

Muscle-specific Transgenic Complementation of GLUT4-deficient Mice

Effects on Glucose But Not Lipid Metabolism

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Abstract

We have taken the approach of introducing the muscle-specific myosin light chain (MLC)-GLUT4 transgene into the GLUT4-null background to assess the relative role of muscle and adipose tissue GLUT4 in the etiology of the GLUT4-null phenotype. The resulting MLC-GLUT4-null mice express GLUT4 predominantly in the fast-twitch extensor digitorum longus (EDL) muscle. GLUT4 is nearly absent in female white adipose tissue (WAT) and slow-twitch soleus muscle of both sexes of MLC-GLUT4-null mice. GLUT4 content in male MLC-GLUT4-null WAT is 20% of that in control mice. In transgenically complemented EDL muscle, 2-deoxyglucose (2-DOG) uptake was restored to normal (male) or above normal (female) levels. In contrast, 2-DOG uptake in slow-twitch soleus muscle of MLC-GLUT4-null mice was not normalized. With the normalization of glucose uptake in fast-twitch skeletal muscle, whole body insulin action was restored in MLC-GLUT4-null mice, as shown by the results of the insulin tolerance test. These results demonstrate that skeletal muscle GLUT4 is a major regulator of skeletal muscle and whole body glucose metabolism. Despite normal skeletal muscle glucose uptake and insulin action, the MLC-GLUT4-null mice exhibited decreased adipose tissue deposits, adipocyte size, and fed plasma FFA levels that are characteristic of GLUT4-null mice. Together these results indicate that the defects in skeletal muscle and whole body glucose metabolism and adipose tissue in GLUT4-null mice arise independently. (*J. Clin. Invest.* 1997. 100:671–677.) Key words: GLUT4 • transgenic mice • knockout mice • glucose metabolism • skeletal muscle

Introduction

GLUT4, the insulin-sensitive glucose transporter, is expressed mainly in heart, skeletal muscle, and adipose tissue, and is redistributed from intracellular stores to the plasma membrane in response to insulin, ischemia, or exercise (1–4). The occurrence of insulin resistance during diabetes and obesity is ac-

companied by decreased GLUT4 expression in the adipose tissue (5). However, this suppression of GLUT4 expression in insulin-resistant states is not seen in the skeletal muscle despite decreased insulin-stimulated glucose uptake (6–9). Rather, the decrease in insulin-stimulated glucose uptake in skeletal muscle is attributed to defects in GLUT4 translocation (6, 10, 11). To examine the significance of the impairment in the expression or the translocation of GLUT4 under insulin-resistant states, we disrupted the GLUT4 gene in mice (GLUT4-null mice) and demonstrated impaired insulin tolerance (12). The disruption of the GLUT4 gene resulted in severely reduced glucose uptake and glycogen synthesis in white fast-twitch skeletal muscle (i.e., extensor digitorum longus [EDL])¹ under insulin-stimulated conditions (13). In contrast, red slow-twitch skeletal muscle (i.e., soleus) could partially compensate for GLUT4 ablation (13). Since fast-twitch muscles are the prevailing muscle type in mice (14), the severely reduced insulin-stimulated glucose uptake and glycogen synthesis leads to a decrease in whole body insulin action. In addition to impaired skeletal muscle glucose metabolism and whole body insulin action, lipid metabolism is also affected by GLUT4 ablation (12). The abnormal lipid metabolism includes decreased plasma FFA levels and a striking reduction in the adipose tissue deposits of GLUT4-null mice.

The first step toward an understanding of how GLUT4 ablation gave rise to altered whole body glucose and lipid metabolism is to delineate the specific contribution of skeletal muscle and adipose tissue GLUT4 to the overall GLUT4-null phenotype. Such analysis may provide further insight into the role of GLUT4 in the regulation of glucose and lipid metabolism under normal and insulin-resistant states. However, the absence of GLUT4 in both skeletal muscle and adipose tissue complicates the analysis of the contribution of GLUT4 in specific tissues to the overall phenotype of the GLUT4-null mice. To circumvent the problems posed by multiple GLUT4 lesion sites, we have complemented the GLUT4-null mice with a transgene that targets GLUT4 expression predominantly to the skeletal muscle (15). The complementation was carried out by mating GLUT4-null mice with the MLC-GLUT4 mice, a line of mice which carries a GLUT4 transgene driven by the myosin light chain (MLC) 1 promoter (15). The MLC-GLUT4 transgenic mice exhibit increased GLUT4 in fast-twitch skeletal muscle, accompanied by an increase in insulin-stimulated glucose uptake in fast-twitch muscle, and an increase in whole body glucose utilization. The MLC-GLUT4-null mice express GLUT4 predominantly in muscle, allowing for the dissection of the individual contributions of skeletal muscle GLUT4 and

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1. Abbreviations used in this paper: 2-DOG, 2-deoxyglucose; EDL, extensor digitorum longus; MLC, myosin light chain; WAT, white adipose tissue.

adipose tissue GLUT4 to the overall phenotype of the GLUT4-null mice.

The MLC-GLUT4-null mice exhibit characteristics of both parent lines. Introduction of the GLUT4 transgene in the MLC-GLUT4-null mice has restored glucose transport *in vitro* in EDL muscle, whole body insulin action, and overall lactate level to that of the control mice. However, the MLC-GLUT4-null mice have overall fat mass, adipocyte size, FFA levels, and reduced glucose transport in soleus muscle similar to that of GLUT4-null mice. These results indicate that (a) GLUT4 expression in muscle primarily affects muscle glucose metabolism and whole body glucose homeostasis; and (b) adipose tissue deposits and circulating FFA levels are strongly affected by GLUT4 expression in adipose tissue. Furthermore, the MLC-GLUT4-null mice demonstrate the utility of transgenic complementation as an alternative to tissue-specific gene knockout strategies for dissecting complex phenotypes and tissue-specific gene functions.

Methods

Animals. The MLC-GLUT4-null mice were obtained by crossing the GLUT4-null mice to the MLC-GLUT4 mice. The GLUT4-null mice were originally generated in mixed genetic background (12). They were transferred to the C57Bl6/CBA, the genetic background of MLC-GLUT4 mice, by successive matings to obtain the MLC-GLUT4-null mice. The GLUT4 locus segregates independently from the MLC-GLUT4 transgene locus. The MLC-GLUT4-null mice were genotyped for MLC-GLUT4 transgene and GLUT4 gene disruption as previously described (12, 15). Mice between 10 and 20 wk old were used in this study. Animals were fed *ad libitum* and maintained in a murine hepatitis virus-free barrier facility on a 14-h light and 10-h 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.

Western blot analysis. Tissues were homogenized in TES buffer (100 mM Tris, pH 7.6, 0.2 mM EDTA, and 255 mM sucrose), and the amount of protein in the homogenate determined by BCA assay (Pierce Chemical Co., Inc., Rockford, IL) with BSA as the standard. Equivalent amounts of protein (50 μ g) from the homogenates were resolved on a 10% SDS-PAGE and electrophoretically transferred onto a supported nitrocellulose membrane (Schleicher & Schuell, Inc., Keene, NH) at 4°C overnight. The filters were stained with Ponceau S (Sigma Chemical Co., St. Louis, MO) to ensure equal loading and transfer. A rabbit polyclonal antiserum (1:1,000 dilution) raised against a peptide corresponding to the GLUT4 carboxy terminus (16, 17) and subsequently a goat anti-rabbit secondary antiserum (1:2,500 dilution) conjugated to horseradish peroxidase (Southern Biotechnology Associates, Inc., Birmingham, AL) were used to detect GLUT4 protein using the ECL reagent (Amersham Corp., Arlington Heights, IL). Filters were exposed to KODAK XAR film (Eastman Kodak Co., Rochester, NY) and subjected to laser scanning densitometry (Molecular Dynamics, Sunnyvale, CA) to quantitate the results.

Isolation of muscles and 2-deoxyglucose (2-DOG) uptake. After cervical dislocation, soleus and EDL muscles were rapidly isolated from the hindlimbs. Muscles from an individual mouse were randomly divided such that one was used for basal and the other for insulin-stimulated 2-DOG uptake. The distal tendon of each muscle was tied to a string to facilitate transfer among different media. All incubation media were prepared from oxygenated (95% O₂; 5% CO₂) Krebs-Ringer bicarbonate buffer (pH 7.4) supplemented with 0.1% BSA (fraction V, RIA grade; Sigma Chemical Co.) and 2 mM sodium pyruvate (Sigma Chemical Co.). The gas phase was maintained throughout all incubations. Muscles were incubated for 1 h (30°C) in the absence or presence of 20 nM porcine insulin (Eli Lilly and Co.,

Indianapolis, IN). Glucose transport was assessed after a 20-min incubation at 30°C using 2-DOG as previously described (18, 19). 2-DOG uptake has been reported to directly reflect glucose transport and not metabolism in mouse muscle when used under these conditions (20).

Insulin tolerance test. After a 6-h fast, porcine insulin was injected intraperitoneally (0.75 U/kg body wt), and blood was withdrawn from the retroorbital sinus at 0, 15, 30, and 60 min after insulin injection. Blood glucose was measured using the One Touch II glucose meter system (Lifescan, Inc., Milpitas, CA).

Adipose cell size and number. Adipocytes were isolated by collagenase (Worthington Biochemical Corp., Freehold, NJ) treatment as described previously (21, 22). Isolated adipocytes were fixed with osmium tetroxide (Stevens Metallurgical Corp., New York) and manually counted using a hemocytometer. Isolated adipocyte size was determined as previously described (23, 24).

Analysis of serum metabolites and hormones. Blood was drawn from the retroorbital sinus using a heparinized microcapillary tube and quickly spun to obtain plasma between 12:00 and 2:00 a.m. in the fed state. Plasma insulin levels were measured using a rat insulin kit (Linco Research, Inc., St. Louis, MO). Plasma glucose, lactate, and β -hydroxybutyrate were measured using kits from Sigma Chemical Co. Plasma FFA was assessed using a kit from Amano Enzyme USA Co. (Troy, VA), with oleic acid as the standard.

Statistical analysis. Data are presented as mean \pm SE. Statistical comparisons of the results of the insulin tolerance test were determined by ANOVA for repeated measurements, with Scheffé's *F* procedure for post-hoc analysis. All other statistical analysis was performed by unpaired, two-tailed, Student's *t* test.

Results

GLUT4 content and 2-DOG uptake in isolated muscles. Western blot analysis was performed to assess the amount of immunoreactive GLUT4 in the isolated soleus and EDL muscles from 10- to 14-wk-old GLUT4-null, MLC-GLUT4-null, and control mice. The EDL is representative of skeletal muscle that consists primarily of fast-twitch fibers, the predominant fiber type of skeletal muscle (25, 26). In contrast, the soleus contains a high proportion of slow-twitch fibers (25, 26). No immunoreactive GLUT4 was detected in the EDL or the soleus muscles of GLUT4-null mice. In female MLC-GLUT4-null mice, there was a tendency for GLUT4 content in EDL to be increased over control mice (Fig. 1 A). The amount of immunoreactive GLUT4 in the EDL of male MLC-GLUT4-null mice was 2.6-fold greater than that observed in control mice (Fig. 2 A). The soleus muscle of both female and male MLC-GLUT4-null mice exhibited negligible (< 2%) GLUT4 levels as compared with controls (Figs. 1 A and Fig. 2 A). These results are consistent with our previous findings that the MLC-GLUT4 transgene is selectively expressed in fast-twitch and not slow-twitch muscles (15). In the MLC-GLUT4-null mice, GLUT4 was restored selectively in the EDL muscle, which represents the predominant fiber type composition of the majority of the muscle mass (25, 26).

To determine the consequences of adding the MLC-GLUT4 transgene on glucose transport properties of individual skeletal muscles, 2-DOG uptake was measured in isolated soleus and EDL muscles of 10–14-wk-old MLC-GLUT4-null, GLUT4-null, and control mice. In MLC-GLUT4-null mice, restoration of GLUT4 to EDL muscle increased insulin-stimulated 2-DOG uptake by 54% in females and 75% in males when compared with control muscles, and by 316% in females and 429% in males when compared with GLUT4-null mice (Figs. 1 B and Fig. 2 B). Under basal condition, 2-DOG uptake

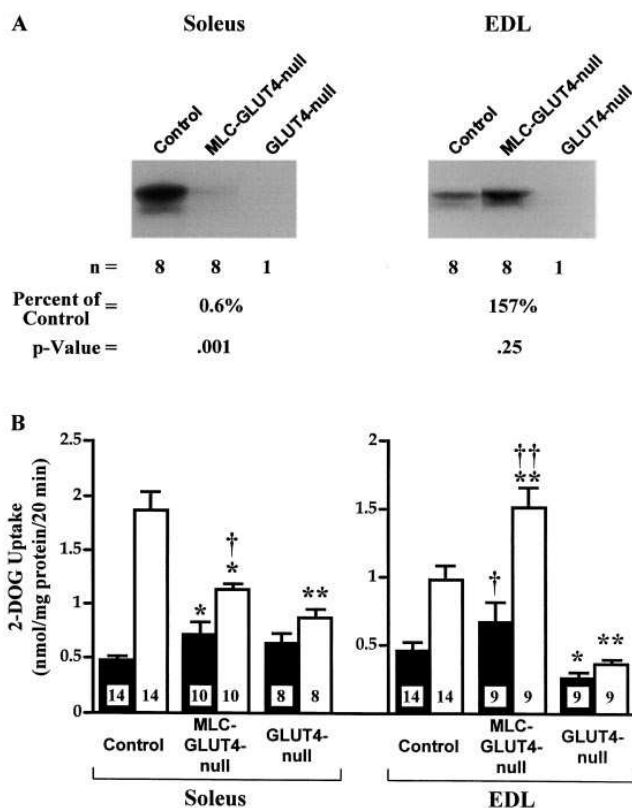


Figure 1. (A) Immunoblot analysis of GLUT4 in isolated soleus and EDL muscles from female control, MLC-GLUT4-null, and GLUT4-null mice. Sonicated soleus and EDL homogenates were prepared. Immunoblot analysis was performed using a GLUT4 anti-carboxy-terminal antibody as described in Methods. Laser scanning densitometry was conducted to quantitate differences between muscles from control and MLC-GLUT4-null mice. Comparisons were made between MLC-GLUT4-null and control mice using unpaired, two-tailed, Student's *t* test. (B) Basal and insulin-stimulated 2-DOG uptake in isolated soleus and EDL muscles from female control, MLC-GLUT4-null, and GLUT4-null mice. Soleus and EDL muscles were isolated and incubated for 60 min in the absence (*open bars*) and presence (*solid bars*) of 20 nM insulin. Uptake was carried out with 0.1 mM 2-DOG (0.5 μ Ci/ml 2,6-[3 H]-2-DOG) for 20 min as described in Methods. Values are means \pm SEM. The number of observations made for each group is indicated inside the bars. Comparisons were made between each group under basal or insulin-stimulated conditions using unpaired, two-tailed, Student's *t* test. Statistical significance is noted at **P* < 0.05 and ***P* < 0.0005, between either MLC-GLUT4-null or GLUT4-null and control muscles; and at †*P* < 0.05, and ††*P* < 0.0005, between MLC-GLUT4-null and GLUT4-null muscles.

in male MLC-GLUT4-null EDL muscles increased by 110% over control muscles (Fig. 2 B). There was no statistically significant increase in basal 2-DOG uptake in female MLC-GLUT4-null EDL muscles (Fig. 1 B). In GLUT4-null mice, basal 2-DOG uptake decreased by 78% in females and 55% in males when compared with control mice (Fig. 1 B and Fig. 2 B). Insulin had little effect in stimulating 2-DOG uptake in the GLUT4-null mice (Fig. 1 B and Fig. 2 B). In the soleus muscles of both female and male MLC-GLUT4-null mice, where GLUT4 is nearly absent, insulin-stimulated 2-DOG uptake decreased by 40 and 50%, respectively, when compared with

control (Fig. 1 B and Fig. 2 B). There was no difference in basal 2-DOG uptake between male MLC-GLUT4-null and control mice (Fig. 2 B). Basal 2-DOG uptake in female MLC-GLUT4-null soleus increased by 50% when compared with control soleus (Fig. 1 B). In the normal soleus muscles, insulin increased 2-DOG uptake by 291% in female and 165% in male mice (Fig. 1 B and Fig. 2 B). In contrast, insulin enhanced 2-DOG uptake 58% in female MLC-GLUT4-null soleus muscle and 21% in male MLC-GLUT4-null soleus muscle (Fig. 1 B and Fig. 2 B). In contrast with male MLC-GLUT4-null soleus, GLUT4-null soleus exhibited a 57% increase in basal

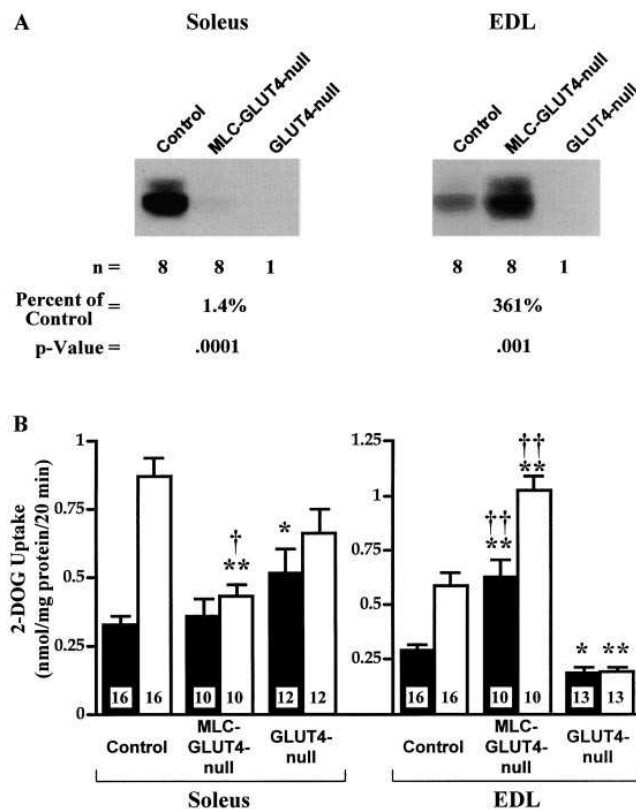


Figure 2. (A) Immunoblot analysis of GLUT4 in isolated soleus and EDL muscles from male control, MLC-GLUT4-null, and GLUT4-null mice. Sonicated soleus and EDL homogenates were prepared. Immunoblot analysis was performed using a GLUT4 anti-carboxy-terminal antibody as described in Methods. Laser scanning densitometry was conducted to quantitate differences between muscles from control and MLC-GLUT4-null mice. Comparisons were made between MLC-GLUT4-null and control mice using unpaired, two-tailed, Student's *t* test. (B) Basal and insulin-stimulated 2-DOG uptake in isolated soleus and EDL muscles from male control, MLC-GLUT4-null, and GLUT4-null mice. Soleus and EDL muscles were isolated and incubated for 60 min in the absence (*open bars*) and presence (*solid bars*) of 20 nM insulin. Uptake was carried out with 0.1 mM 2-DOG (0.5 μ Ci/ml 2,6-[3 H]-2-DOG) for 20 min as described in Methods. Values are means \pm SEM. The number of observations made for each group is indicated inside the bars. Comparisons were made between each group under basal or insulin-stimulated conditions using unpaired, two-tailed, Student's *t* test. Statistical significance is noted at **P* < 0.05, and ***P* < 0.0005, between either MLC-GLUT4-null or GLUT4-null and control muscles; and at †*P* < 0.05, and ††*P* < 0.0005, between MLC-GLUT4-null and GLUT4-null muscles.

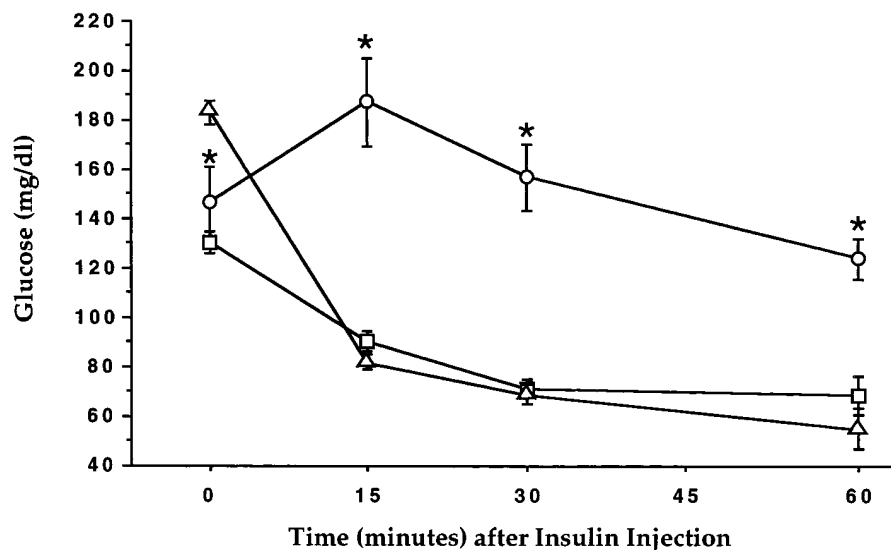


Figure 3. Insulin tolerance test. Porcine insulin (0.75 U/kg body wt) was injected intraperitoneally in conscious 10–14-wk-old female MLC-GLUT4-null (\square , $n = 6$), GLUT4-null (\circ , $n = 5$), and control (\triangle , $n = 6$) mice. Blood was withdrawn from retroorbital sinus at 0, 15, 30, and 60 min after injection, and blood glucose level was monitored by One Touch II glucose meter (Lifescan, Inc.). The glucose clearance curves are significantly different between MLC-GLUT4-null and GLUT4-null mice ($*P < 0.0001$), as well as between GLUT4-null and control mice ($*P < 0.0001$), as determined by ANOVA for repeated measures using Scheffé's F posthoc analysis.

2-DOG uptake over control soleus (Fig. 2B). The basal 2-DOG uptake activity observed in male GLUT4-null soleus was not significantly stimulated by insulin (Fig. 2B). In contrast, basal 2-DOG uptake in female GLUT4-null soleus was not significantly elevated when compared with control soleus (Fig. 1B). Insulin elicited a modest increase in 2-DOG uptake (38% over basal values) in female GLUT4-null soleus (Fig. 1B).

Whole body insulin action. Since skeletal muscle is the major site of insulin-stimulated glucose disposal (27–29), we assessed the impact of restored cellular glucose transport in fast-twitch muscle on whole body insulin action using an insulin tolerance test. The glucose levels in the female control mice decreased after intraperitoneal injection of insulin (Fig. 3). Unlike the control mice, the female GLUT4-null mice did not clear glucose in response to insulin (Fig. 3). In contrast, female

MLC-GLUT4-null mice were able to clear glucose in response to insulin as efficiently as female control mice (Fig. 3).

The effect of the restoration of GLUT4 in fast-twitch muscle of MLC-GLUT4-null mice on whole body insulin action was further assessed by measuring fed plasma glucose, lactate, and insulin levels (Table I). The male MLC-GLUT4-null mice exhibited an 18% decrease in plasma glucose when compared to control mice, and a 28% decrease when compared to GLUT4-null mice, while female MLC-GLUT4-null mice showed no significant changes in glucose levels. The three different groups of male mice exhibited a gradation in plasma lactate levels. Male GLUT4-null mice showed a 59% decrease when compared to control mice, while the MLC-GLUT4-null mice exhibited a 31% increase in lactate levels when compared to control mice. Overall, the male MLC-GLUT4-null mice displayed a 218% increase in fed plasma lactate levels when com-

Table I. Fed Plasma Metabolites and Insulin Levels, Body Weights, and Adipose Tissue Characteristics

	Female			Male		
	Control	MLC-GLUT4-null	GLUT4-null	Control	MLC-GLUT4-null	GLUT4-null
Glucose (mg/dl)	158.1 ± 4.8 (14)	160.5 ± 3.5 (25)	175.8 ± 9.7 (8)	193.7 ± 10.7 (24)	160.0 ± 7.7* [‡] (16)	222.1 ± 9.8 (22)
Insulin (ng/ml)	2.29 ± .65 (14)	3.07 ± 0.57 (24)	3.75 ± 1.10 (8)	16.10 ± 3.67 (24)	12.27 ± 2.03 (20)	14.89 ± 4.10 (26)
Lactate (mg/dl)	54.5 ± 4.0 (9)	50.5 ± 2.3 [‡] (25)	26.3 ± 2.9* (8)	67.7 ± 5.4 (21)	89.2 ± 9.6* [‡] (14)	28.3 ± 5.3* (15)
FFA (μeq/liter)	664 ± 55 (14)	476 ± 45* (25)	421 ± 44* (8)	641 ± 35 (21)	451 ± 38* (14)	375 ± 30* (17)
Body weight (g)	28.9 ± 0.9 (14)	24.3 ± 0.9* (15)	23.4 ± 1.0* (8)	40.3 ± 1.1 (16)	35.6 ± 1.0* [‡] (18)	30.7 ± 0.8* (12)
Fat pad weight (mg)	1340 ± 139 (14)	233 ± 55* (15)	126 ± 49* (8)	1711 ± 103 (16)	853 ± 94* [‡] (16)	359 ± 51* (12)
Adipocyte size (μg lipid/cell)	0.382 ± 0.103 (11)	0.119 ± 0.050 [§] (8)	0.165 ± 0.051 [§] (13)	0.586 ± 0.117 (8)	0.245 ± 0.067* (8)	0.235 ± 0.063* (10)

Data are means ± SE (n). *Statistical significance with $P < 0.05$ vs. control. [§]Statistical significance with $P < 0.06$ vs. control. [‡]Statistical significance with $P < 0.005$ between MLC-GLUT4-null and GLUT4-null mice.

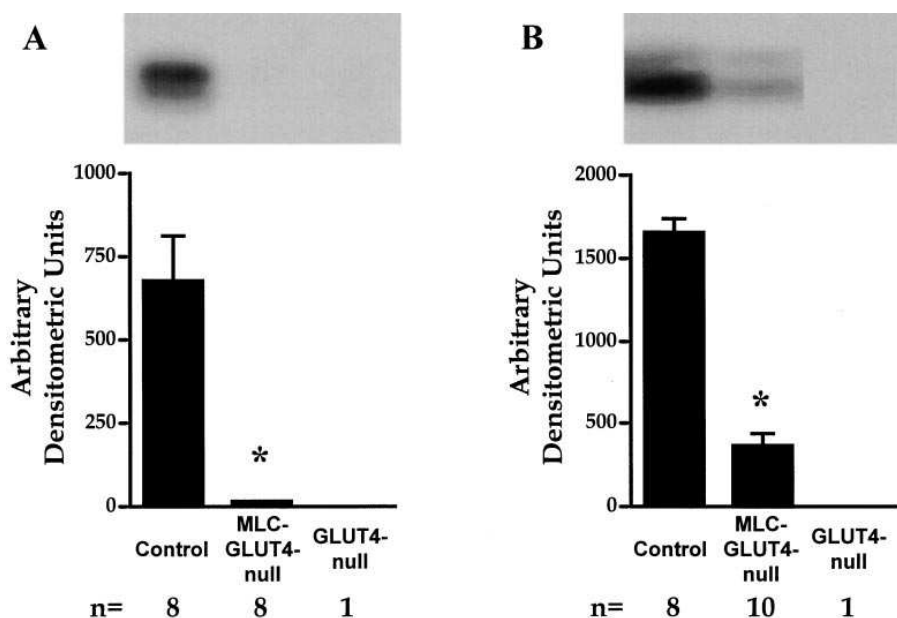


Figure 4. Immunoblot analysis of GLUT4 in adipose tissue of female (A) and male (B) MLC-GLUT4-null mice. Immunoblot analysis was performed as described in Methods. Comparisons were made between MLC-GLUT4-null and control mice using unpaired, two-tailed, Student's *t* test. Statistical significance is noted at **P* < 0.0005.

pared with GLUT4-null mice. When compared to both female MLC-GLUT4-null and control mice, the female GLUT4-null mice showed an ~50% decrease in fed plasma lactate levels. Female MLC-GLUT4-null mice exhibited the same fed plasma lactate levels as control. No significant changes in insulin levels were observed in the control, GLUT4-null, and MLC-GLUT4-null mice of either sex.

Fed plasma FFA levels, and characteristics of white adipose tissue (WAT). Immunoreactive GLUT4 levels in WAT were assessed by Western blot analysis (Fig. 4). In female MLC-GLUT4-null WAT, a band corresponding to GLUT4 can be observed upon prolonged exposure of the autoradiogram. The level of immunoreactive GLUT4 in female MLC-GLUT4-null WAT is estimated to be < 3% that of controls (Fig. 4A). Male MLC-GLUT4-null mice exhibited comparatively higher levels of GLUT4 immunoreactivity than female MLC-GLUT4-null mice. The level of immunoreactive GLUT4 in male MLC-GLUT4-null WAT is ~20% of controls (Fig. 4B).

GLUT4-null mice exhibited a decrease in fed plasma FFA levels (12). Female GLUT4-null mice FFA levels decreased by 37% when compared to controls under fed conditions (Table I). Similarly, MLC-GLUT4-null mice exhibited a 28% decrease in FFA levels compared to control mice (Table I). A similar pattern was observed for male GLUT4-null and MLC-GLUT4-null mice which exhibited decreased fed FFA levels by 41 and 30%, respectively, when compared to control mice (Table I).

A major manifestation of abnormal lipid metabolism in GLUT4-null mice is the decreased size of the gonadal adipose tissue deposits (12). Furthermore, upon visual inspection the size of the perirenal, intrascapular, and perimuscular adipose deposits was similarly decreased. Ovarian fat pad weight of female GLUT4-null mice was 10% that of control mice (Table I). Similarly, female MLC-GLUT4-null mice had ovarian fat pad weights 17% of control mice (Table I). The size of adipocytes from female GLUT4-null and MLC-GLUT4 null mice is decreased by 57 and 69%, respectively, when compared to control mice (Table I). Male GLUT4-null and MLC-GLUT4-

null mice had epididymal fat pad weights that were 21 and 50% that of control mice, respectively (Table I). In addition, the adipocyte cell size of male GLUT4-null and MLC-GLUT4-null mice was ~40% of control values (Table I). Both male and female GLUT4-null and MLC-GLUT4-null mice had lower body mass than the control mice (Table I).

Discussion

The GLUT4-null mice suffer from systemic defects in glucose and lipid metabolism (12). Alterations in glucose metabolism of GLUT4-null mice include decreased insulin-stimulated skeletal muscle glucose uptake, decreased fed plasma lactate levels, and impaired insulin tolerance (12, 13). The disruption in lipid metabolism in GLUT4-null mice is evidenced by decreased fed plasma FFA levels and adipose tissue weight. Since GLUT4 is absent from all tissues that normally express GLUT4, it is difficult to determine the respective contribution of the lack of GLUT4 in any specific tissue to these diverse defects.

The first aim of this study was to assess the effects of restored GLUT4 expression in fast-twitch muscle on glucose transport characteristics and whole body insulin action. The complementation of GLUT4 in fast-twitch EDL muscle restored insulin-stimulated glucose transport in that muscle. This emphasizes the central role of GLUT4 in the regulation of skeletal muscle glucose uptake and utilization. Restoration of insulin-stimulated glucose transport was concomitant with normalization of whole body insulin action, shown by the efficient clearance of glucose from the blood of MLC-GLUT4-null mice after an insulin injection. Results of the insulin tolerance test were supported by the fed plasma lactate levels, which returned to normal (female) and above normal (male) levels. Fed plasma lactate levels of MLC-GLUT4-null mice were consistent with a possible acceleration of glucose metabolism as a result of GLUT4 overexpression in fast-twitch skeletal muscle. Collectively, these data demonstrate the important contribution of skeletal muscle glucose metabolism to whole body insu-

lin action. In addition, defects in glucose metabolism associated with GLUT4 ablation can be largely corrected by complementation of GLUT4 in fast-twitch skeletal muscle. In contrast to previous results (12), we did not observe a significant increase in fed insulin levels in GLUT4-null mice. This result may be due to the genetic background in which the GLUT4 deletion resides (30). In this study, the GLUT4-null mutation was transferred from a predominantly CD1 background to a predominantly C57Bl6/CBA background similar to that used to carry the MLC-GLUT4 transgene (15). Since mice of C57Bl6/CBA background exhibited higher fed insulin levels than mice of CD1 background, it is possible that the penetrance and/or expression of fed hyperinsulinemia associated with the GLUT4 knockout is reduced in the C57Bl6/CBA background.

Previously, we demonstrated that insulin-stimulated 2-DOG uptake in the soleus of female GLUT4-null mice decreased relative to controls (13). This observation was repeated here, albeit the magnitude of the increase in insulin-stimulated 2-DOG uptake was lower in this genetic background. The small difference between these results is attributed to the small increases in insulin-stimulated 2-DOG uptake in the controls, and basal 2-DOG uptake in the GLUT4-nulls. Interestingly, female MLC-GLUT4-null soleus retained the insulin-stimulatable glucose uptake activity observed in GLUT4-null soleus. This suggests that the compensatory response seen in female GLUT4-null soleus does not depend upon relative hyperinsulinemia, since glucose metabolism was restored in MLC-GLUT4-null mice. We reported previously that male GLUT4-null soleus exhibited increased basal 2-DOG uptake which could not be further stimulated by insulin (13). Here, we confirm that basal 2-DOG uptake was increased in male GLUT4-null soleus. In contrast with previous work, we observed a tendency to further increase 2-DOG uptake upon insulin stimulation of GLUT4-null soleus. Strikingly, male MLC-GLUT4-null soleus lacks the compensatory increase in basal 2-DOG uptake seen in male GLUT4-null soleus. It is likely that the compensatory mechanism in male GLUT4-null soleus is responding to the altered metabolic milieu of GLUT4-null mice.

The second aim of this study was to assess if adipose tissue abnormality in GLUT4-null mice is independent of the altered global metabolic milieu. It has been shown that transgenic overexpression of GLUT4 in adipose tissue resulted in increased glucose transport and triglyceride synthesis in adipose tissue *in vitro* (31). It is possible that GLUT4 ablation in adipose tissue may lead to decreased glucose transport and triglyceride synthesis.

The study of the effects of skeletal muscle GLUT4 complementation on alterations in adipose tissue is complicated by the ectopic expression of the MLC-GLUT4 transgene in adipose tissue. This ectopic expression was negligible in female MLC-GLUT4-null mice; however, in adipose tissue of male mice, the transgene was expressed at a higher level. This is a surprising finding, since previously the MLC 1 promoter and enhancer used in the construction of the MLC-GLUT4 transgene did not drive expression of a heterologous reporter gene construct in WAT (32). However, despite the ectopic expression of the transgene, fed FFA levels, adipose tissue deposits, and adipocyte size remained abnormal in both female and male MLC-GLUT4-null mice. In female MLC-GLUT4-null mice, decreases in adipose tissue deposits, adipocyte size, and fed FFA levels were as severe as in female GLUT4-null mice.

Since whole body glucose homeostasis was fully restored in female MLC-GLUT4-null mice, these results suggest that the near absence of GLUT4 in adipose tissue of female MLC-GLUT4-null mice is responsible for abnormal adipose tissue deposits, smaller adipocyte size, and reduced fed plasma FFA levels. This hypothesis is supported by the observation that the size of the adipose tissue deposits and fed plasma FFA levels in the male MLC-GLUT4-null mice were intermediate between GLUT4-null and control mice. The alteration of the adipose tissue abnormalities and fed plasma FFA levels closely correlates with the level of ectopic expression of the MLC-GLUT4 transgene in adipose tissue. Male MLC-GLUT4-null mice exhibited a moderate level of MLC-GLUT4 transgene expression in the adipose tissue, and partially normalized adipose tissue deposits and fed plasma FFA levels. In contrast, female MLC-GLUT4-null mice exhibited a negligible amount of MLC-GLUT4 transgene expression in adipose tissue, and no normalization in adipose tissue deposits, adipocyte cell size, and fed plasma FFA levels. In both skeletal muscle and adipose tissue, the defect in glucose uptake leads to alteration in glucose metabolism, and in the adipose tissue, this results in a defect in lipid metabolism, thus affecting whole body energy metabolism.

The results of this study indicate that the alterations in skeletal muscle and whole body glucose metabolism and the adipose tissue abnormality noted in GLUT4-null mice can be precisely defined by tissue-specific GLUT4 complementation. GLUT4 in skeletal muscle plays a central role in the regulation of skeletal muscle glucose metabolism. In addition to providing energy, glucose can serve as precursor for both the glycerol and the fatty acid moiety in triglyceride biosynthesis in adipose tissue. Overexpression of GLUT4 in the adipose tissue of transgenic mice results in increased glucose uptake and incorporation of glucose into lipid (31). Together, the former and this study suggest that GLUT4 plays a regulatory role in lipid metabolism. This hypothesis is consistent with previous studies demonstrating tissue-specific, differential regulation of GLUT4 expression under altered metabolic states (5). In insulin-resistant conditions such as obesity and starvation, adipose tissue GLUT4 mRNA and protein expression are downregulated, while skeletal muscle GLUT4 levels are not affected. The reason for the divergence in GLUT4 expression is unknown. In view of the fact that insulin resistance is as much a manifestation of abnormal lipid metabolism as abnormal glucose metabolism, the downregulation of GLUT4 in adipose tissue may be responsible for decreased FFA reesterification and elevated circulating FFA levels (33). Decreased FFA reesterification and elevated circulating FFA levels are major defects in insulin resistance, and may further exacerbate impaired glucose uptake by skeletal muscle (33).

In summary, we have used a transgenic complementation strategy to reintroduce GLUT4 into the fast-twitch skeletal muscle of GLUT4-null mice to delineate the individual contributions of adipose tissue and skeletal muscle GLUT4 to the overall phenotype of the GLUT4-null mice. Our results suggest that the absence of GLUT4 in skeletal muscle is sufficient to cause the impaired glucose metabolism seen in the GLUT4-null mice. Despite low level ectopic GLUT4 expression in adipose tissue, the MLC-GLUT4-null mice retain the defects in lipid metabolism seen in GLUT4-null mice. Additionally, preliminary study suggests that partial restoration of GLUT4 in hearts of MLC-GLUT4-null mice is associated with less severe

cardiac hypertrophy than previously observed in GLUT4-null mice (12). Taken together, these results strongly suggest that the defects in glucose and lipid metabolism in GLUT4-null mice arise independently, and that adipose tissue GLUT4 is a major regulator of adipose tissue storage deposits, adipocyte size, and circulating FFA levels. The transgenic complementation strategy to study tissue-specific gene disruption serves as a powerful tool in analyzing the complex phenotype of the GLUT4-null mouse.

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