GLUT4 Overexpression in db/db Mice Dose-Dependently Ameliorates Diabetes But Is Not a Lifelong Cure

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We previously reported that overexpression of GLUT4 in lean, non-diabetic C57BL/KsJ-learnertm/(db/+) mice resulted in improved glucose tolerance associated with increased basal and insulin-stimulated glucose transport in isolated skeletal muscle. We used the diabetic (db/db) litter mates of these mice to examine the effects of GLUT4 overexpression on in vivo glucose utilization and on in vitro glucose transport and GLUT4 translocation in diabetic mice. We examined in vivo glucose disposal by oral glucose challenge and hyperinsulinemic-hyperglycemic clamps. We also evaluated the in vitro relationship between glucose transport activity and cell surface GLUT4 levels as assessed by photolabeling with the membrane-impermeant reagent 2-N-(4-(1-azido-2,2,2-trifluoroethyl)benzoyl)-1,3-bis(o-mannose-4-xyloxy)-2-propylamine in extensor digitorum longus (EDL) muscles. All parameters were examined as functions of animal age and the level of GLUT4 overexpression. In young mice (age 10–12 weeks), both lower (two- to threefold) and higher (four- to fivefold) levels of GLUT4 overexpression were associated with improved glucose tolerance compared to age-matched nontransgenic (NTG) mice. However, glucose tolerance deteriorated with age in db/db mice, although less rapidly in transgenic mice expressing the higher level of GLUT4. Glucose infusion rates during hyperinsulinemic-hyperglycemic clamps were increased with GLUT4 overexpression, compared with NTG mice in both lower and higher levels of GLUT4 overexpression, even in the older mice. Surprisingly, isolated EDL muscles from diabetic db/db mice did not exhibit alterations in either basal or insulin-stimulated glucose transport activity or cell surface GLUT4 compared to nondiabetic db/+ mice. Furthermore, both GLUT4 overexpression levels and animal age are associated with increased basal and insulin-stimulated glucose transport activities and cell surface GLUT4. However, the observed increased glucose transport activity in older db/db mice was not accompanied by an equivalent increase in cell surface GLUT4 compared to younger animals. Thus, although in vivo glucose tolerance is improved with GLUT4 overexpression in young animals, it deteriorates with age; in contrast, insulin responsiveness as assessed by the clamp technique remains improved with GLUT4 overexpression, as does in vitro insulin action. In summary, despite an impairment in whole-body glucose tolerance, skeletal muscle of the old transgenic GLUT4 db/db mice is still insulin responsive in vitro and in vivo. Diabetes 50:593–600, 2001

Skeletal muscle is the primary tissue responsible for the insulin-stimulated disposal of a glucose load and is a major site of insulin resistance in type 2 diabetes (1). Increases in glucose transport in skeletal muscle are mediated by the GLUT4 transporter isoform, which translocates to the plasma membrane from an intracellular storage site in response to insulin (2,3). It has been previously shown that the defect that causes type 2 diabetes is not a result of a decrease in skeletal muscle GLUT4 gene expression, but may be related to a defect in GLUT4 intracellular trafficking (4). However, increased expression of GLUT4 in skeletal muscle produces changes (lowered blood glucose, increased insulin, and contractile-stimulated glucose transport) that could be of benefit in the treatment of type 2 diabetes (5–9).

Recently, several lines of transgenic (TG) mice that overexpress the human GLUT4 gene in a tissue-specific manner (adipose tissue and cardiac and skeletal muscle) have been described (7–12). These animals are hypoglycemic and hypoinsulinemic relative to their nontransgenic (NTG) litter mates and show a markedly increased ability to dispose an intravenous or oral glucose load (7,8,11,12). Additionally, increased basal and insulin-stimulated glucose transport is observed in isolated adipocytes (12) and also in isolated skeletal muscle in TG mice compared to NTG mice (8,9).

More recently, we demonstrated that the increased glucose transport rates observed in skeletal muscle of GLUT4 TG mice correlate with increased cell surface GLUT4, as assessed by photolabeling with the membrane-impermeant reagent 2-N-(4-(1-azido-2,2,2-trifluoroethyl)benzoyl)-1,3-bis(o-mannose-4-xyloxy)-2-propylamine (ATBBMPA) (10). This earlier study was performed on GLUT4 TG mice produced on the inbred C57BL/KsJ-leprdb/db (db/db) background (7,10). Because these mice express only a single copy of the recessive db gene, they express a normal lean, nondiabetic phenotype. On the other hand, mice that are homozygous for the db gene possess a...
mutation in the leptin receptor and display many of the characteristics of human type 2 diabetes, such as hyperglycemia, hyperlipidemia, obesity, and marked in vivo insulin resistance (13–15). Furthermore, several studies have reported that insulin-stimulated glucose transport in skeletal muscle is impaired in db/db mice (16,17). However, it is largely unknown what effect GLUT4 overexpression would have on glucose transport and glucose transporter translocation in skeletal muscle in the insulin-resistant state, in which insulin-stimulated glucose transporter trafficking is defective.

In the present study, we used GLUT4 TG mice that were homozygous for the db gene to address this issue. Our initial report on GLUT4 overexpression in db/db mice demonstrated a marked improvement in whole-body glucose homeostasis and glucose disposal in young db/db mice (7) that overexpress various levels of GLUT4. More recently, we have observed that the improved glycemic control of hGLUT4/db/db TG mice was somewhat associated with aging in an expression level–dependent manner (E.M. Gibbs, S.C. McCoid, unpublished observations). Because the long-term effect of GLUT4 overexpression on diabetic progression is unclear, the purposes of the present study were to assess the effects of GLUT4 overexpression on whole-body glucose homeostasis with aging and to examine the effects of increased GLUT4 expression on insulin-stimulated glucose transport and GLUT4 translocation in skeletal muscle of insulin-resistant db/db mice.

**RESEARCH DESIGN AND METHODS**

**Transgenic mice.** TG mice carrying 11.8 kb (Novel fragment) of the human GLUT4 transporter genomic DNA were produced as described previously (7). Two lines of TG mice (hGLUT4/db/db line 6 and line 8), ages 10–12 (young), 20–25 (middle-aged), and 28–30 weeks (old) and homozygous for the hGLUT4 transgene and obese NTG C57BL/KsJ-lepr(db/db) of the same ages were used in this study; hGLUT4/db/db line 6 (TG6) displays a two- to threefold overexpression of GLUT4 in skeletal muscle, cardiac muscle, and white adipose tissue, whereas hGLUT4/db/db line 8 (TG8) displays a fivefold overexpression in these tissues. All mice used in this study were homozygous for the db gene and therefore exhibited an obese, diabetic phenotype, with the exception that NTG C57BL/KsJ-1 lept(db) mice were used as diabetic controls for direct comparison to NTG db/db mice in the experiments described in Fig. 5.

**Muscle preparation and incubation.** Mice in the postprandial state were anesthetized with 5 mg/100 g body weight of sodium pentobarbital. The extensor digitorum longus (EDL) muscles were used for all transport and photolabeling measurements. This muscle is composed of a fiber type (~50% fast-twitch oxidative and 50% fast-twitch glycolytic) that is representative of the hind limb muscle fiber composition of the rat (18). The EDL muscles were dissected out, blotted on gauze, and transferred to 25-ml Erlenmeyer flasks. Muscle slices were trimmed of their tendons, blotted, and frozen between tongs cooled to 5,000 °C until they were homogenized in 1 ml 0.6 N perchloric acid. Homogenates were centrifuged at 70°C until they were 100%, and aliquots of the supernatant were counted for radioactivity in a standard (50 μCi rat skeletal muscle plasma membrane protein) run on each gel.

**Measurement of glucose transport activity.** Glucose transport activity was measured using 2-deoxyglucose (2DG) as described in detail previously (8). After the incubations, the muscles were frozen between tongs cooled to the temperature of liquid nitrogen and stored at −70°C until they were processed for measurement of 2DG transport. Frozen muscle tissue was homogenized in 1 ml 0.6% perchloric acid. Homogenates were centrifuged at 5,000g, and aliquots of the supernatant were counted for radioactivity in a liquid scintillation counter.

**Statistical analysis.** The data were analyzed by analysis of variance (ANOVA) to test the effects of group (control versus transgenic, young versus old) and treatment (basal, insulin) on muscle glucose uptake and glucose transporter distribution. When a significant F ratio was obtained, Fisher's protected least significant difference (PLSD) post-hoc test was used to identify statistically significant differences (P < 0.05) between the means. Statistical analysis was performed using the StatView 4.5 for Macintosh (Abdott Laboratories, North Chicago, IL). Plasma insulin was determined using a radioimmunoassay (Linco Research, St. Charles, MO).

**RESULTS**

GLUT4 overexpression in hGLUT4/db/db mice. Levels of GLUT4 expression in NTG and TG mice are shown in Fig. 1. Young TG6 mice overexpressed skeletal muscle GLUT4 twofold as compared with NTG, and the overexpression level increased slightly in 20- to 25-week-old mice (threefold). Young TG8 mice exhibited a fivefold overexpression of GLUT4 in skeletal muscle, which declined slightly in 28- to 30-week-old mice to approximately fourfold.
and clearance that is to be expected in the more insulin-responsive TG mice. In young TG8 mice (age 10–12 weeks), the GIR required to maintain steady-state plasma glucose was elevated 18-fold compared with NTG db/db mice of the same age (102 vs. 56 \( \mu \text{mol} \cdot \text{min}^{-1} \cdot \text{kg}^{-1} \), respectively; Table 1 and Fig. 3B). In old NTG mice (28–30 weeks of age), the GIR required to maintain plasma glucose at \( \sim 17 \text{ mmol/l} \) decreased 70% relative to the young NTG mice (18 vs. 56 \( \mu \text{mol} \cdot \text{min}^{-1} \cdot \text{kg}^{-1} \) for old and young NTG, respectively; Table 1). The low GIR in both the young and old NTG mice (Figs. 3B and 4B) indicates that these mice are extremely insulin resistant in vivo. The GIR in old TG8 and TG6 mice was increased >5.5-fold relative to the age-matched NTG mice (Table 1 and Fig. 4B). GIR during most of the clamp was elevated in the TG8 relative to the TG6; however, during the last 30 min of the clamp, when the glucose values were relatively steady, the GIR was only slightly increased and this difference was not statistically significant (Fig. 4A and B, Table 1). These data suggest that both lines of TG mice remain responsive to high levels of exogenous insulin with increasing age.

**Pancreatic weight.** Pancreases from some mice were excised and weighed; the total pancreatic weight in TG8 mice at age 10 and 30 weeks was significantly greater than in NTG or TG6 mice (Table 2), which is consistent with the diminished pancreatic atrophy in the more insulin-sensitive TG8 mice.

**Cell surface GLUT4 photolabeling and 2DG uptake.** Cell surface GLUT4 labeling and 2DG uptake are shown in Fig. 5A and B, respectively. Insulin stimulation increased 2DG transport approximately threefold in EDL muscles from both NTG db/+ and db/db mice. Among the db/db mice, both basal and insulin-stimulated 2DG was greater in EDL muscles from old NTG db/db than young NTG db/db mice. In young and old TG6 and TG8 mice, both basal and insulin-stimulated 2DG transport was elevated above that in NTG mice, with the exception of basal transport in old TG6 mice. In young and old mice, both basal and insulin-stimulated 2DG transport was greater in TG8 mice than TG6 mice, with the exception of basal 2DG transport in young mice, but not in proportion to the greater levels of GLUT4 expression. In old TG6 and TG8 mice, both basal and insulin-stimulated 2DG transport was increased as compared with young mice. In general, the changes in 2DG transport were paralleled by similar changes in cell surface GLUT4 photolabeling. However, a discrepancy was noted between insulin-stimulated 2DG transport and cell surface GLUT4 photolabeling in the old TG6 and TG8 mice, in that whereas 2DG transport tended to increase with aging, cell surface GLUT4 photolabeling either remained unchanged (TG6 mice) or declined (TG8 mice).

**Correlation between ATB-BMPA labeling and 2DG transport in isolated EDL muscles under basal and insulin-stimulated conditions.** Linear regression analyses of GLUT4 ATB-BMPA labeling and 2DG transport are shown in Fig. 6. ATB-BMPA surface-labeled GLUT4 was significantly correlated with 2DG transport (\( r^2 = 0.66 \)) for all samples. This correlation improved when the data are subdivided into young (\( r^2 = 0.92 \)) and old (\( r^2 = 0.70 \)) mice. However, the slopes of the regression lines differ between young and old mice, such that less of an increase in cell surface GLUT4 is observed in old mice compared with young mice at the same level of insulin stimulation in vivo.
CELL SURFACE GLUT4 IN TRANSGENIC MICE

DISCUSSION
In the present study, we investigated the effects of different levels of GLUT4 overexpression on insulin resistance in two lines of db/db transgenic mice: hGLUT4 line 6 (two- to threefold overexpression) and hGLUT4 line 8 (four- to fivefold overexpression). In young hGLUT4/db/db of both lines, glucose disposal after an oral glucose challenge was markedly improved, similar to previous results (7). Despite the significantly higher levels of GLUT4 in the muscles of the TG8 mice, the improved ability to dispose an oral glucose load was comparable in young TG6 and TG8 mice. However, in older TG6 mice, the enhanced glycemic control observed in the young mice was no longer evident. In contrast, TG8 mice maintained their enhanced glucose tolerance completely until at least age 21 weeks and to a slightly lesser extent until age 30 weeks. Thus, although it seems that the greater level of GLUT4 young mice for the same incremental increase in 2DG transport.

FIG. 2. Effect of an oral glucose challenge on plasma glucose levels in 10- to 12-week-old (A), 21-week-old (B), and 29- to 32-week-old (C) TG and NTG db/db mice. Mice were fasted overnight and then given 1 g glucose per kg by oral gavage. Plasma glucose levels were determined at the times indicated. Data are means ± SE from 3–7 mice per group. TG6 (○), TG8 (■), and NTG (●) mice. A: All values for TG mice are significantly different from NTG mice (P < 0.05). B: All values for TG8 mice are significantly different from NTG mice (P < 0.05). C: Values for t = 0 min and t = 120 min for TG8 are significantly different from NTG mice (P < 0.05).

FIG. 3. Blood glucose concentrations (A) and GIRs (B) in 10- to 12-week-old TG8 (○) and NTG (●) db/db mice. Anesthetized NTG and TG mice received an intravenous bolus dose of insulin (10 mU/mouse) and were then infused with insulin continuously at a rate of 1 mU/min and glucose at a variable rate to maintain a blood glucose concentration of ~17 mmol/l for 2 h. Blood glucose levels were recorded every 5 min. Data are means ± SE of 7 TG8 mice and 10 NTG mice.

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DISCUSSION
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overexpression in the TG8 mice does not cause a proportional improvement in glucose tolerance in young mice, it does dramatically reduce the decline in glucose tolerance with aging observed in the TG6 mice.

To further assess the effects of GLUT4 overexpression in vivo, we conducted hyperglycemic-hyperinsulinemic clamp studies in mice of age 10 and 30 weeks. The severe insulin resistance in the NTG db/db mice forced us to conduct these experiments at a blood glucose level of 17 mmol/l. At both ages, TG8 mice had increased GIR compared with NTG db/db mice. Glucose disposal was also increased in 30-week-old TG6 mice relative to NTG mice. Although TG6 mice have a lower level of GLUT4 overexpression relative to TG8 mice, during the last 30 min of the clamp, their GIR was not significantly lower than the GIR of the TG8 mice. These data directly demonstrate that overexpression of GLUT4 on an insulin-resistant background results in improved glucose homeostasis as a result of enhanced peripheral glucose utilization. However, the level of GLUT4 overexpression and insulin-stimulated glucose disposal in vivo at high insulin concentrations does not show a one-to-one correspondence. In addition, the response to insulin in TG mice in the glucose clamp studies did not diminish. This finding differs from the results obtained in NTG mice, in which the GIRs required to maintain a blood glucose level of 17 mmol/l decreased ~70% between age 10 and 30 weeks. The glucose clamp data suggest that despite the in vivo impair-

TABLE 1
Glucose infusion rates during hyperinsulinemic-hyperglycemic clamp in young (age 10–12 weeks) NTG and TG8 mice and old (age 28–30 weeks) NTG, TG8, and TG6 mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Initial glucose concentration (mmol/l)</th>
<th>Steady-state (90–120 min) glucose concentration (mmol/l)</th>
<th>Steady-state (90–120 min) GIR (μmol · min⁻¹ · kg⁻¹)</th>
<th>Final (120 min) insulin concentration (pmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NTG (n = 10)</td>
<td>18.5 ± 1.05</td>
<td>16.1 ± 0.4</td>
<td>55.5 ± 10</td>
<td>23,950 ± 1,825</td>
</tr>
<tr>
<td>TG8 (n = 7)</td>
<td>7.4 ± 1.7*</td>
<td>17.0 ± 0.8</td>
<td>102 ± 13.3*</td>
<td>16,790 ± 2,510†</td>
</tr>
<tr>
<td>Old</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NTG (n = 7)</td>
<td>22.6 ± 1.4</td>
<td>18.3 ± 0.7</td>
<td>17.8 ± 12.7</td>
<td>23,585 ± 3,560</td>
</tr>
<tr>
<td>TG8 (n = 9)</td>
<td>10.6 ± 1.9*</td>
<td>18.3 ± 0.5</td>
<td>130 ± 32.7*</td>
<td>13,295 ± 2,250*</td>
</tr>
<tr>
<td>TG6 (n = 8)</td>
<td>18.2 ± 2.6*</td>
<td>16.7 ± 0.3</td>
<td>100 ± 8.3*</td>
<td>15,220 ± 2,450</td>
</tr>
</tbody>
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Data are means ± SE. *Significantly different from corresponding NTG value (P < 0.05); †significantly different from corresponding TG8 value (P < 0.05).
ment in glucose tolerance demonstrated by the results of the oral glucose challenge, skeletal muscle of both TG6 and TG8 mice maintain their maximal capacity to respond to insulin during aging. The apparent discrepancy between the oral glucose tolerance test and glucose clamp data might be explained if the secretion of endogenous insulin from the pancreas during the oral glucose challenge did not reach the high levels infused during the clamps. Therefore, this could indicate that the higher level of expression of GLUT4 in skeletal muscle may preserve pancreatic function in the TG8 mice through chronically lowered blood glucose. This hypothesis is supported by our findings that pancreatic atrophy was reduced in TG8 mice (Table 2).

One potential explanation for the age-dependent waning of the improved glucose disposal after an oral glucose challenge was that the expression of the hGLUT4 transgene diminished as the mice aged. However, we demonstrated that total cellular GLUT4 protein expression actually increased slightly in 20- to 25-week-old TG6 mice compared with 10-week-old TG6 mice (Fig. 1). Although there was a small decrease (14%; \( P < 0.05 \)) in GLUT4 expression in TG8, GLUT4 remained more than fourfold overexpressed in this transgenic line of mice. Furthermore, continued functional expression of the GLUT4 transgene in vivo was confirmed in 30-week-old TG8 mice by quantitating cardiac glycogen content; these mice maintained a similar fourfold increase in cardiac glycogen content compared with NTG mice, as was observed in 10-week-old mice (W.J. Zavadoski, S.C. McCoid, and E.M. Gibbs, unpublished observations). The minimal changes in GLUT4 expression during the period in which there was a dramatic decline in glycemic control (most notably in TG6 mice) strongly suggests that a factor independent of the level of total cellular GLUT4 expression was responsible for the diabetic progression. Along this line, pancreatic atrophy is known to occur in NTG \( db/db \) mice as they age (13,14), and here we demonstrated that the atrophy is diminished in TG8 but not TG6 mice at age \( >30 \) weeks. This finding offers a potential explanation for the more prolonged improvement in glucose disposal observed in TG8 mice compared with TG6 mice, in that secretion of endogenous insulin after an oral glucose challenge seems to remain sufficient to promote enhanced glucose disposal in TG8 mice until a later age than in TG6 mice. In turn, the smaller effect on glucose disposal after a glucose challenge in TG6 mice compared with TG8 may be attributable to the higher ambient plasma glucose levels and induction of a more severe insulin resistance (4,22,23).

![FIG. 5. ATB-BMPA GLUT4 labeling (A) and 2DG transport (B) in isolated EDL muscles from \( db/+ \) NTG, \( db/db \) NTG, \( db/db \) TG6, and \( db/db \) TG8 mice under basal and insulin-stimulated conditions. Number of observations are shown in parentheses above each bar. Data are means ± SE. *Significantly different from basal (\( P < 0.05 \)); †significantly different from corresponding young value (\( P < 0.05 \)); ‡significantly different from corresponding NTG value (\( P < 0.05 \)); §significantly different from corresponding TG6 value (\( P < 0.05 \)).](image-url)

![FIG. 6. Correlation between ATB-BMPA labeling and 2DG transport in isolated EDL muscles from \( db/+ \) NTG, \( db/db \) NTG, \( db/db \) TG6, and \( db/db \) TG8 mice under basal and insulin-stimulated conditions. Values from Fig. 5 were plotted, photolabeling is shown on the y axis, and 2DG transport is shown on the x axis. Results of linear regression for all values (solid line) show that \( r^2 = 0.67 \), significant at \( P < 0.05 \). The dashed line shows regression for young animals (\( r^2 = 0.92 \)), and the dash-dotted line shows regression for old animals (\( r^2 = 0.76 \)). Data are means ± SE.](image-url)
In our initial test of insulin action in vitro using EDL muscles, we were surprised to discover that neither basal nor insulin-stimulated glucose transport was abnormal in muscles isolated from NTG db/db mice compared with NTG db/+ mice (10) (Fig. 5). Moreover, the glucose transport activities were roughly paralleled by the levels of cell surface GLUT4. Thus, in contrast to the expected result, isolated EDL muscles from db/db mice do not exhibit an insulin-resistant glucose transport response to a maximally stimulating concentration of insulin. Furthermore, the lack of insulin resistance was also observed in epitrochlearis and soleus muscles isolated from db/db mice (G.J. Etgen, Jr., and E.M. Gibbs, unpublished observations). Compared with NTG db/db mice, progressively increasing GLUT4 overexpression results in progressively increasing basal and insulin-stimulated transport activities and cell surface GLUT4 levels. Moreover, the cell surface GLUT4 labeling data are highly correlated with the 2DG transport rates ($r^2 = 0.92$) (Fig. 6), indicating that the elevations in basal and insulin-stimulated glucose transport can be fully explained by increased appearance of GLUT4 at the cell surface. This conclusion agrees with results of our earlier study in transgenic GLUT db/+ mice (10). A similar correlation between cell surface GLUT4 and glucose transport activity was also observed in older mice, although when the surface labeling data were plotted as a function of transport activity, the slope of the regression line was less steep and the correlation was not as good ($r^2 = 0.76$) as observed in younger mice. The reduction in the slope occurs because of the discrepancy between insulin-stimulated 2DG transport activity and cell surface GLUT4 in the older mice (i.e., there is a greater increase in 2DG transport activity than in cell surface labeling). Whatever the reason for this discrepancy, it is apparently not due to an alteration in GLUT1 levels, because previous studies have shown that GLUT1 levels are unaltered in transgenic mice in which GLUT4 has been ablated (24). Furthermore, the amount of GLUT1 in skeletal muscle is small in comparison with GLUT4 and functions only in regulation of basal transport (25), whereas the discrepancy in the present work occurred under insulin-stimulated conditions. One potential explanation for this finding is that in the older mice, GLUT4 may exist in an occluded state and may transport glucose but may not be accessible to photolabel (26), although a previous report indicated that occluded transporters do not function in glucose transport (26). Alternatively, there may be some unknown alteration in either GLUT4 or some factor that affects GLUT4 activity with aging that makes it more intrinsically active. Although previous studies have indicated that there is a one-to-one relationship between transport activity and cell surface labeling, this hypothesis has not been tested previously in older animals (10,19,20,27). However, an explanation for this apparent change in the relationship between cell surface GLUT4 and glucose transport remains to be determined by further study.

It is notable that despite the marked in vivo insulin resistance observed for the NTG db/db mice during hyperinsulinemic clamps, their muscles are completely insulin responsive in vitro. This finding suggests that the factor that causes impaired in vivo insulin action in NTG db/db mice is not intrinsic to the muscle itself but rather is a humoral factor that may be related to the fasting hyperglycemia observed in db/db NTG mice and could, in fact, be glucose itself. Recent evidence has shown that chronic exposure of muscle to high circulating levels of glucose can downregulate the glucose transport system (28,29). It has also been shown that incubating muscles from patients with diabetes in a medium containing a lower glucose concentration rapidly reverses the impaired insulin-stimulated glucose transport to normal levels (23). Furthermore, we have recently observed a similar phenomenon in ob/ob mice, which like db/db mice, are hyperglycemic and fail to show insulin resistance in isolated skeletal muscle preparations (G.J. Etgen and E.M. Gibbs, unpublished observations). Importantly, this result differs from the situation with the obese, insulin-resistant Zucker diabetic fatty (ZDF) rat, which is not hyperglycemic and whose muscles remain insulin resistant in vitro (27,30). The speculation that hyperglycemia per se causes the in vivo skeletal muscle insulin resistance that can be reversed in vitro is strengthened by the very recent observation by Dolan et al. (27), who found that skeletal muscle isolated from ZDF rats, which are hyperglycemic, is not insulin resistant in vivo. Thus, it is possible that the impaired in vivo insulin action in all three of the hyperglycemic rodent models (db/db, ob/ob, and ZDF) is attributable to the chronically elevated blood glucose that occurs in these animals and that incubation in normoglycemic buffer in vitro restores insulin sensitivity.

Old TG6 mice displayed an abnormal glucose tolerance that was identical to that of the NTG db/db mice. However, during hyperinsulinemic-hyperglycemic clamps, the GIRs of these mice were significantly higher than that of NTG mice. This observation indicates that the muscles of these mice were still able to respond to insulin, because under the clamp conditions used, most of the infused glucose is taken up by skeletal muscle, and the high circulating levels of insulin should fully suppress hepatic glucose production (31). Therefore, it is possible that the impaired glucose tolerance in the old TG6 mice was attributable to a relative reduction of circulating insulin or to a change in hepatic insulin sensitivity. Because insulin levels were markedly lower during the glucose tolerance test than the clamp, it is likely that hepatic glucose production would not be suppressed in the old animals. Furthermore, as mentioned above, the pancreatic weights of TG6 mice were significantly smaller than those of old TG8 mice, which supports this hypothesis. However, it is possible that a decrement in insulin action at a lower blood insulin concentration (i.e., a change in sensitivity) could contribute to the impairment in glucose tolerance in old TG mice. In other words, if the hyperglycemic clamps were performed at a lower insulin concentration, the GIR for old TG6 mice may not differ from that of old NTG mice. Which of these possibilities is correct remains to be determined.

In summary, the present study demonstrates that overexpression of GLUT4 in an insulin-resistant animal model improves both in vivo and in vitro skeletal muscle glucose transport in db/db mice. Moderate (twofold) overexpression resulted in proportional increases in 2DG transport, but overexpression to a higher level (fivefold) did not increase 2DG transport proportionally. Despite the loss of in vivo glucose tolerance in the TG6 mice, GLUT4 overex-
pression prevented the decline in skeletal muscle insulin responsiveness observed in the NTG mice. Thus, these data suggest that increasing GLUT4 at the plasma membrane may be an effective therapy for type 2 diabetes, even in the face of severe insulin resistance.

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REFERENCES


