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Research Article

The effects of increased GLUT4 (insulin-regulatable muscle/fat glucose transporter) expression on glucose homeostasis in a genetic model of non-insulin-dependent diabetes mellitus were determined by expressing a human GLUT4 transgene (hGLUT4) in diabetic C57BL/KsJ-db/db mice. A genomic hGLUT4 construct was microinjected directly into pronuclear murine embryos of db/+ matings to maintain the inbred background. Four lines of hGLUT4 transgenic mice were bred to homozygosity at the db locus and all showed a marked reduction of both fasted and fed plasma glucose levels (to approximately 50 and 360 mg/dl, respectively) compared with age-matched nontransgenic db/db mice (approximately 215 and 550 mg/dl, respectively), as well as an enhanced disposal of an oral glucose challenge. In situ immunocytochemical localization of GLUT4 protein in muscle from hGLUT4 db/db mice showed elevated plasma membrane-associated GLUT4 protein in the basal state, which markedly increased after an insulin/glucose injection. In contrast, nontransgenic db/db mice had low levels of plasma membrane-associated GLUT4 protein in the basal state with a relatively small increase after an insulin/glucose challenge. Since the intracellular GLUT4 levels in db/db mice were similar to nontransgenic db/+ mice, the glucose transport defect in db/db mice is at the level of glucose transporter translocation. Together, these data demonstrate that GLUT4 upregulation overcomes the glucose transporter translocation defect and alleviates insulin resistance in genetically diabetic mice, thus resulting in […]

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Glycemic Improvement in Diabetic *db/db* Mice by Overexpression of the Human Insulin-regulatable Glucose Transporter (GLUT4)

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Abstract

The effects of increased GLUT4 (insulin-regulatable muscle/fat glucose transporter) expression on glucose homeostasis in a genetic model of non–insulin-dependent diabetes mellitus were determined by expressing a human GLUT4 transgene (hGLUT4) in diabetic C57BL/KsJ-*db/db* mice. A genomic hGLUT4 construct was microinjected directly into pronuclear murine embryos of *db/+* matings to maintain the inbred background. Four lines of hGLUT4 transgenic mice were bred to homozygosity at the *db* locus and all showed a marked reduction of both fasted and fed plasma glucose levels (to ~ 50 and 360 mg/dl, respectively) compared with age-matched nontransgenic *db/db* mice (~ 215 and 550 mg/dl, respectively), as well as an enhanced disposal of an oral glucose challenge. In situ immunocytochemical localization of GLUT4 protein in muscle from hGLUT4 *db/db* mice showed elevated plasma membrane–associated GLUT4 protein in the basal state, which markedly increased after an insulin/glucose injection. In contrast, nontransgenic *db/db* mice had low levels of plasma membrane–associated GLUT4 protein in the basal state with a relatively small increase after an insulin/glucose challenge. Since the intracellular GLUT4 levels in *db/db* mice were similar to nontransgenic *db/+* mice, the glucose transport defect in *db/db* mice is at the level of glucose transporter translocation. Together, these data demonstrate that GLUT4 upregulation overcomes the glucose transporter translocation defect and alleviates insulin resistance in genetically diabetic mice, thus resulting in markedly improved glycemic control. (*J. Clin. Invest.* 1995. 95:1512–1518.) Key words: non–insulin-dependent diabetes mellitus • genetic models • basal metabolism • gene expression • upregulation

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Introduction

Non–insulin-dependent diabetes mellitus (NIDDM)[1] is a metabolic disease that affects ~5% of the population of the Western world. NIDDM is characterized by hyperglycemia in both the fed and fasted states and is predominantly associated with obesity. The disease is manifested by defects in both insulin secretion from the pancreas and insulin action in peripheral target tissues (i.e., liver, skeletal muscle, and adipose tissue). Skeletal muscle is the major site of post-prandial peripheral glucose disposal, and muscle from NIDDM patients displays markedly impaired glucose uptake in response to insulin, although the precise nature of the defect is unknown (1–3). The defect in glucose transport cannot be explained by a simple reduction in the level of gene expression of the major muscle glucose transporter isoform (GLUT4), as little or no reduction in skeletal muscle GLUT4 content has been found in NIDDM patients (3–6). Several lines of evidence suggest that elevated glucose levels directly affect insulin sensitivity in target tissues and contribute to the dire long-term diabetic complications such as cardiovascular disease, retinopathy, neuropathy, and nephropathy (7, 8).

db/db mice are a genetic model of NIDDM that display many of the characteristics of the human disease including hyperglycemia, insulin resistance, and obesity (9–11). Importantly, as in human NIDDM, *db/db* mice have a marked decrease in skeletal muscle glucose utilization due to a major defect in glucose transport that is not accompanied by significant alterations in GLUT4 expression (12, 13). In this study, the possible beneficial effects of GLUT4 overexpression in this murine NIDDM model were tested by producing transgenic mice that express a human GLUT4 transgene (14). These data indicate that GLUT4 upregulation in this genetic model of NIDDM reduced hyperglycemia and resulted in improved glycemic control.

Methods

Transgenic mice. Pronuclear transgenic mice were produced by established protocols (15, 16), however, embryos were obtained from the inbred, mutant mouse strain, C57BL/KsJ-*m+/+db* (Jackson Laboratories, Bar Harbor, ME), in which the recessive diabetic mutation, *db*, is linked in repulsion to the recessive coat color mutation, misty, *m* (17). The linking of *db* and *m* mutations in repulsion allows for the identification of offspring genotypically wild-type (+/+) at the *db* locus by the presence of the recessive misty (*m/m*) coat color (17). An

1. *Abbreviations used in this paper: db,* diabetes; GLUT4, insulin-regulatable muscle/fat glucose transporter; hGLUT4, human GLUT4; *m,* misty; NIDDM, non–insulin-dependent diabetes mellitus; nt, nucleotide.

11.8-kb (NotI fragment) human genomic GLUT4 transgene including 5.3 kb of 5′ flanking sequence and an SV40 polyadenylation sequence (14) was microinjected at ~ 2.0 μg/ml into pronuclei of embryos derived from matings of C57BL/KsJ-*m+/+db* males and superovulated females. 400 injected embryos were transferred to pseudopregnant CD-1 females. Of 135 offspring, 13 founder transgenic mice were identified by the polymerase chain reaction (PCR) of total genomic DNA (data not shown). We observed two founders with the recessive, obese phenotype (*+db/+db*) and two founders with the recessive misty coat color (*m+/m+*). The remaining nine founders were *m+/+db* with black coat and lean phenotype. Independent lines were established by backcrossing lean founder mice with C57BL/KsJ-*m+/+db* mates.

RNase protection assay. Total RNA was isolated from heart tissue using RNAzol™B (BIOTECX, Inc, Houston, TX) following the manufacturer's directions. The human-specific GLUT4 probe, PJW3 (14), was linearized with EcoR1 and its T3 promoter was used to generate a [^{32}P]UTP-labeled antisense probe. The RNA from each sample was hybridized for 16 h at 55°C, treated with RNase A, and electrophoresed on a 6% polyacrylamide/7.5 M urea gel.

Immunoblotting. Postnuclear membrane fractions were prepared from frozen hearts and adipose tissue as described previously (18). Samples were solubilized in Laemmli loading buffer, and proteins (50 μg protein) were separated on 10% reducing SDS-polyacrylamide gels and then transferred to nitrocellulose membranes. The membranes were incubated with affinity-purified rabbit anti-GLUT4 polyclonal antibodies (19) overnight at 4°C. Immunoreactive bands were visualized using enhanced chemiluminescence (ECL; Amersham Corp., Arlington Heights, IL) according to the manufacturer's instructions. Bands were quantitated using a MasterScan interpretive densitometer (Scanalytics, a Division of CSPI, San Diego, CA).

Oral glucose tolerance test and metabolite measurements. Nonanesthetized GLUT4 transgenic (*db/+*, and *db/db*) and age-matched nontransgenic mice were fasted overnight and bled via the orbital sinus (0.025 ml) immediately before administration of an oral glucose load (1 g glucose per kg of body wt) by gavage using a syringe equipped with a murine oral feeding needle (20 gauge; Popper & Sons, Inc., New Hyde Park, NY). Mice were subsequently bled after 30, 75, and 120 min. Plasma glucose levels were measured using the VP Super System Autoanalyzer (Abbott Laboratories, North Chicago, IL). Plasma insulin was determined using a radioimmunoassay (Binax, Portland, ME) with porcine insulin as standard.

Immunofluorescence. Nontransgenic and GLUT4 transgenic *db/db* mice were either fasted overnight and left untreated or allowed to eat ad libitum and then given an intraperitoneal injection of glucose (1 g/kg) and insulin (8 U/kg) as previously described (20, 21). 30 min after the glucose/insulin challenge, mice were anesthetized and immediately 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. After fixation in situ, cardiac and quadriceps muscle and pancreas were removed, fixed by immersion for 1 h, and processed for frozen sectioning as previously described (22). Briefly, 0.5-μm frozen sections were incubated at 23°C with 4.8 μg/ml rabbit anti–human GLUT4 IgG (19) for 1 h followed by incubation with 5 μg/ml donkey anti–rabbit IgG conjugated to Texas red (Jackson Laboratories). Optimal exposure time was determined for the insulin/glucose-treated transgenic mice, and all other conditions were photographed with an identical exposure time on a Nikon FXA microscope.

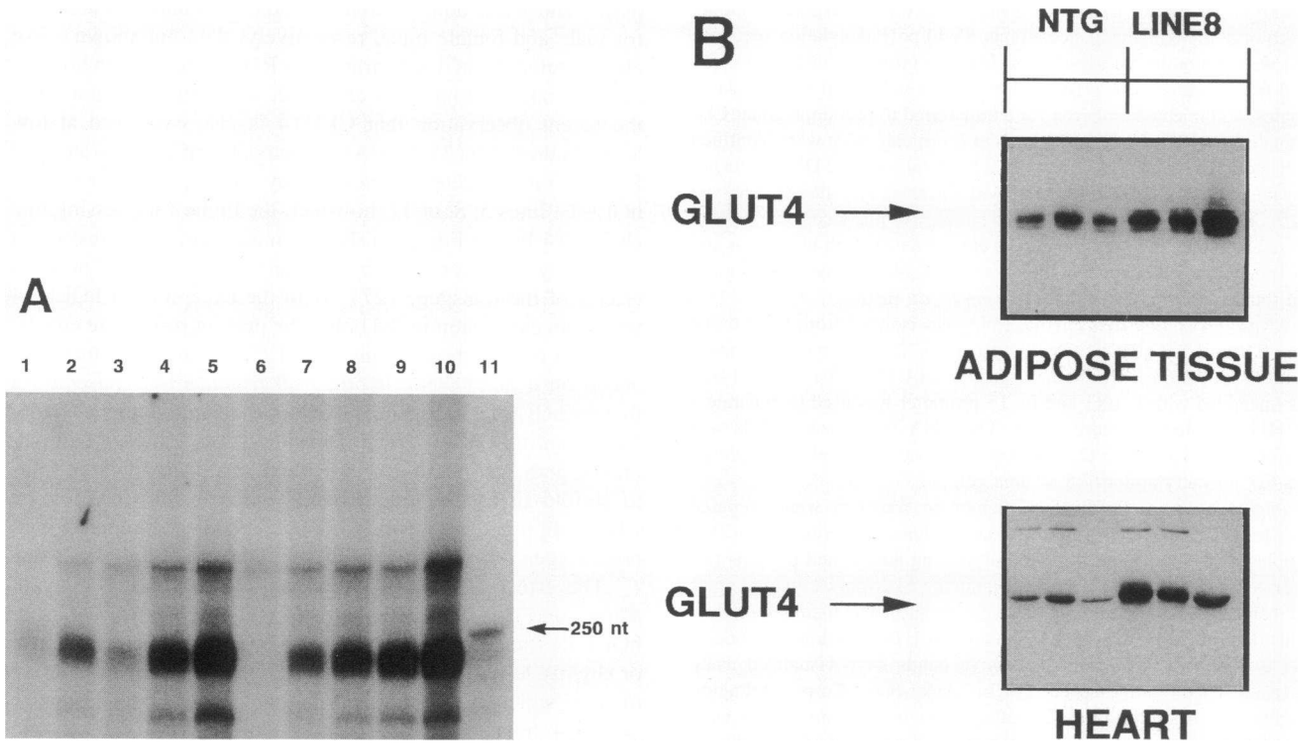
Results and Discussion

The relative expression of human GLUT4 (hGLUT4) in eight lines of F1 transgenic mice was determined by RNase protection of total RNA from cardiac tissue (Fig. 1 *A*). Tissue-specific expression of hGLUT4 RNA was examined by reverse transcriptase-PCR of purified total RNA from four of the lines expressing high hGLUT4 levels. In addition to cardiac muscle, appropriate expression (23, 24) of the hGLUT4 transgene was detected in hindquarter skeletal muscle, perirenal brown adipose tissue, and white adipose tissue (epididymal and subcutaneous for male and female mice, respectively; data not shown). We also detected the hGLUT4 transgene RNA in total brain homogenates from the four lines analyzed, which is consistent with the recent observation that GLUT4 is also expressed at low levels in the brain (25, 26). As expected, the hGLUT4 transgene RNA was not detected in liver, kidney, spleen, or pancreas from hGLUT4 lines 5, 8, or 12, however, the highest expressing line (hGLUT4 line 6; Fig. 1 *A*) showed inappropriate expression in the kidney and liver, presumably due to chromosomal position effects of the transgene (27). With the exception of hGLUT4 transgene expression in the brain, the present results are consistent with earlier observations in hGLUT4 transgenic mice produced on a nondiabetic hybrid background (14) and confirm that the hGLUT4 transgene is expressed in parallel to the endogenous murine GLUT4 gene. Densitometric analysis of immunoblots indicated that GLUT4 protein was overexpressed four- to sixfold in cardiac muscle and approximately threefold in white adipose tissue in hGLUT4*db/db* mice compared with nontransgenic *db/db* mice (Fig. 1, *B* and *C*).

The effect of GLUT4 overexpression on body weight of *db/+* and *db/db* mice is shown in Fig. 2. Body weights of hGLUT4 transgenic *db/+* mice of both sexes were similar to or slightly less than for nontransgenic *db/+* mice up to 40 wk of age, which is consistent with our earlier observations in hGLUT4 transgenic mice produced on a hybrid background (21). Body weights of both female (Fig. 2 *A*) and male (Fig. 2 *B*) hGLUT4 transgenic *db/db* mice were comparable with nontransgenic *db/db* mice up to ~ 10 wk of age. However, the nontransgenic *db/db* mice stopped gaining weight at this age, and in fact slowly lost weight over the remaining course of their lives, consistent with progression of the diabetic phenotype (10). In marked contrast, the hGLUT4*db/db* transgenic mice continued to gain weight until ~ 15 wk of age and then maintained this weight until at least 35 wk of age. Since overexpression of GLUT4 in muscle and fat is not a direct cause of obesity as evidenced by the results in hGLUT4*db/+* transgenic mice (Fig. 2) and hGLUT4 transgenic hybrid mice (14, 21), it is likely that GLUT4 overexpression in severely insulin-resistant *db/db* mutant mice at least partially overcomes the insulin resistance and thus enhances the animals' ability to thrive.

The improved weight gain of the hGLUT4*db/db* transgenic mice was in direct contrast with a previous study in which GLUT4 was selectively overexpressed in adipose tissue using the aP2 promoter in a different genetic mouse background (28). Although there was a marked increase in adiposity in these animals, this did not occur when GLUT4 was overexpressed in both muscle and adipose tissue using the GLUT4 promoter (21). Thus, we hypothesize that either the overexpression of GLUT4 in both muscle and adipose tissue prevents fat cell hyperplasia or that these differences in adiposity arise due to the use of an early developmental aP2 promoter (29) versus the late developmental GLUT4 promoter (30).

In any case, we next determined the functional expression of the hGLUT4 transgene on glucose homeostasis in vivo by assessing the physiological responses of both nondiabetic (*db/+*) and diabetic (*db/db*) mice to an oral glucose challenge (Fig. 3). 30 min after an oral glucose load (1 g/kg), a substantial increase in plasma glucose levels (approximately twofold) occurred in nontransgenic, nondiabetic *db/+* mice (Fig. 3 *A*). This increase in circulating glucose levels was transient and gradually declined toward basal levels by 120 min. In contrast, hGLUT4 transgenic nondiabetic *db/+* mice from three overex-



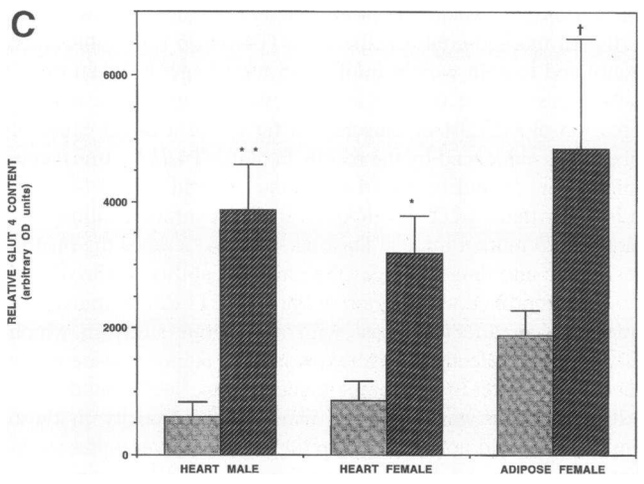


Figure 1. Expression of hGLUT4 in transgenic *db/db* mice. (*A*) RNase protection assay of hGLUT4 lines. 15 μg of heart total RNA was probed with 5×10^5 cpm of the human GLUT4 specific probe, pJW3, to determine hGLUT4 mRNA expression as described in Methods. Lanes *1, 2, 3, 4, 5, 7, 8,* and *9* correspond to individual mice (7–15 wk of age) from hGLUT4 lines 4, 3, 7, 5, 6, 12, 8, and 11, respectively. Lane *6* is a nontransgenic negative control mouse, while lane *10* is a positive control mouse from the previously characterized hGLUT4C line (14, 21, 31). The transgenic GLUT4 protected fragment is 241 nucleotides (nt) and migrates just ahead of a 250-nt size marker (lane *11*). The dried gel was exposed to x-ray film at −70°C with an intensifying screen for 3 d. (*B*) Immunoblot analysis of hGLUT4 transgenic line 8. 50 μg of adipose tissue (*top*) or cardiac muscle (*bottom*) postnuclear membrane protein was analyzed for GLUT4 protein content using anti-GLUT4 antibodies as described in Methods. NTG indicates three samples from female *db/db* nontransgenic mice, and LINE8 indicates three samples from female hGLUT4 line 8 *db/db* transgenic mice. (*C*) Relative expression of GLUT4 protein. GLUT4 bands from the immunoblots of female mice shown in *B* and also from immunoblots of cardiac muscle of male nontransgenic ($n = 3$) and hGLUT4*db/db* transgenic ($n = 5$) mice were quantitated by densitometric analysis. Lightly shaded bars represent nontransgenic mice, and hatched bars represent transgenic mice. All mice analyzed were between 9 and 12 wk of age. Significant differences from sex-matched nontransgenic mice are indicated, $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{\dagger}P = 0.07$.

pressing lines displayed reduced fasting plasma glucose, which remained below 100 mg/dl after the glucose load. One hGLUT4 transgenic line did not have reduced fasting plasma glucose compared with the nontransgenic mice, but showed essentially no increase in plasma glucose after the glucose load (hGLUT4 line 8; Fig. 3 *A*). The male mice used in the experiment shown in Fig. 3 *A* ranged from 7 to 16 wk of age; similar results were obtained in female mice and also in one line of hGLUT4 *db/+* mice (line 8) that was tested at 6 and 90 wk of age (data not shown). Further, as was observed in our previous studies (21, 31), functional expression of the GLUT4 transgene in skeletal and cardiac muscle was confirmed by a two- to fourfold increase in glycogen content in hGLUT4 transgenic mice (Gibbs, E. M., S. C. McCoid, W. J. Zavadoski, J. L. Stock, J. D. McNeish, and R. W. Stevenson, unpublished observations). Thus, high level expression of hGLUT4 protein in nondiabetic, *db/+* heterozygotes produced a phenotype similar to GLUT4 transgenic mice on a nondiabetic hybrid background (21, 31), and the effect of the transgene was maintained over the course of the animals' lives.

Nontransgenic *db/db* mice (8–9 wk of age) had fasting hyperglycemia (~ 250 mg/dl) that markedly increased up to ~ 600–700 mg/dl, after the glucose challenge (Fig. 3 *B*). Remarkably, age-matched *db/db* mice expressing the hGLUT4

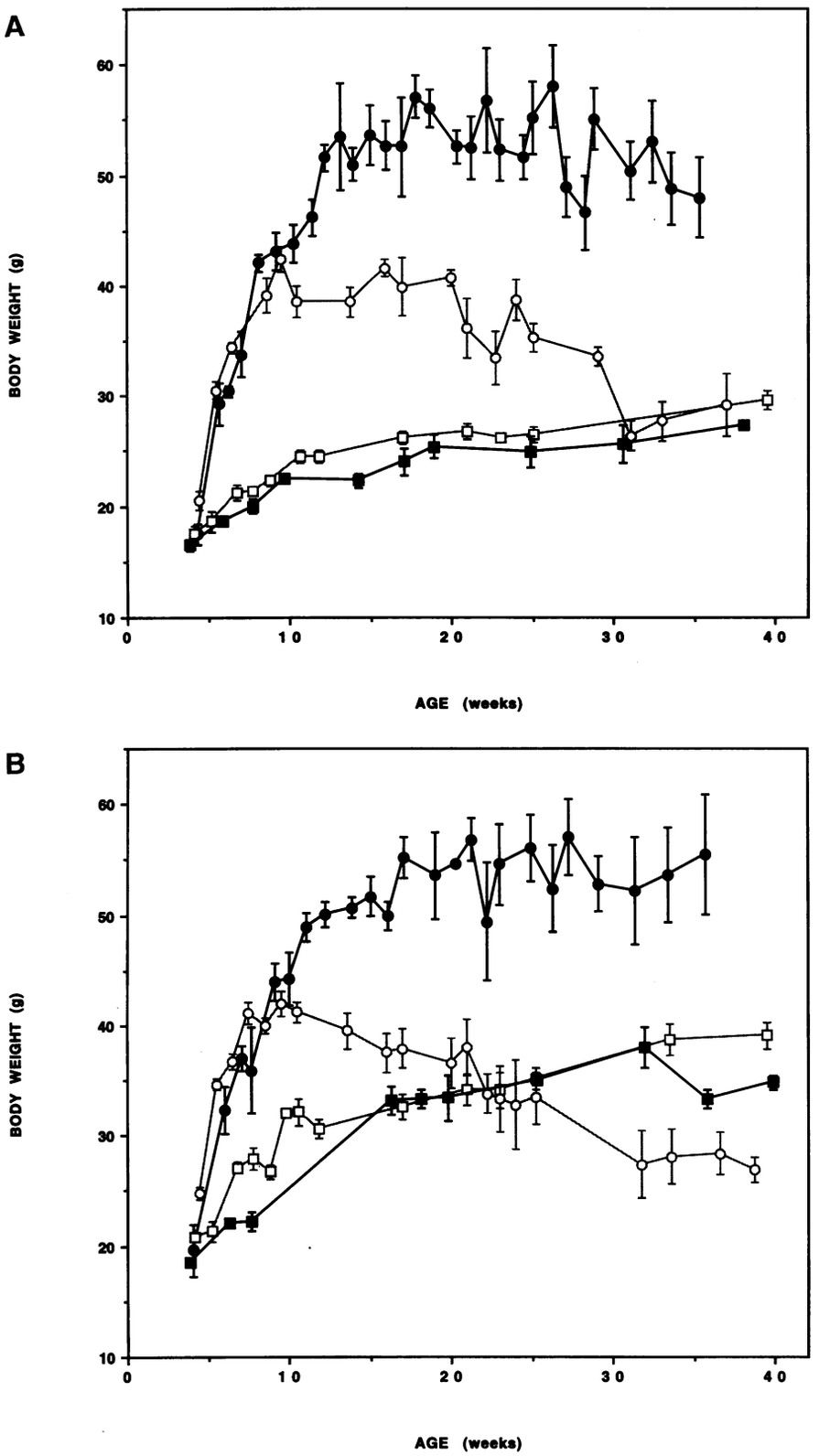


Figure 2. Body weight versus age in nontransgenic and transgenic hGLUT4*db*/+ and *db/db* mice. Body weights of female (*A*) and male (*B*) *db*/+ (*squares*) and *db/db* (*circles*) nontransgenic (*open symbols*) and hGLUT4 transgenic (*closed symbols*) mice are presented as means±SEM (n = 3–15, with a median n = 5). Most data points represent means obtained from single measurements of individual mice. However, because of the limiting number of hGLUT4*db/db* transgenic mice that was available, a subgroup of this phenotype was set aside for this study (n = 22 and 17, for female and male mice, respectively) and was reweighed at 2-wk intervals covering the 15–35 wk age range.

transgene (line 12) had fasting plasma glucose levels of ~ 50 mg/dl, which typically remained below 100 mg/dl even after the oral glucose challenge (Fig. 3*B*; similar results were obtained for three other hGLUT4 lines, not shown).

As expected, fed plasma insulin levels were reduced in female transgenic *db*/+ mice compared with female nontransgenic *db*/+ mice (28.3±2.5 vs 39.5±3.4 μU/ml; $P < 0.025$; mice were 6–11 wk of age) and in male transgenic mice *db*/+ compared with nontransgenic *db*/+ mice (37.1±3.1 vs 65.5±9.3 μU/ml; $P < 0.005$; mice were 6–10 wk of age). The insulin-lowering effect in the transgenic mice presumably occurs in response to the hypoglycemia produced by increased

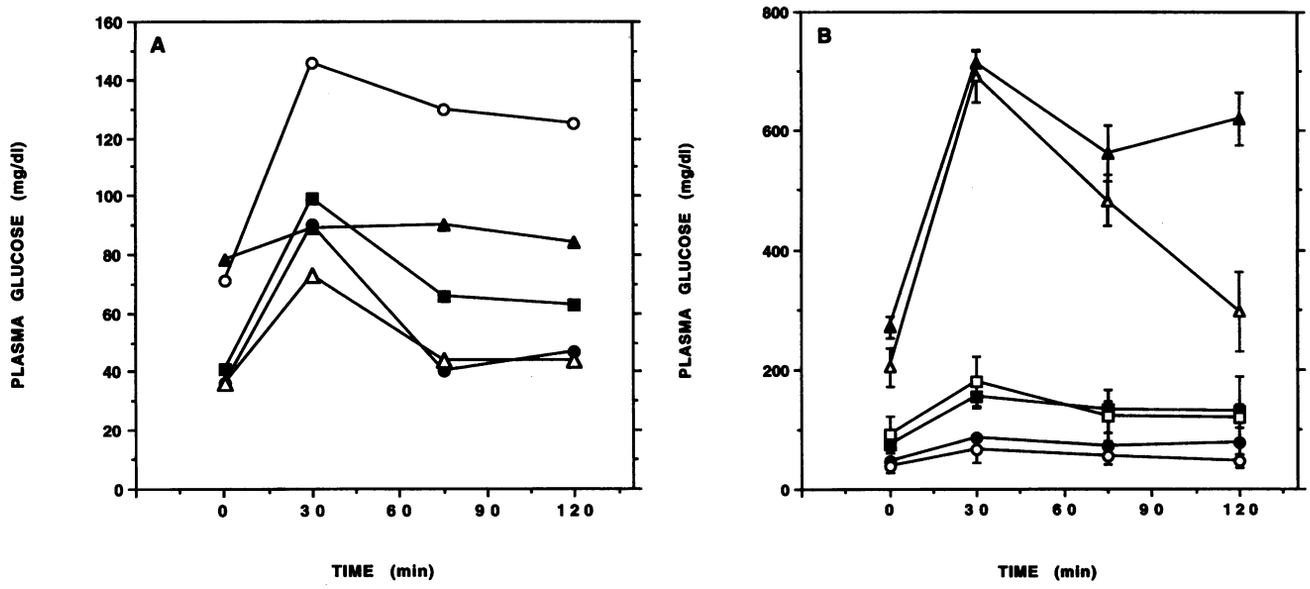


Figure 3. Effect of an oral glucose challenge on plasma glucose levels in hGLUT4 transgenic mice. Mice were fasted overnight and bled via the orbital sinus (0.025 ml) immediately before administration of an oral glucose load (1 g glucose/kg body wt) and at 30, 75, and 120 min thereafter. (*A*) Oral glucose tolerance in male nontransgenic and hGLUT4 transgenic *db/+* mice. Values are expressed as means obtained from the analysis of $n = 5$ *db/+* (*open circles*), $n = 4$ hGLUT4*db/+* line 5 (*closed circles*), $n = 4$ hGLUT4*db/+* line 6 (*closed squares*), $n = 3$ hGLUT4*db/+* line 8 (*closed triangles*), and $n = 3$ hGLUT4*db/+* line 12 (*open triangles*). Error bars are omitted for clarity. Plasma glucose values for transgenic mice significantly different from nontransgenic mice are as follows: all 4 lines were lower at both the 75- and 120-min time points ($P < 0.005$), lines 8 and 12 were lower at the 30-min time point ($P < 0.05$), and lines 5, 6, and 12 had lower fasting plasma glucose ($P < 0.025$). (*B*) Oral glucose tolerance in nontransgenic and hGLUT4 transgenic *db/db* mice. Values are expressed as means±SEM obtained from the analysis of $n = 5$ *db/+* (*squares*), $n = 5$ *db/db* (*triangles*), and $n = 3$ hGLUT4*db/db* line 12 (*circles*) male (*closed symbols*) and female (*open symbols*) mice, respectively. All glucose values were significantly lower in the transgenic mice compared with both *db/db* mice ($P < 0.005$) except for the 120-min time point for the female mice ($P < 0.025$), and with *db/+* mice ($P < 0.05$).

glucose disposal. Surprisingly, fed plasma insulin levels in hGLUT4*db/db* transgenic male and female mice were significantly higher than nontransgenic *db/db* mice, whereas fasting plasma insulin levels were lower in transgenic *db/db* mice of both sexes (Table I). The increased fed plasma insulin levels in the transgenic *db/db* mice likely result from a (partial) protection from the glucose-dependent pancreatic atrophy that occurs in nontransgenic *db/db* mice as they age (9–11, 32). Along this line, we have observed that the islet surface area in paraffin sections of pancreatic tissue is approximately threefold greater in hGLUT4*db/db* mice compared with age-matched (~ 30 wk) nontransgenic *db/db* mice (Milici, A. J., D. N. Scampoli, S. C. McCoid, J. L. Stock, J. D. McNeish, R. W. Stevenson, and E. M. Gibbs, unpublished observations). Since inappropriate GLUT4 expression in β cells might be expected to alter insulin secretion, it was important to demonstrate that this did not occur. hGLUT4 RNA was not detected in pancreatic RNA using the sensitive reverse transcriptase-PCR methodology. In addition, no difference in GLUT4 expression was observed in frozen sections of pancreatic tissue from transgenic mice compared with nontransgenic mice as assessed by immunofluorescence (data not shown). Taken together, these data suggest that expression of the hGLUT4 transgene in an appropriate tissue-specific manner in *db/db* mice results in more functional pancreatic β cells that can efficiently respond to the partially elevated fed plasma glucose levels than nontransgenic *db/db* mice. These data suggest that lowering plasma glucose by increased GLUT4 expression reduces stress on the pancreas and maintains its functional integrity, thus preventing the mice from progressing into the late hypoinsulinemic stage of NIDDM.

Table I. Comparison of Plasma Glucose and Insulin Levels between Nontransgenic db/db and hGLUT4 Transgenic db/db Mice

	Male		Female	
	db/db	hGLUT4 *db/db*	*db/db*	hGLUT4 *db/db*
Fed glucose (mg/dl)	537±18	363±44*	566±30	352±40*
(*n*)	(22)	(12)	(16)	(14)
Fasting glucose (mg/dl)	246±19	60±10*	185±17	36±5*
(*n*)	(11)	(6)	(11)	(6)
Fed insulin (μU/ml)	118±8	263±38*	80±6	174±40*
(*n*)	(29)	(19)	(25)	(12)
Fasting insulin (μU/ml)	101±7	69±9‡	109±7	81±5‡
(*n*)	(10)	(9)	(15)	(14)

Data from transgenic mice between 7 and 11 wk of age from hGLUT4 lines 5, 6, 8, and 12 were pooled for these analyses. The value in parentheses indicates the number of animals used for each analysis. Significant differences from age- and sex-matched nontransgenic mice are indicated, ‡ $P < 0.005$, * $P < 0.0005$.

To assess GLUT4 subcellular localization in vivo under fasting and maximally insulin-stimulated states (20, 21), we examined the cardiac myocyte distribution of the GLUT4 protein by immunofluorescence using a GLUT4 specific antibody (19) coupled with a Texas red–conjugated secondary antibody

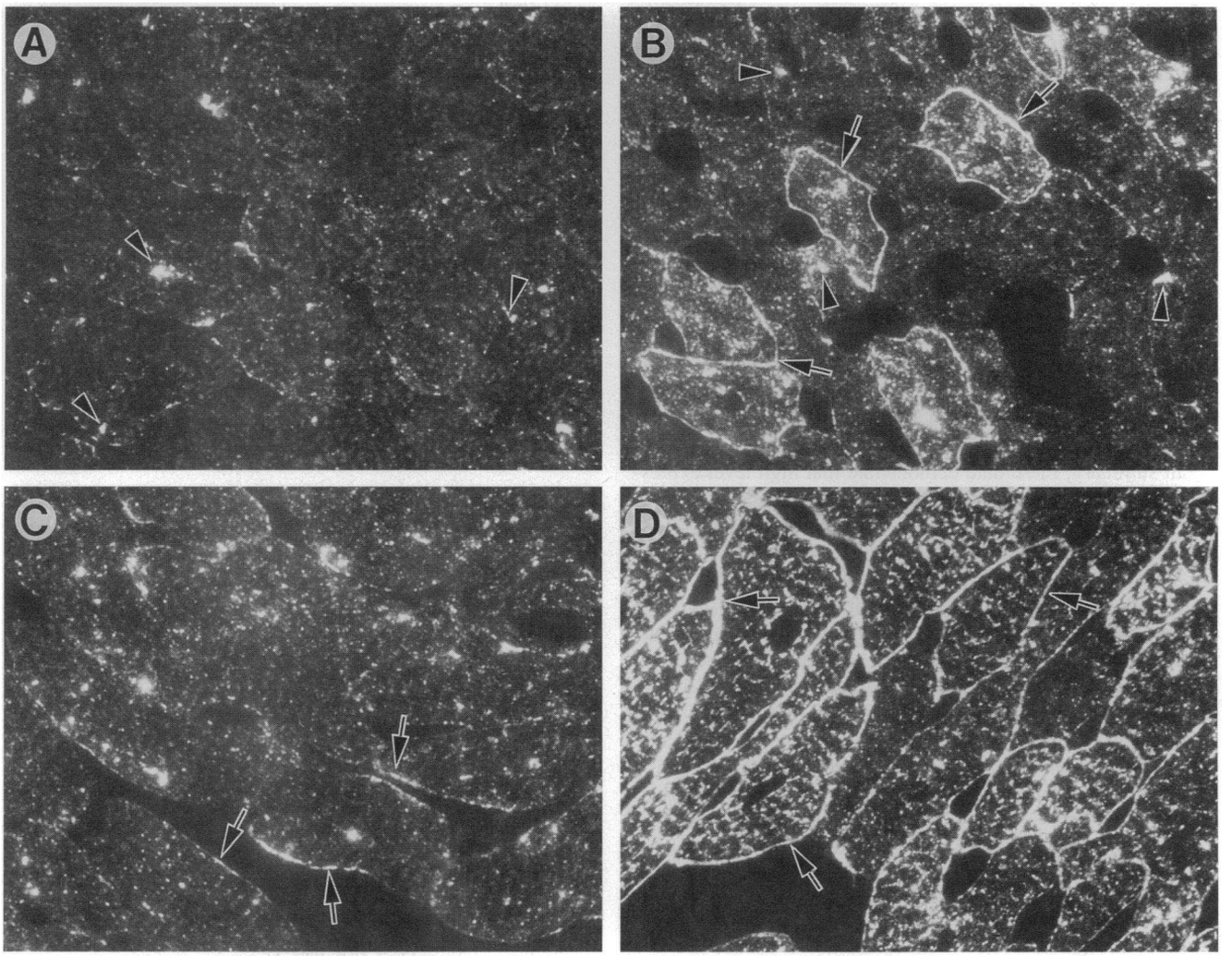


Figure 4. Immunofluorescent localization of cardiac myocyte GLUT4 protein expression in nontransgenic and transgenic hGLUT4 *db/db* mice. Female nontransgenic (*A* and *C*) and hGLUT4 line 8 transgenic (*B* and *D*) *db/db* mice (9 wk of age) were either fasted overnight and left untreated (*A* and *B*) or allowed to feed ad libitum and given an intraperitoneal injection of insulin (8 U/kg) and glucose (1 g/kg) (*C* and *D*). Arrowheads mark several locations of punctate cytoplasmic staining (*A* and *B*) in myocytes from the fasted animals, and arrows mark isolated myocytes in which the entire membrane was labeled in a fasted transgenic animal (*B*). Arrows mark locations of membrane-associated GLUT4 (*C* and *D*) in myocytes after insulin/glucose treatment. Insulin induced a very patchy translocation of GLUT4 in the nontransgenic mice (*C*), whereas in the transgenic mice (*D*) there was an intense uniform labeling of the entire myocyte plasmalemma. Essentially identical results were also obtained from *db/db* hGLUT4 line 12 transgenic mice (8 wk of age; data not shown). No significant fluorescent signal could be detected in any tissue sections from either nontransgenic or transgenic mice labeled with equal concentrations of nonimmune rabbit IgG (data not shown). ×900.

(Fig. 4). Cardiac muscle tissue from fasted nontransgenic *db/db* mice exhibited a punctate cytoplasmic GLUT4 immunofluorescence (Fig. 4 *A*). Both the level of GLUT4 immunofluorescence and localization in the basal state were similar to our previous observations in normal mice (21). With insulin and glucose treatment, nontransgenic *db/db* mice showed a minimal redistribution of GLUT4 immunofluorescence from the condensed cytoplasmic vesicles to the plasmalemma (Fig. 4 *C*). This contrasts with our previous data indicating substantial GLUT4 translocation in nondiabetic mice (21) and is a direct demonstration that one manifestation of the insulin resistance in the *db/db* mice is a GLUT4 translocation defect. Overexpression of GLUT4 protein in vivo resulted in a specific immunofluorescent signal that was readily detected in both the plasma membrane and intracellular vesicles from cardiac myocytes of the fasted non–insulin-treated hGLUT4*db/db* line 8 transgenic mice (Fig. 4 *B*). Furthermore, GLUT4 *db/db* 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 (Fig. 4 *D*). Qualitatively and quantitatively similar immunofluorescent data were obtained in quadriceps muscles obtained from the same mice used in these experiments (data not shown), which indicates that the cardiac myocyte analysis is representative of GLUT4 translocation in skeletal muscle.

Quantitative data obtained by image analysis of cardiac myocytes from nontransgenic *db/db* mice demonstrated only a 1.2-fold increase in plasmalemma GLUT4 immunofluorescence after insulin stimulation. In contrast, basal levels of plasmalemma GLUT4 in the transgenic *db/db* mice were much greater than those observed in insulin-treated nontransgenic mice and increased more than threefold after the insulin chal-

lenge. These data indicate that in the hGLUT4*db/db* transgenic mice relatively high levels of GLUT4 protein reside at the plasma membrane in the basal state and that insulin directly induces an increase in plasma membrane–associated human GLUT4 protein in a manner consistent with translocation from an intracellular vesicular pool. This GLUT4 redistribution occurs in spite of the insulin resistance (9–13) and translocation defect (see above) in the nontransgenic *db/db* mice.

Thus, if the defect in insulin action in *db/db* mice occurs in the insulin signaling pathway, it does not appear to be rate limiting in preventing GLUT4 translocation from the intracellular pool to the plasma membrane. Since the defect in the insulin signaling pathway in *db/db* mice appears to be overcome by increased GLUT4 expression, it is possible that increased glucose disposal reduces hyperglycemia and the "glucose toxicity" proposed by DeFronzo (7) as a contributing factor to insulin resistance.

In summary, these data demonstrate that high level expression of the human insulin responsive GLUT4/muscle-fat specific facilitative glucose transporter transgene in a genetic model of NIDDM results in a high level of cell surface GLUT4 protein localization. Additionally, the increased GLUT4 expression resulted in a restoration of insulin-stimulated GLUT4 translocation from the intracellular storage site to the plasma membrane. As a consequence, after a large oral glucose load, circulating glucose levels were markedly reduced in the hGLUT4 transgenic *db/db* mice compared with the nontransgenic *db/db* mice, indicating a substantially greater degree of glycemic control. Most importantly, these results suggest that increasing GLUT4 levels at the plasma membrane by either genetic manipulation or by pharmacologic intervention may be an effective therapy for human NIDDM as improved glycemic control occurs even in the presence of severe insulin resistance and pancreatic defects.

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References

1. Baron, A. D., G. Brechtel, P. Wallace, and S. V. Edelman. 1988. Rates and tissue sites of non-insulin- and insulin-mediated glucose uptake in humans. *Am. J. Physiol.* 255:E769–E774.

2. Shulman, G. I., D. L. Rothman, T. Jue, P. Stein, R. A. DeFronzo, and R. G. Shulman. 1990. Quantitation of muscle glycogen synthesis in normal subjects and subjects with non-insulin-dependent diabetes by ^{13}C nuclear magnetic resonance spectroscopy. *N. Engl. J. Med.* 322:223–228.

3. Garvey, W. T., L. Maianu, J. A. Hancock, A. M. Golichowski, and A. Baron. 1992. Gene expression of GLUT4 in skeletal muscle from insulin-resistant patients with obesity, IGT, GDM, and NIDDM. *Diabetes.* 41:465–475.

4. Pedersen, O., J. F. Bak, P. H. Andersen, S. Lund, D. E. Moller, J. S. Flier, and B. B. Kahn. 1990. Evidence against altered expression of GLUT1 or GLUT4 in skeletal muscle of patients with obesity of NIDDM. *Diabetes.* 39:865–870.

5. Handberg, A., A. Vaag, P. Damsbo, H. Beck-Nielsen, and J. Vinten. 1990. Expression of insulin regulatable glucose transporters in skeletal muscle from type 2 (non-insulin-dependent) diabetic patients. *Diabetologia.* 33:625–627.

6. Eriksson, J., L. Koranyi, R. Bourey, C. Schalin-Jantti, E. Widen, M. Mueckler, A. M. Permutt, and L. C. Groop. 1992. Insulin resistance in type 2 (non-insulin-dependent) diabetic patients and their relatives is not associated with a defect in the expression of the insulin-responsive glucose transporter (GLUT4) gene in human skeletal muscle. *Diabetologia.* 35:143–147.

7. DeFronzo, R. A. 1988. The triumvirate: β-cell, muscle, liver a collusion responsible for NIDDM. *Diabetes.* 37:667–687.

8. Santiago, J. V. 1993. Lessons from the diabetes control and complications trial. *Diabetes.* 42:1549–1554.

9. Hummel, K. P., M. M. Dickie, and D. L. Coleman. 1966. Diabetes, a new mutation in the mouse. *Science (Wash. DC).* 153:1127–1128.

10. Herberg, L., and D. L. Coleman. 1977. Laboratory animals exhibiting obesity and diabetes syndromes. *Metab. Clin. Exp.* 26:59–99.

11. Leiter, E. H. 1989. The genetics of diabetes susceptibility in mice. *FASEB (Fed. Am. Soc. Exp. Biol.) J.* 3:2231–2241.

12. Chan, T. M., and J. P. Dehaye. 1981. Hormone regulation of glucose metabolism in the genetically obese-diabetic mouse (*db/db*): glucose metabolism in the perfused hindquarters of lean and obese mice. *Diabetes.* 30:211–218.

13. Koranyi, L., D. James, M. Mueckler, and M. A. Permutt. 1990. Glucose transporter levels in spontaneously obese (*db/db*) insulin-resistant mice. *J. Clin. Invest.* 85:962–967.

14. Olson, A. L., M.-L. Liu, W. S. Moye-Rowley, J. B. Buse, G. I. Bell, and J. E. Pessin. 1993. Hormonal/metabolic regulation of the human GLUT4/muscle-fat facilitative glucose transporter gene in transgenic mice. *J. Biol. Chem.* 268:9839–9846.

15. Hogan, B., F. Costantini, and E. Lacy. 1986. Manipulating the Mouse Embryo: A Laboratory Manual. Cold Spring Harbor Press, Cold Spring Harbor, NY. 89–173.

16. DePamphilis, M. L., S. A. Herman, E. Martinez-Salas, L. E. Chalifour, D. O. Wirak, D. Y. Cupo, and M. Miranda. 1988. Microinjecting DNA into mouse ova to study DNA replication and gene expression and to produce transgenic mice. *Biotechniques.* 6:662–688.

17. Chick, W. L., and A. A. Like. 1970. Studies in the diabetic mutant mouse. IV. DBM, a modified diabetic mutant produced by outcrossing of the original strain. *Diabetologia.* 6:252–256.

18. Shepherd, P. R., E. M. Gibbs, C. Wesslau, G. W. Gould, and B. B. Kahn. 1992. Human small intestine facilitative fructose/glucose transporter (GLUT5) is also present in insulin-responsive tissues and brain. *Diabetes.* 41:1360–1365.

19. Brant, A. M., S. C. McCoid, H. M. Thomas, S. A. Baldwin, A. Davies, J. C. Parker, E. M. Gibbs, and G. W. Gould. 1992. Analysis of the glucose transporter content of islet cell lines: implications for glucose-stimulated insulin release. *Cell. Signalling.* 4:641–650.

20. Slot, J. W., H. J. Geuze, S. Gigengack, D. E. James, and G. E. Lienhard. 1991. Translocation of the glucose transporter GLUT4 in cardiac myocytes of the rat. *Proc. Natl. Acad. Sci. USA.* 88:7815–7819.

21. Liu, M.-L., E. M. Gibbs, S. C. McCoid, A. J. Milici, H. A. Stukenbrok, R. K. McPherson, J. L. Treadway, and J. E. Pessin. 1993. Transgenic mice expressing the human GLUT4/muscle-fat facilitative glucose transporter protein exhibit efficient glycemic control. *Proc. Natl. Acad. Sci. USA.* 90:11346–11350.

22. Milici, A. J., and G. A. Porter. 1991. Lectin and immunolabeling of microvascular endothelia. *J. Electron Microsc. Tech.* 19:305–315.

23. Pessin, J. E., and G. I. Bell. 1992. Mammalian facilitative glucose transporter family: structure and regulation. *Annu. Rev. Physiol.* 54:911–930.

24. Kahn, B. B. 1992. Facilitative glucose transporters: regulatory mechanisms and dysregulation in diabetes. *J. Clin. Invest.* 89:1367–1374.

25. Brant, A. M., T. J. Jess, G. Milligan, C. M. Brown, and G. W. Gould. 1993. Immunological analysis of glucose transporters expressed in different regions of the rat brain and central nervous system. *Biochem. Biophys. Res. Commun.* 192:1297–1302.

26. Rayner, D. V., M. E. A. Thomas, and P. Trayhurn. 1994. Glucose transporters (GLUTs1-4) and their mRNAs in regions of the rat brain: insulin-sensitive transporter expression in the cerebellum. *Can. J. Physiol. Pharmacol.* 72:476–479.

27. Palmiter, R. D., and R. L. Brinster. 1986. Germ-line transformation of mice. *Annu. Rev. Genet.* 20:465–499.

28. Shepherd, P. R., L. Gnudi, E. Tozzo, H. Yang, F. Leach, and B. B. Kahn. 1993. Adipose cell hyperplasia and enhanced glucose disposal in transgenic mice overexpressing GLUT4 selectively in adipose tissue. *J. Biol. Chem.* 268:22243–22246.

29. Ailhaud, G., P. Grimaldi, and R. Negrel. 1992. Cellular and molecular aspects of adipose tissue development. *Annu. Rev. Nutr.* 12:207–233.

30. Santalucia, T., M. Camps, A. Castello, P. Munoz, A. Nuel, X. Testar, M. Palacin, and A. Zorzano. 1992. Developmental regulation of GLUT1 (erythroid/HepG2) and GLUT4 (muscle/fat) glucose transporter expression in rat heart, skeletal muscle, and brown adipose tissue. *Endocrinology.* 130:837–846.

31. Treadway, J. L., D. M. Hargrove, N. A. Nardone, R. K. McPherson, J. F. Russo, A. J. Milici, H. A. Stukenbrok, E. M. Gibbs, R. W. Stevenson, and J. E. Pessin. 1994. Enhanced peripheral glucose utilization in transgenic mice expressing the human GLUT4 gene. *J. Biol. Chem.* 269:29956–29961.

32. Chick, W. L., and A. A. Like. 1970. Studies in the diabetic mutant mouse. III. Physiological factors associated with alterations in beta cell proliferation. *Diabetologia.* 6:243–251.