Prevention of Insulin Resistance and Diabetes in Mice Heterozygous for GLUT4 Ablation by Transgenic Complementation of GLUT4 in Skeletal Muscle

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Impaired skeletal muscle glucose utilization under insulin action is a major defect in the etiology of type 2 diabetes. This is underscored by a new mouse model of type 2 diabetes generated by genetic disruption of one allele of glucose transporter 4 (GLUT4$^{+/-}$), the insulin-responsive glucose transporter in muscle and adipose tissue. Male GLUT4$^{+/-}$ mice exhibited decreased GLUT4 expression and glucose uptake in muscle that accompanied impaired whole-body glucose utilization, hyperinsulinemia, hyperglycemia, and heart histopathology. To determine whether development of the diabetic phenotype in GLUT4$^{+/-}$ mice can be forestalled by preventing the onset of impaired muscle GLUT4 expression and glucose utilization, standard genetic crossing was performed to introduce a fast-twitch muscle-specific GLUT4 transgene—the myosin light chain (MLC) promoter–driven transgene MLC-GLUT4—into GLUT4$^{+/-}$ mice (MLC-GLUT4$^{+/-}$ mice). GLUT4 expression and 2-deoxyglucose uptake levels were normalized in fast-twitch muscles of MLC-GLUT4$^{+/-}$ mice. In contrast to GLUT4$^{+/-}$ mice, MLC-GLUT4$^{+/-}$ mice exhibited normal whole-body glucose utilization. In addition, development of hyperinsulinemia and hyperglycemia observed in GLUT4$^{+/-}$ mice was prevented in MLC-GLUT4$^{+/-}$ mice. The occurrence of diabetic heart histopathology in MLC-GLUT4$^{+/-}$ mice was reduced to control levels. Based on these results, we propose that the onset of a diabetic phenotype in GLUT4$^{+/-}$ mice can be avoided by preventing decreases in muscle GLUT4 expression and glucose uptake. *Diabetes* 48:775–782, 1999

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ANOVA, analysis of variance; 2-DOG, 2-deoxyglucose; EDL, extensor digitorum longus; FFA, free fatty acid; PLSD, protected least significant difference; R_d, rate of whole-body glucose utilization; WAT, white adipose tissue.

Skeletal muscle plays a prominent role in glucose homeostasis because it is the major site of insulin-stimulated glucose disposal (1,2). Glucose transport is the rate-limiting step in glucose utilization under most physiological conditions (3,4). Impairment of glucose transport under insulin action plays a major role in the pathogenesis of type 2 diabetes (1,5). Glucose transporter 4 (GLUT4) is the major facilitative glucose transporter isoform in skeletal muscle (6). GLUT4 is redistributed from intracellular stores to the sarcolemma and transverse tubule system on stimulation by insulin, ischemia, or exercise (7–11). Defects in skeletal muscle GLUT4 translocation have been consistently observed in various animal models of insulin resistance and type 2 diabetes (12–14).

It has been proposed that a rational therapeutic regimen for type 2 diabetic patients should include both inhibition of basal hepatic glucose production and stimulation of muscle glucose uptake (1). Because GLUT4 is a major regulator of muscle glucose metabolism, a new mode of therapy based on increased GLUT4 expression and/or activity may prove effective for type 2 diabetic patients. This hypothesis is supported by recent studies demonstrating that overexpression of GLUT4 in transgenic mice improves whole-body insulin action (15–17). Moreover, we have demonstrated that the use of the myosin light chain (MLC) promoter–driven MLC-GLUT4 transgene to achieve selective overexpression of GLUT4 in muscle increased by 2.5-fold the in vivo insulin-stimulated 2-deoxyglucose (2-DOG) uptake in fast-twitch skeletal muscles (18). Consequently, MLC-GLUT4 mice exhibited increased whole-body glucose utilization under euglycemic-hyperinsulinemic clamp conditions (18).

We have generated a new model of type 2 diabetes by genetic disruption of one allele of GLUT4 (19,20). Male mice heterozygous for GLUT4 disruption (GLUT4$^{+/-}$) exhibit decreased GLUT4 expression, decreased glucose uptake in muscle, hyperglycemia, hyperinsulinemia, and diabetic cardiomyopathy. Because normal body weight is maintained in GLUT4$^{+/-}$ mice, they provide an excellent model for studying insulin resistance and diabetes without obesity-related complications. Blood glucose and insulin levels are normal in young GLUT4$^{+/-}$ mice (19). As they age, these mice develop hyperinsulinemia, followed by hyperglycemia (19). Before the onset of hyperglycemia, GLUT4$^{+/-}$ mice are characterized by decreased muscle GLUT4 content and peripheral insulin action but normal hepatic insulin response (19,20).

To examine whether the diabetic phenotype exhibited by GLUT4$^{+/-}$ mice can be prevented by restoring normal muscle but not adipose tissue GLUT4 expression, we introduced a muscle-specific GLUT4 transgene into GLUT4$^{+/-}$ mice by standard genetic crossing. GLUT4 expression and glucose transport in fast-twitch skeletal muscle was restored in the resulting MLC-GLUT4$^{+/-}$ mice. In addition, results of euglycemic hyperinsulinemic clamps indicated that whole-body insulin action was normalized by expression of the MLC-GLUT4 transgene. Introduction of the MLC-GLUT4 transgene prevented the development of hyperglycemia and hyperinsulinemia seen in GLUT4$^{+/-}$ mice. Histopathology of the heart was partially normalized in MLC-GLUT4$^{+/-}$ mice. These results indicate that expression of the MLC-GLUT4 transgene in skeletal muscle of GLUT4$^{+/-}$ mice can prevent development of the type 2 diabetes phenotype in GLUT4$^{+/-}$ mice.

RESEARCH DESIGN AND METHODS

Animals. MLC-GLUT4$^{+/-}$ mice were obtained by crossing GLUT4$^{+/-}$ mice to MLC-GLUT4 mice, which were maintained in a CBA/C57Bl6 hybrid background (18,21). The GLUT4 locus segregates independently of the MLC-GLUT4 transgene locus. MLC-GLUT4$^{+/-}$ mice were genotyped for the MLC-GLUT4 transgene and GLUT4 gene disruption as previously described (18,21). Control and GLUT4$^{+/-}$ mice used in plasma hormone and metabolite analysis were maintained in the CBA/C57Bl6 background. For 2-DOG uptake experiments and immunoblot analysis, GLUT4$^{+/-}$ and control mice from either CD1 or CBA/C57Bl6 hybrid backgrounds were used. For euglycemic clamp studies, GLUT4$^{+/-}$ mice from CD1 background and control mice from both CD1 and CBA/C57Bl6 backgrounds were used. We have determined that differences in genetic backgrounds contribute very little to variations in muscle uptake rates of 2-DOG and whole-body glucose utilization rates (data not shown). Mice from different genetic backgrounds were pooled to account for phenotypic variations between strains carrying the GLUT4$^{+/-}$ mutation and the MLC-GLUT4 transgene. Male mice were used in all studies. Animals were fed ad libitum and were maintained in a murine hepatitis virus–free barrier facility on a 12-h light and dark cycle. All protocols have been approved by the Animal Care and Use Committee of the Albert Einstein College of Medicine, in accordance with the Public Health Service Animal Welfare Policy.

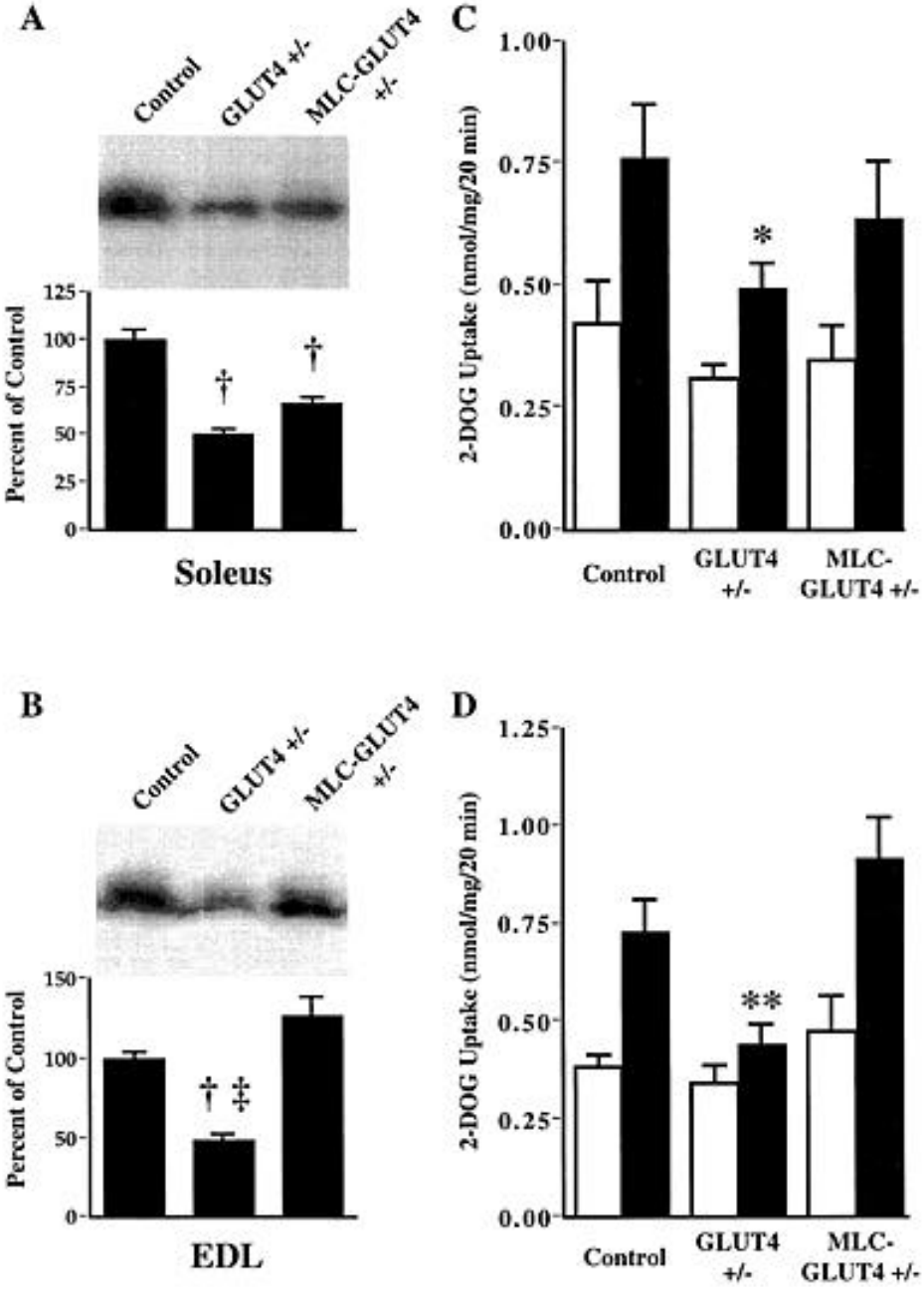

FIG. 1. Immunoblot analysis of GLUT4 was performed in isolated soleus (A) and EDL (B) muscle from control, GLUT4$^{+/-}$, and MLC-GLUT4$^{+/-}$ mice. Sonicated soleus and EDL muscle homogenates were prepared. Immunoblot analysis was performed using a GLUT4 anti–COOH-terminal antibody (control, n = 8; GLUT4$^{+/-}$, n = 7; and MLC-GLUT4$^{+/-}$, n = 12 or 13). Laser scanning densitometry was conducted to quantitate the differences. Densitometric units of the control group were set to 100%. Comparisons were made using ANOVA and Fisher's PLSD post hoc analysis. Basal and insulin-stimulated 2-DOG uptake rates were assessed in isolated soleus (C) and EDL (D) muscle from control, GLUT4$^{+/-}$, and MLC-GLUT4$^{+/-}$ mice. 2-DOG uptake in soleus and EDL muscle was measured in the absence (□) and presence (■) of 20 nmol/l insulin as described in METHODS. Comparisons were made under basal or insulin-stimulated conditions (control, n = 7; GLUT4$^{+/-}$, n = 6; and MLC-GLUT4$^{+/-}$, n = 11). Statistical significance is indicated: *P < 0.05, †P < 0.001 vs. controls; **P < 0.01 vs. either control or MLC-GLUT4$^{+/-}$; ‡P < 0.0001 vs. MLC-GLUT4$^{+/-}$.

TABLE 1
Fed plasma metabolites and insulin levels in 4- to 6-month-old control, GLUT4$^{+/-}$, and MLC-GLUT4$^{+/-}$ mice

	Control	GLUT4$^{+/-}$	MLC-GLUT4$^{+/-}$
Glucose (mmol/l)	10.1 ± 0.6 (20)	12.8 ± 1.5 (13)*	8.9 ± 0.8 (16)†
Insulin (ng/ml)	23.5 ± 6.8 (21)	74.8 ± 20.8 (11)‡	6.0 ± 1.4 (12)†
Lactate (mmol/l)	8.5 ± 0.6 (15)	7.9 ± 0.9 (10)	7.4 ± 0.8 (11)
FFA (μEq/l)	633 ± 46 (15)	415 ± 39 (10)‡	472 ± 56 (11)*

Data are means ± SE (n) of mice analyzed. Statistically significant values are indicated: *P < 0.05 vs. controls; †P < 0.01 vs. GLUT4$^{+/-}$ mice; ‡P < 0.005 vs. controls.

Immunoblot analysis of GLUT4. Hearts were homogenized in TES buffer (100 mmol/l Tris [pH 7.6], 0.2 mmol/l EDTA, and 255 mmol/l sucrose) supplemented with a cocktail of protease inhibitors (Boehringer Mannheim, Indianapolis, IN), containing 1 mmol/l 4-(2-aminoethyl)-benzenesulfonyl-fluoride, 0.3 μmol/l aprotinin, 1 μmol/l pepstatin, and 1 μmol/l leupeptin. Individual extensor digitorum longus (EDL) and soleus muscles were disrupted by sonication in 20 mmol/l Tris, pH 7.6, 1 mmol/l EDTA, and 250 mmol/l sucrose supplemented with protease inhibitors as described above. Immunoblot analysis was performed as previously described (18,22). The COOH-terminal peptide anti-GLUT4 antibody used in the present study has been described previously (23).

2-DOG uptake in isolated muscles. After cervical dislocation, soleus and EDL muscles were rapidly isolated from the hindlimbs. The distal tendon of each muscle was tied to a piece of suture to facilitate transfer among different media. Muscles from an individual mouse were randomly divided such that one was used for basal and the other for insulin-stimulated 2-DOG uptake. All incubations were carried out at 30°C in oxygenated (95% O_2; 5% CO_2) Krebs-Henseleit bicarbonate buffer supplemented with 0.1% bovine serum albumin (fraction V, radioimmunoassay grade; Sigma, St. Louis, MO) and 2 mmol/l pyruvic acid (Sigma). After a short incubation, muscles were incubated for 45 min in the absence or presence of 20 nmol/l porcine insulin (Eli Lilly, Indianapolis, IN). The rate of 2-DOG uptake was measured at 0.1 mmol/l 2-DOG concentration as previously described (22,24).

Conscious euglycemic-hyperinsulinemic clamp. Male mice 5–6 months of age were used in this study. Details of the euglycemic-hyperinsulinemic clamp procedure used in this study have been described previously (25,26). In summary, an indwelling catheter was inserted into the right internal jugular vein of anesthetized mice, and euglycemic clamps were performed in conscious, unrestrained mice 4–5 days after surgery, to allow the animal to recover. The rate of whole-body glucose utilization (R_d) was determined by enrichment of [3-^{3}H]glucose in plasma. The rate of glycolysis was determined from the accumulation of ^3H_2O in plasma as a result of [3-^{3}H]glucose breakdown. The rate of glycogen synthesis was calculated as the difference between the R_d and the glycolysis rate. Insulin was infused at a rate of 18 mU · kg^{-1} · min^{-1}, and plasma glucose concentration was clamped at 100 mg/dl by variable glucose infusion. Various analytic procedures for processing tissue and blood samples have been described previously (25,26).

Analysis of plasma metabolites and hormones. Fed plasma from control, GLUT4$^{+/-}$, and MLC-GLUT4$^{+/-}$ male mice, 3–7 months old, were analyzed. With animals in the fed state, blood was drawn between midnight and 2:00 A.M. from the retro-orbital sinus using a heparinized microcapillary tube and was quickly spun in a microcentrifuge to obtain plasma. Insulin levels were measured using a rat insulin kit (Linco, St. Louis, MO). Glucose and lactate were measured using kits from Sigma. Free fatty acid (FFA) was assessed using a kit from Amano Enzyme (Troy, VA), with oleic acid used as the standard.

Heart histology. After mice were anesthetized with an intraperitoneal injection of pentobarbital (1 ml/kg body wt), Trump's fixative was used to perfuse hearts as previously described (19). After perfusion, transverse sections of hearts were prepared, embedded in paraffin, sectioned at 5-μm thickness, and stained with hematoxylin and eosin as previously described (19).

Statistical analysis. Data are presented as means ± SE of multiple determinations. Statistical significance of all results except heart GLUT4 immunoblot analysis were determined by analysis of variance (ANOVA) using Fisher's protected least significant difference (PLSD) procedure for post hoc analysis. An unpaired, two-tailed, Student's t test was used to assess statistical significance of heart GLUT4 immunoblot analysis. Relationships between the incidence of heart histopathology and plasma glucose and insulin were analyzed using contingency tables, and statistical significance was evaluated using χ^2 tests. In these analyses, plasma glucose and insulin levels of mice analyzed for heart histopathology were designated as either high (>2 SE of control values) or normal (≤2 SE of control values).

RESULTS

Immunoblot analysis of GLUT4. GLUT4 protein in soleus and EDL muscles of 4- to 6-month-old male control, GLUT4$^{+/-}$, and MLC-GLUT4$^{+/-}$ mice was determined by immunoblot analysis. We previously showed that expression of the MLC-GLUT4 transgene is targeted to muscles rich in fast-twitch fibers (EDL and gastrocnemius) and not to muscles containing significant amounts of slow-twitch fibers (soleus) (18,22). Muscles rich in fast-twitch fibers are the predominant muscle type (27). Compared with control soleus muscle, GLUT4$^{+/-}$ soleus muscle showed a 50% decrease in immunoreactive GLUT4 (Fig. 1*A*). In transgene-negative MLC-GLUT4$^{+/-}$ soleus muscle, GLUT4 content was only 66% of control soleus muscle (Fig. 1*A*). Results of the EDL comparison were similar to those of the soleus muscle comparison: the amount of immunoreactive GLUT4 in the EDL of GLUT4$^{+/-}$ mice was 48% of control EDL (Fig. 1*B*). In contrast to soleus muscle, the MLC-GLUT4$^{+/-}$ EDL did not have significantly increased GLUT4 content compared with control EDL (Fig. 1*B*). This result is consistent with our previous finding that MLC-GLUT4 transgene is negligibly expressed in soleus muscle (18,22).

TABLE 2
Body weights of 4- to 5-month-old control, GLUT4$^{+/-}$, and MLC-GLUT4$^{+/-}$ mice and their steady-state plasma glucose and insulin concentrations during euglycemic clamp studies

	Control	GLUT4$^{+/-}$*	MLC-GLUT4$^{+/-}$
n	9	6	9
Body weight (g)	38.3 ± 1.2	37.0 ± 1.8	39.7 ± 3.3
Glucose (mmol/l)	6.6 ± 0.3	5.6 ± 0.5	5.8 ± 0.4
Insulin (ng/ml)	52 ± 8	54 ± 7	51 ± 7

Data are means ± SE. *Data for GLUT4$^{+/-}$ mice are from Rossetti et al. (20).

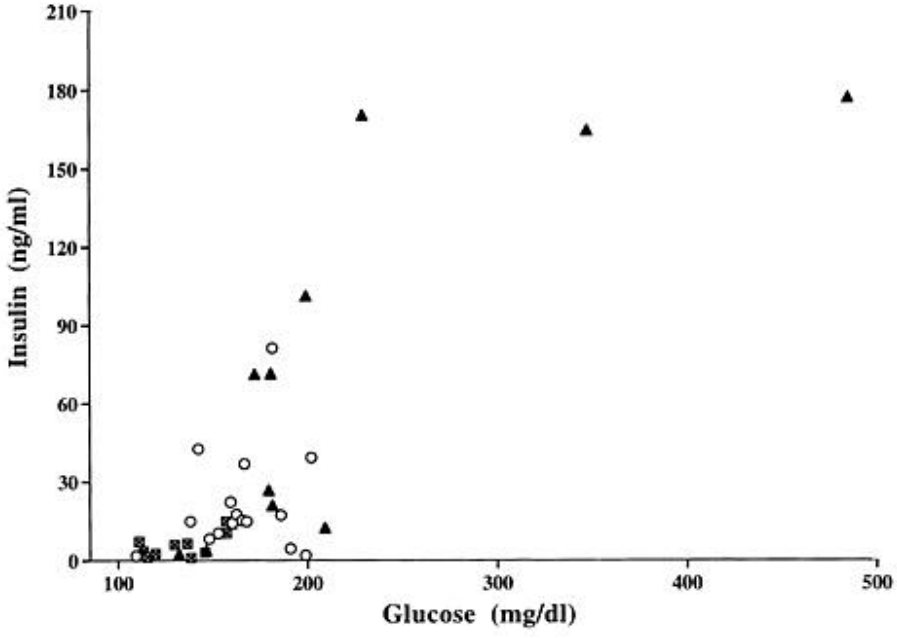

FIG. 2. Relationship between fed glucose and insulin from individual control (○), GLUT4$^{+/-}$(▲), and MLC-GLUT4$^{+/-}$ (☒) mice. Glucose levels were plotted against insulin levels from the same individuals (control, *n* = 16; GLUT4$^{+/-}$, *n* = 11; and MLC-GLUT4$^{+/-}$, *n* = 12). Symbols representing each group are shown in the insert.

Immunoreactive GLUT4 in white adipose tissue (WAT) of MLC-GLUT4$^{+/-}$ mice was 60% compared with control mice, which is similar to the magnitude of reduction in GLUT4$^{+/-}$ mice (data not shown).

2-DOG uptake in isolated soleus and EDL muscle. We examined the consequences of introducing the MLC-GLUT4 transgene into a GLUT4$^{+/-}$ genetic background on glucose uptake in skeletal muscles. 2-DOG uptake was measured in soleus and EDL muscle of 4- to 5-month-old male control, GLUT4$^{+/-}$, and MLC-GLUT4$^{+/-}$ mice. Basal 2-DOG uptake rates in both soleus and EDL muscle were similar in all groups (Fig. 1*C* and *D*). Insulin-stimulated 2-DOG uptake rates in GLUT4$^{+/-}$ soleus and EDL muscle were decreased by 35 and 39%, respectively, when compared with those of controls (Fig. 1*C* and *D*). In MLC-GLUT4$^{+/-}$ EDL, in which GLUT4 expression was normalized, insulin-stimulated 2-DOG uptake was similar to that in controls (Fig. 1*D*). This finding represented a 108% increase in MLC-GLUT4$^{+/-}$ EDL 2-DOG uptake compared with that in GLUT4$^{+/-}$ EDL (Fig. 1*D*). In soleus muscle of MLC-GLUT4$^{+/-}$ mice, in which GLUT4 protein levels remained below that of controls, both basal and insulin-stimulated 2-DOG uptake were intermediate between those of control and GLUT4$^{+/-}$ soleus muscle (Fig. 1*C*).

Plasma hormone and metabolite profile of control, GLUT4$^{+/-}$, and MLC-GLUT4$^{+/-}$ mice. A hallmark of disturbed glucose homeostasis in type 2 diabetes is the development of hyperinsulinemia and hyperglycemia. To assess the effects of reduced muscle glucose transport, as well as the effects of the restoration of glucose uptake by MLC-GLUT4 transgene expression on the maintenance of glucose homeostasis, we measured fed plasma insulin, glucose, lactate, and FFA levels. Male GLUT4$^{+/-}$ mice displayed a 27% increase in fed glucose levels versus those of controls (Table 1). Development of this moderate hyperglycemia in male GLUT4$^{+/-}$ mice was prevented by introduction of the MLC-GLUT4 transgene in MLC-GLUT4$^{+/-}$ mice (Table 1). In fact, MLC-GLUT4$^{+/-}$ mice exhibited fed glucose levels that were 30% lower than those of GLUT4$^{+/-}$ mice (Table 1). GLUT4$^{+/-}$ mice exhibited a 3.2-fold increase in fed insulin levels when compared with controls (Table 1). Consistent with the fed glucose results, this hyperinsulinemia associated with the GLUT4$^{+/-}$ mutation was not observed in MLC-GLUT4$^{+/-}$ mice (Table 1). A large dispersion exists in individual fed glucose levels of GLUT4$^{+/-}$ mice. This resulted in a mean fed glucose value that was only slightly higher than that of controls. A more accurate representation of the hyperinsulinemia and hyperglycemia present in GLUT4$^{+/-}$ mice is shown in Fig. 2. Nearly one half of GLUT4$^{+/-}$ mice exhibited higher fed glucose levels than the controls, with a few GLUT4$^{+/-}$ mice showing severe hyperglycemia (≥350 mg/dl). Although 64% of GLUT4$^{+/-}$ mice had higher insulin levels than those of control mice, 27% exhibited extreme hyperinsulinemia (≥150 ng/ml), with values six times higher than the average for controls (Fig. 2). The efficacy with which the MLC-GLUT4 transgene prevented the onset of hyperglycemia and hyperinsulinemia associated with the GLUT4$^{+/-}$ lesion is also illustrated in Fig. 2. All MLC-GLUT4$^{+/-}$ mice exhibited fed glucose and insulin levels within or below the control range.

Fed plasma lactate levels were similar among GLUT4$^{+/-}$, MLC-GLUT4$^{+/-}$, and control mice (Table 1). GLUT4$^{+/-}$ mice exhibited a 34% decrease in fed FFA levels compared with controls (Table 1). Similar to GLUT4$^{+/-}$ mice, MLC-GLUT4$^{+/-}$ had fed FFA levels that were 25% lower than those of controls (Table 1). MLC-GLUT4$^{+/-}$ mice behave more like GLUT4$^{+/-}$ mice when fed FFA levels are considered, as opposed to fed glucose and insulin levels. The restoration of muscle GLUT4 content and glucose uptake in MLC-GLUT4$^{+/-}$ mice was contrasted with lack of normalization in WAT GLUT4 content and plasma FFA levels.

Euglycemic-hyperinsulinemic clamp. The return of fed insulin and glucose to control levels indicated the restoration of normal glucose homeostasis in MLC-GLUT4$^{+/-}$ mice. To determine whether establishment of normal glucose homeostasis in MLC-GLUT4$^{+/-}$ mice was associated with an improvement in whole-body insulin action over that of GLUT4$^{+/-}$ mice, euglycemic-hyperinsulinemic clamp studies were performed on male mice. The mice studied had similar body weights (Table 2). All three groups of mice were main-

tained at plasma glucose concentrations of ~100 mg/dl and at the same level of hyperinsulinemia (Table 2). Steady-state glucose utilization rates of GLUT4$^{+/-}$ mice were decreased by 56% compared with controls (Fig. 3*A*). In GLUT4$^{+/-}$ mice, the exogenous glucose infusion rate required to maintain constant plasma glucose during the euglycemic clamp was similarly decreased by 64% (Fig. 3*B*). With the introduction of the MLC-GLUT4 transgene and restoration of 2-DOG uptake in fast-twitch skeletal muscle (Fig. 1*D*), glucose utilization rate and glucose infusion rate of MLC-GLUT4$^{+/-}$ mice returned to control levels (Fig. 3*A* and *B*). Endogenous glucose production rates during euglycemic-hyperinsulinemic clamps were similar in all groups (Fig. 3*C*).

To understand the metabolic basis responsible for decreased insulin-stimulated glucose utilization in GLUT4$^{+/-}$ mice and restoration of glucose utilization in MLC-GLUT4$^{+/-}$ mice, we performed whole-body assessment of glucose fluxes into glycolysis and glycogen synthesis during euglycemic clamps (Fig. 4*A* and *B*). In GLUT4$^{+/-}$ mice, both the rate of glucose fluxes into glycolysis and the rate of glycogen synthesis were decreased by 55% compared with controls (Fig. 4*A* and *B*). The extent of reduction in the glucose utilization rate in GLUT4$^{+/-}$ mice (56%) matched the extent of reduction in glycolysis and glycogen synthesis (Figs. 3*A* and 4*A* and *B*). In accordance with the glucose utilization and glucose infusion rates, both the glycolysis and glycogen synthesis rates in MLC-GLUT4$^{+/-}$ mice returned to control levels (Fig. 4*A* and *B*).

Heart histopathology and GLUT4 expression. Because cardiomyopathy is a major complication of type 2 diabetes (28), hearts from control, GLUT4$^{+/-}$, and MLC-GLUT4$^{+/-}$ mice were examined for possible histopathologic features. Four of eight GLUT4$^{+/-}$ hearts examined exhibited significant abnormalities similar to those previously noted in GLUT4$^{+/-}$ mice (19). The observed histopathologic conditions associated with GLUT4$^{+/-}$ hearts included cardiomyocytic hypertrophy, focal interstitial and replacement fibrosis, and interstitial inflammation. In contrast, of the eight MLC-GLUT4$^{+/-}$ and eight control hearts, only one from each group displayed focal interstitial fibrosis.

The high frequency of histopathology in GLUT4$^{+/-}$ hearts and the low incidence in MLC-GLUT4$^{+/-}$ hearts was significantly correlated with differences in glucose ($P < 0.05$) and insulin ($P < 0.02$) levels between the two groups (data not shown). When MLC-GLUT4$^{+/-}$ hearts were compared with control hearts for GLUT4 protein expression, a modest 25% reduction was observed (Fig. 5). We reported previously that GLUT4 protein levels were normal in GLUT4$^{+/-}$ hearts (19). The incidence of heart histopathologic conditions is correlated with high glucose and insulin levels, and not with the heart's expression of GLUT4 protein. These results indicate that cardiomyopathy in GLUT4$^{+/-}$ mice is due to insulin resistance, not to small variations in heart GLUT4 protein levels.

DISCUSSION

Alterations in skeletal muscle GLUT4 expression and/or function have been implicated in insulin-resistant states (12,29,30). However, it remains to be elucidated whether these alterations can cause abnormal glucose homeostasis and are not simply the consequence of insulin resistance. To address this, we disrupted the murine GLUT4 gene (21) and demonstrated that male mice heterozygous for the GLUT4 disruption in a CD1 background develop insulin resistance and hyperglycemia (19). A transgenic complementation strategy was employed to reintroduce GLUT4 to fast-twitch muscle of GLUT4$^{+/-}$ mice by standard genetic crossing. The resulting MLC-GLUT4$^{+/-}$ mice exhibited normal levels of immunoreactive GLUT4 and glucose uptake in fast-twitch EDL muscle. Results of euglycemic hyperinsulinemic clamp studies indicated that the rates of insulin-stimulated whole body glucose utilization were restored to normal levels in MLC-GLUT4$^{+/-}$

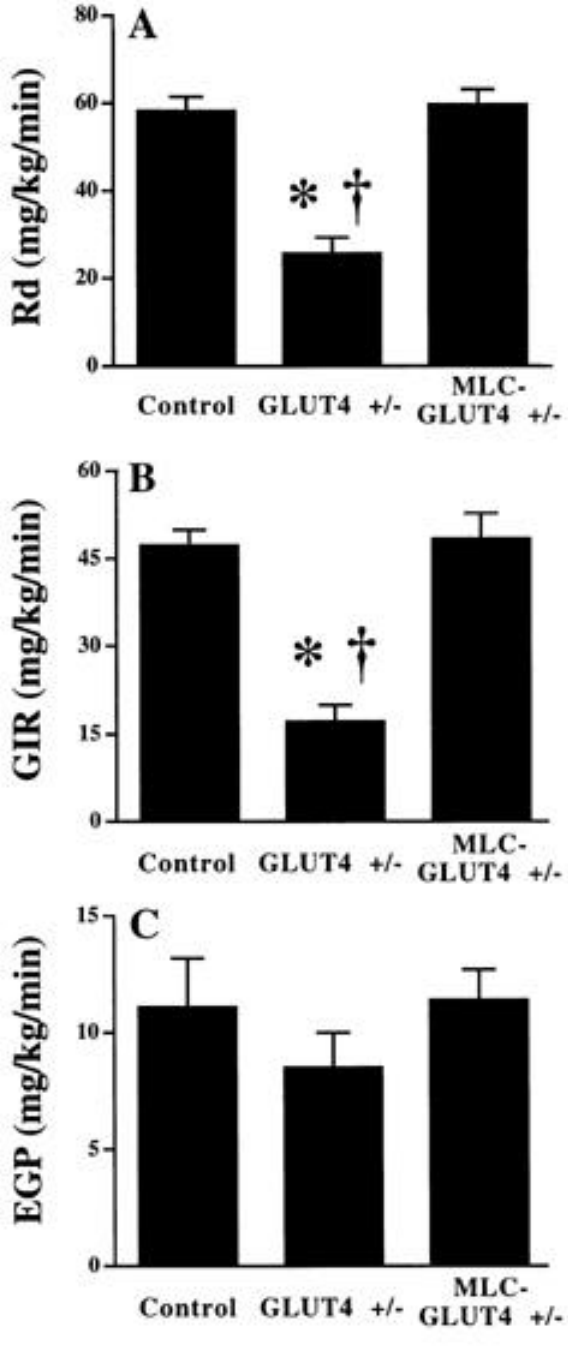

FIG. 3. Whole-body glucose utilization rate (*A*), glucose infusion rate (GIR) (*B*), and endogenous glucose production (EGP) (*C*) rates during euglycemic-hyperinsulinemic clamps. Clamp studies included 6 GLUT4$^{+/-}$ and 9 each of control and MLC-GLUT4$^{+/-}$ mice. Results for GLUT4$^{+/-}$ mice were previously presented (20). Comparisons were made using ANOVA and Fisher's PLSD post hoc analysis. Statistical significance is indicated: *$P < 0.0001$ vs. MLC-GLUT4$^{+/-}$ mice; †$P < 0.0001$ vs. control mice.

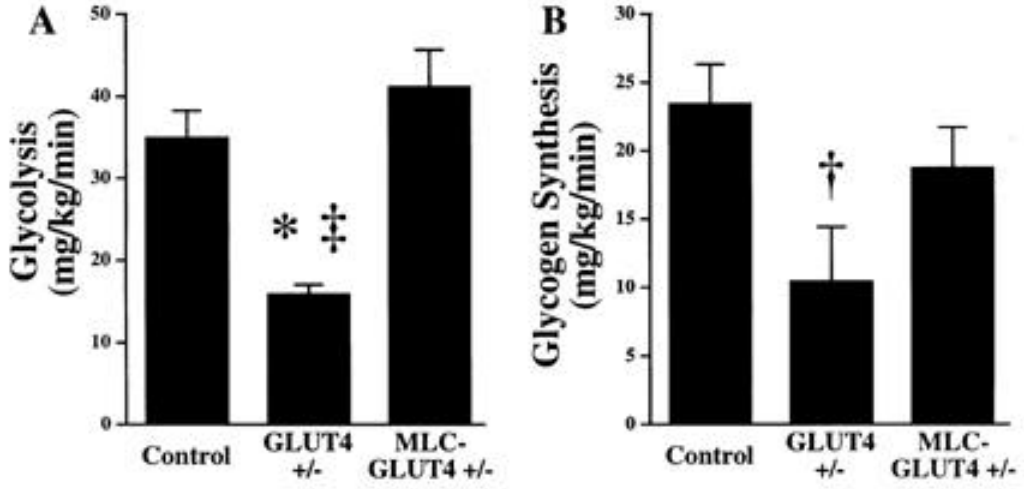

FIG. 4. Rates of glucose fluxes into glycolysis (*A*) and glycogen synthesis (*B*) pathways in whole body during euglycemic-hyperinsulinemic clamps. Clamp studies included 6 GLUT4$^{+/-}$ and 9 each of control and MLC-GLUT4$^{+/-}$ mice. Results for GLUT4$^{+/-}$ mice were previously presented (20). Comparisons were made using ANOVA. Statistical significance is indicated: *P < 0.0001 vs. MLC-GLUT4$^{+/-}$ mice; †P < 0.03, ‡P < 0.005 vs. controls.

mice. In addition, MLC-GLUT4$^{+/-}$ mice displayed normal fed glucose and insulin levels, which were associated with amelioration of histopathologic heart conditions.

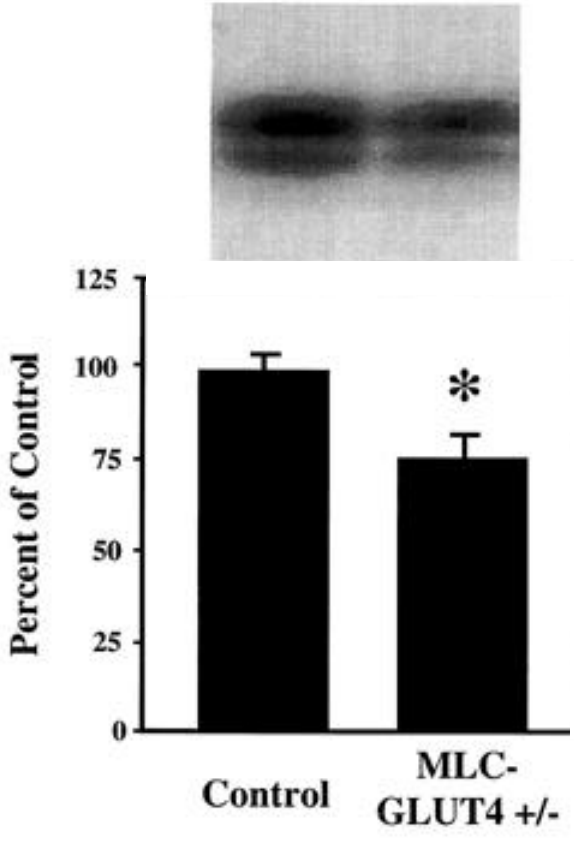

FIG. 5. Immunoblot analysis of GLUT4 was performed in hearts from control and MLC-GLUT4$^{+/-}$ mice. Heart homogenates were prepared as described in METHODS. Immunoblot analysis was performed using a GLUT4 anti–COOH-terminal antibody (control, n = 4; MLC-GLUT4$^{+/-}$, n = 8). Laser scanning densitometry was conducted to quantitate the differences. Densitometric units of the control group were set to 100%. Comparisons were made using an unpaired, two-tailed, Student's t test. *Statistical significance is indicated: P < 0.05 vs. controls.

The alteration of normal glucose homeostasis in type 2 diabetes is often associated with impaired insulin-stimulated glucose utilization in skeletal muscle (1,5,31,32). Whether a defect in insulin-stimulated glucose utilization in skeletal muscle can result in hyperglycemia in type 2 diabetes remains unknown. Our previous results in GLUT4$^{+/-}$ mice associate decreased GLUT4 expression with impaired insulin-stimulated muscle glucose uptake, decreased whole-body glucose utilization, hyperglycemia, and hyperinsulinemia (19,20). The present study shows that introduction of GLUT4 to fast-twitch muscles of GLUT4$^{+/-}$ mice normalizes glucose uptake in EDL and whole-body glucose utilization under insulin action. As a result, development of fed hyperglycemia and hyperinsulinemia was prevented in MLC-GLUT4$^{+/-}$ mice. Collectively, the results suggest that decreased GLUT4 expression and skeletal muscle glucose uptake was the initial cause responsible for the onset of whole-body insulin resistance, hyperinsulinemia, and eventual hyperglycemia in GLUT4$^{+/-}$ mice. However, because we have used a strategy of stable transgene expression in this study, it cannot be ruled out that the observations in MLC-GLUT4$^{+/-}$ mice were due to a gain of function rather than to true complementation of a loss of function. Therefore, it is still possible that altered glucose homeostasis reported in the GLUT4$^{+/-}$ mice was caused by decreased GLUT4 expression in WAT of GLUT4$^{+/-}$ mice. We and others have shown that the level of GLUT4 expression in WAT may affect both local and global lipid metabolism (22,33). It has been proposed that abnormal lipid metabolism may play a significant role in the development of altered glucose homeostasis in type 2 diabetes (34). In addition, WAT has recently emerged as an important endocrine tissue that regulates energy balance and coordination of glucose and lipid metabolism (35).

In this study, we demonstrated that targeted expression of the MLC-GLUT4 transgene in skeletal muscle provided sufficient protection from the development of aberrant glucose homeostasis in GLUT4$^{+/-}$ mice. This result showed that if decreased insulin-stimulated skeletal muscle glucose uptake can be prevented, it is possible to avoid the onset of insulin resistance, hyperglycemia, and hyperinsulinemia. This finding may have implications for the prevention and treatment of type 2 diabetes. During the development of type 2 diabetes,

peripheral insulin resistance often precedes overt hyperglycemia (32,36). It was also shown previously that the initial onset of fasting hyperglycemia is correlated with decreased glucose clearance and not increased hepatic glucose production (1). Our results suggest that if the defect in skeletal muscle glucose uptake can be diagnosed early and treated, it may be possible to prevent the onset of hyperglycemia and the associated vascular complications. This study also suggests that a gene-based therapy involving GLUT4 may correct impaired skeletal muscle glucose uptake (15,16,18,37,38). The basis of impaired muscle glucose uptake in type 2 diabetic patients is defective GLUT4 trafficking, resulting in decreased GLUT4 content at the plasma membrane, even though total GLUT4 content in all cellular compartments remains normal (39,40). GLUT4 overexpression may not correct the cellular defects responsible for impaired GLUT4 trafficking, but it may normalize GLUT4 content at the plasma membrane because of an overwhelming increase in transporters at intracellular locations. It remains to be determined whether it is possible to reverse impaired skeletal muscle glucose uptake and hyperglycemia after type 2 diabetes has fully developed by restoring normal or above-normal GLUT4 expression to skeletal muscle. In addition, because central nervous system and non–insulin-sensitive tissues are responsible for a large proportion of postabsorptive glucose utilization in humans (41,42), the effectiveness of muscle-based GLUT4 gene therapy in humans remains to be tested.

In contrast to the GLUT4$^{+/-}$ mice, MLC-GLUT4$^{+/-}$ and control mice exhibited similar whole-body glucose disposal rates, muscle glucose uptake rates, and plasma glucose and insulin levels. However, as in GLUT4$^{+/-}$ mice, plasma FFA levels were decreased in MLC-GLUT4$^{+/-}$ mice compared with controls. These results are similar to those from our previous study of GLUT4^{null} mice expressing the MLC-GLUT4 transgene (MLC-GLUT4^{null} mice) (22). Impaired insulin action and glucose homeostasis in GLUT4^{null} mice was prevented by the expression of MLC-GLUT4 transgene in MLC-GLUT4^{null} mice. In contrast to controls, MLC-GLUT4^{null} mice exhibited decreased plasma FFA levels and WAT mass similar to that of GLUT4^{null} mice. Collectively, the results of the present and the previous studies indicate that whereas MLC-GLUT4 transgene expression can restore normal insulin action and glucose homeostasis, it cannot correct defects in FFA homeostasis.

An issue currently under debate is whether glucose transport or other intracellular defects, most notably glucose phosphorylation and glycogen synthesis, are responsible for impaired skeletal muscle glucose utilization in type 2 diabetes (2,31). Under euglycemic-hyperinsulinemic clamp conditions, the reduced whole-body glucose utilization in GLUT4$^{+/-}$ mice could be attributed to decreases in glycolysis and glycogen synthesis. Given that both glycolysis and glycogen synthesis were reduced in GLUT4$^{+/-}$ mice, the observed defect in skeletal muscle glucose transport was most likely responsible for decreased glucose utilization. Even though the concentration of 2-DOG selected for glucose uptake experiments (0.1 mmol/l) is rate-limiting for transport (43), it cannot be ruled out that impaired glucose phosphorylation may play a role in GLUT4$^{+/-}$ muscle. Impaired glucose phosphorylation has been shown to contribute to decreased glucose utilization in type 2 diabetes (31). In MLC-GLUT4$^{+/-}$ mice, normalization of whole-body glucose utilization was accompanied by the return of both glycolysis and glycogen synthesis rates to control levels. Similarly, restoration of fast-twitch skeletal muscle glucose transport to control levels was most likely responsible for the normalization of glycolysis and glycogen synthesis in MLC-GLUT4$^{+/-}$ mice. These results do not rule out the possibility that a defect in glycogen synthesis can result in impaired glucose utilization in skeletal muscle. However, they do indicate that glucose fluxes into both glycolysis and glycogen synthesis pathways are highly dependent on glucose transport rates.

In conclusion, we have demonstrated that transgenic expression of GLUT4 in fast-twitch skeletal muscle of GLUT4$^{+/-}$ mice can prevent the development of impaired skeletal muscle insulin-stimulated glucose transport and whole-body glucose utilization, thereby avoiding the onset of hyperglycemia, hyperinsulinemia, and histopathologic heart conditions. These results emphasize the decisive role of GLUT4-mediated skeletal muscle glucose uptake in the determination of insulin-stimulated whole-body glucose utilization. Based on these results, we propose that the prevention of impaired skeletal muscle glucose uptake using muscle GLUT4 gene therapy in individuals genetically prone to type 2 diabetes may prevent whole-body glucose utilization defects and cardiovascular complications.

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