild-type (WT) and hGLUT4-11.5B female transgenic (TG) mice. The membranes were subjected to Western blotting using the carboxy-terminal-specific GLUT4 antibody (IRGT). (A) Autoradiographic analysis of the GLUT4 protein. (B) Relative quantitation of GLUT4 protein determined by excision of the bands in A and γ counting. This is a representative experiment independently performed three times for the wild-type and two times for the transgenic adipocytes.
cytes (Fig. 3, compare lanes 1 and 3). Consistent with the high level of GLUT4 protein previously reported (30), the isolated intracellular vesicle fraction (low density microsomes) also contained a substantially increased amount of GLUT4 protein compared to the intracellular vesicle fraction of the wild-type adipocytes (Fig. 3, compare lanes 5 and 7). As expected, insulin treatment of the wild-type adipocytes resulted in a 6-fold translocation of the intracellular pool of GLUT4 transporter protein to the plasma membrane fraction (Fig. 3, lanes 1 and 2). Although a similar phenomenon also occurred following insulin stimulation of the transgenic adipocytes (Fig. 3, lanes 3 and 4), the extent of translocation was reduced due to the relatively high level of GLUT4 protein already residing at the plasma membrane in the basal state. Interestingly, the basal and insulin-stimulated increase in 2-deoxyglucose glucose transport activity in the GLUT4 transgenic adipocytes (Fig. 2) was substantially less than the increase in plasma membrane-associated GLUT4 protein (Fig. 3). As controls, no significant differences in GLUT1 protein expression or insulin-stimulated translocation was detected in adipocytes isolated from either the wild-type or transgenic mice (data not shown).

As an independent assessment of GLUT4 subcellular localization, we also examined the adipocyte and cardiac myocyte distribution of the GLUT4 protein by immunofluorescence using a GLUT4-specific antibody coupled with a Texas Red-conjugated secondary antibody (Figs. 4 and 5). Adipose and muscle tissue from fasted wild-type mice exhibited a punctate cytoplasmic GLUT4 immunofluorescence as well as a weak immunofluorescence associated with the plasmalemma (Figs. 4A and 5A). In contrast, both tissues displayed a redistribution of the GLUT4 immunofluorescence from the condensed cytoplasmic vesicles to the plasmalemma in wild-type mice that were insulin and glucose treated (Figs. 4B and 5B). Overexpression of GLUT4 protein in vivo resulted in a specific immunofluorescent signal that was readily detected in the plasma membrane and intracellular vesicles from adipocytes and cardiac myocytes of the

Fig. 4. Immunofluorescence localization of adipocyte GLUT4 protein expression in wild-type and transgenic mice. Wild-type (A and B) and hGLUT4-11.5C transgenic (C and D) female mice were either fasted overnight and left untreated (A and C) or allowed to feed ad lib and given an intraperitoneal injection of insulin and glucose (B and D). Perirenal adipose tissue was cryopreserved and processed for GLUT4 protein immunofluorescence. Optimal exposure time was determined for the insulin/glucose-treated transgenic mice and all other conditions were photographed with an identical exposure time. Arrowheads mark several locations of punctate cytoplasmic staining (A and C) in adipocytes from the fasted animals. Arrows mark locations of extensive membrane-associated GLUT4 (B and D) in adipocytes following insulin/glucose treatment. (×320.)

Fig. 5. Immunofluorescence localization of cardiac myocyte GLUT4 protein expression in wild-type and transgenic mice. Wild-type (A and B) and hGLUT4-11.5C transgenic (C and D) female mice were either fasted overnight and left untreated (A and C) or allowed to feed ad lib and given an intraperitoneal injection of insulin and glucose (B and D). Left ventricular tissue was cryopreserved and processed for GLUT4 protein immunofluorescence. Optimal exposure time was determined for the insulin/glucose-treated transgenic mice and all other conditions were photographed with an identical exposure time. Arrowheads mark several locations of punctate cytoplasmic staining (A and C) in myocytes from the fasted animals and a large arrow marks an area of plasma membrane-associated GLUT4 in a fasted transgenic animal (C). Large arrows mark locations of extensive membrane-associated GLUT4 (B and D) in myocytes following insulin/glucose treatment. Small arrows mark translocation of GLUT4 to the t tubular system (D). (×520.)

fasted GLUT4 transgenic mice (Figs. 4C and 5C). Consistent with the subcellular fractionation results (Fig. 3), exposure-matched photographs clearly demonstrated that the GLUT4 transgenic mice had an increase in the total immunofluorescent signal compared to the wild-type controls (compare Fig. 4 A–C and 5 A–C). In addition, GLUT4 transgenic mice treated with insulin and glucose demonstrated a marked increase in cell surface GLUT4 protein content with an accompanying decrease in the punctate intracellular GLUT4 immunofluorescence (Figs. 4D and 5D). No significant fluorescent signal could be detected in any tissue sections from either wild-type or transgenic mice labeled with equal concentrations of nonimmune rabbit IgG (data not shown). Essentially identical results were also obtained in adipose tissue and skeletal muscle from the hGLUT4-11.5B transgenic mice (data not shown).

DISCUSSION

To assess the role of the GLUT4 protein in regulating glucose homeostasis we have generated transgenic mice expressing the human GLUT4 protein. This was accomplished by using a GLUT4 minigene (hGLUT4-11.5) containing 5.3 kb of 5'-flanking sequence and extending downstream into exon 10. In addition to the appropriate tissue-specific expression of the GLUT4 protein, this construct was also found to direct hormonal/metabolic regulation that was identical to that observed for the endogenous mouse GLUT4 gene (30). During the course of these studies, we observed that transgenic mice expressing a high level of GLUT4 protein (~10-fold greater than wild-type mice) were hypoglycemic relative to wild-type controls and that female mice had increased adipose tissue mass and adipocyte cell size. In contrast, male epididymal adipose tissue from the transgenic mice was not significantly different from that of wild-type animals. This
observation is consistent with our previous studies demonstrating relatively low-level expression of the human GLUT4 transgene in male epididymal adipose tissue compared to female parametrial adipose tissue (30). The fact that fasting plasma glucose and the ability to dispose of a glucose load were essentially identical in male and female mice argues strongly that overexpression of the transgene in muscle is sufficient to enhance glycemic control.

To investigate the functional basis for the observed fasting hypoglycemia in these transgenic animals, we examined GLUT4 subcellular localization and insulin-responsive glucose transport activity in parametrial adipocytes and cardiac muscle. These data demonstrated that the hypoglycemic state was accounted for by a marked increase in basal glucose uptake, which correlated with increased plasma membrane-associated GLUT4 protein. Similarly, the 3- to 5-fold increased cardiac glycogen content in the transgenic mice is reflective of increased glucose uptake into muscle via the increased cell surface GLUT4 protein. The increased content of GLUT4 protein at the plasma membrane could occur via several mechanisms, including saturation of either the intra-cellular targeting pathway or overloading of the capacity of the intracellular vesicular compartment and/or the basolateral endocytic pathway. Regardless of the mechanism(s) involved, insulin treatment of the transgenic mice was still capable of inducing GLUT4 translocation from the intracellular pool to the cell surface in both adipocytes and cardiac muscle.

The reduced plasma glucose levels displayed by these GLUT4 transgenic mice would be expected to reduce the rate of pancreatic β-cell insulin secretion. Indeed, insulin as well as circulating glucose levels were remarkably refractory to an oral glucose challenge. In addition, the increased circulating levels of triglycerides, free fatty acids, and β-hydroxybutyrate were also indicative of increased lipolysis due to insulinopenia. Despite the relative insulinopenic state of these animals, postprandial glucose disposal was not compromised as these GLUT4 transgenic mice displayed a highly efficient mechanism for glucose disposal. Thus, although glucose transport has been well documented to be the rate-limiting step in basal whole body glucose disposal (40–44), high levels of tissue-specific GLUT4 protein expression resulted in glucose transport activity that is unlikely to be rate limiting for whole body glucose disposal.

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