Minireview

THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 274, No. 6, Issue of February 5, pp. 3253–3256, 1999

GLUT4 Gene Regulation and Manipulation*

Maureen J. Charron‡§, Ellen B. Katz‡, and Ann Louise Olson¶

From the ‡Department of Biochemistry, Albert Einstein College of Medicine, Bronx, New York 10461 and ¶Department of Biochemistry and Molecular Biology, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma 73190

A decade has passed since the cloning of the insulin-responsive glucose transporter, *GLUT4*. Numerous studies have demonstrated the complex hormonal and metabolic regulation of *GLUT4* gene expression in adipose tissue and muscle. Careful dissection of the regulatory elements in the *GLUT4* promoter has provided insight into the intricate control of this central gene of glucose homeostasis. Genetic manipulation of mice has provided further insight into the role of GLUT4 in carbohydrate and lipid metabolism at the whole body and tissue-specific levels. Analysis of *GLUT4*+/−, *GLUT4* null, and muscle-complemented *GLUT4* knockout mice has furthered our understanding of peripheral insulin sensitivity. Additional studies on *GLUT4* gene regulation and *GLUT4* knockout models are likely to lead to novel therapies for type II diabetes and other diseases of insulin resistance.

Regulation of GLUT4 mRNA Expression in Vivo and in Vitro

Proof that the facilitative glucose transporter, GLUT4, is the primary effector molecule for insulin-mediated glucose disposal comes from the use of transgenic animals. Mice that are genetically engineered to generally overexpress an exogenous *GLUT4* gene, or specifically in skeletal muscle or adipose tissue, display enhanced insulin responsiveness and peripheral glucose utilization (for review see Ref. 1). The high levels of transporters are able to enhance insulin responsiveness in genetic and experimental models of diabetes. Thus, expression of the *GLUT4* gene is a clinically relevant molecule to target for treatment of insulin-resistant disease states.

Expression of *GLUT4* mRNA is subject to tissue-specific, hormonal, and metabolic regulation (for review see Ref. 2). *GLUT4* mRNA expression is largely restricted to both brown and white adipose tissue, skeletal and cardiac muscle, although *GLUT4* mRNA have been detected in specialized cell types of other tissues. Changes in *GLUT4* gene expression are observed in physiologic states of altered glucose homeostasis and vary in a tissue-specific manner, occurring much more rapidly in adipose tissue than skeletal muscle (3). In general, *GLUT4* mRNA expression is down-regulated in states of relative insulin deficiency such as streptozotocin (STZ)[1]-induced diabetes and chronic fasting (for review see Ref. 2). Chronic fasting markedly reduces *GLUT4* mRNA levels in adipose tissue, while having either no effect or slightly increasing *GLUT4* mRNA in skeletal muscle (4). Changes in steady state levels of *GLUT4* mRNA result from changes in the rate of synthesis of *GLUT4* mRNA (gene transcription) and changes in degradation of the mRNA (5, 6). Transcription rates using nuclear run-on transcription assays have demonstrated that the *GLUT4* mRNA transcription rate is decreased in both adipose tissue and skeletal muscle in STZ-induced diabetic animals (5, 6), whereas it is increased in skeletal muscle of fasted animals (6). Thus, changes in *GLUT4* mRNA steady state levels reflect changes in the rate of mRNA synthesis.

The molecular basis for regulation of *GLUT4* gene expression in states of relative insulin deficiency *in vivo* has been very difficult to solve. Insulin deficiency *in vivo* is complicated by the fact that compensatory counter-regulatory hormones are elevated. In addition, insulin deficiency is tightly coupled to plasma glucose levels and intracellular glucose utilization. For instance, STZ-diabetic animals are hyperglycemic and insulinopenic whereas fasted animals are hypoglycemic and insulinopenic, suggesting that insulin rather than circulating glucose levels are responsible for regulation of adipose tissue *GLUT4* expression. This hypothesis was supported in studies using phlorizin to increase urinary output of glucose in diabetic rats (2). In contrast to insulin, phlorizin-induced normalization of glycemia in these insulin-deficient animals was unable to restore *GLUT4* mRNA expression in adipose tissue. On the other hand, skeletal muscle *GLUT4* mRNA is not down-regulated by insulinopenia associated with fasting, implying that insulin levels do not directly regulate *GLUT4* gene expression (4).

Both chronically fasted and STZ-diabetic animals represent states of insulin deficiency where peripheral glucose metabolism is inhibited. With the production of transgenic mice overexpressing the *GLUT4* gene, a model of insulinopenia in which peripheral glucose utilization is enhanced has been made available. Overexpression of human GLUT4 protein markedly enhanced glucose uptake and utilization in the fed state resulting in hypoglycemia and hypoinsulinemia (7). Despite the relative hypoinsulinemia, expression of the endogenous mouse *GLUT4* mRNA was unaffected by the presence of the human GLUT4 protein (8).[2] This suggests the predominate metabolic control of *GLUT4* gene expression is linked to intracellular glucose metabolism. The divergent effect of hypoinsulinemia of fasting compared with STZ-diabetes in skeletal muscle may be linked to differences in energy metabolism that occur in muscle in these different states. *In vitro* models for studying *GLUT4* expression are limited by the small number of cultured cell models that express the *GLUT4* gene. Differentiated murine 3T3-L1 and F442A adipocytes express relatively high levels of *GLUT4* mRNA and protein similar to that found in primary isolated adipocytes or adipose tissue (9–11). The appearance of *GLUT4* mRNA in these cells first occurs about 4 days after the onset of differentiation (9). Unlike primary adipocytes or adipose tissue, 3T3-L1 adipocytes also express high levels of the GLUT1 glucose transporter isoform (10). These cell lines have been used as *in vitro* models to study several aspects of glucose transporter regulation, including regulation of gene expression. Interestingly, chronic exposure of 3T3-L1 *in vitro* cells or adipose tissue *in vivo* to insulin has differential effects on *GLUT4* gene expression. Animals chronically treated with insulin show increased *GLUT4* mRNA in adipose tissue (12, 13) whereas chronic insulin treatment of 3T3-L1 adipocytes has resulted in either no change or in a marked reduction in *GLUT4* mRNA levels (14, 15). The different responses of *GLUT4* mRNA to chronic insulin treatment *in vivo* and *in vitro* suggest *GLUT4* mRNA does not respond directly to insulin action on adipose tissue. On the other hand, incubation of 3T3-L1 adipocytes in glucose-free medium down-regulates *GLUT4* mRNA about 10-fold and is accompanied by the up-regulation of *GLUT1* mRNA (16). Re-addition of glucose to the starved adipocytes restored *GLUT4* mRNA levels. Supplementation of glucose-free medium with either fructose or pyruvate as an alternative energy source maintained the steady state level of *GLUT4* mRNA. These data are

* This minireview will be reprinted in the 1999 Minireview Compendium, which will be available in December, 1999. This is the third article of three in the "Insulin-stimulated Glucose Transport Minireview Series." This work was supported by National Institutes of Health Grants DK47425 and HL58119 (to M. J. C.) and DK47894 (to A. L. O.) and by grants from the American Diabetes Association (to M. J. C. and A. L. O.) and the American Heart Association (to M. J. C.).

§ Recipient of an Irma T. Hirschl career scientist award. To whom correspondence should be addressed: Dept. of Biochemistry, Albert Einstein College of Medicine, 1300 Morris Park Ave., Bronx, NY 10461. Tel.: 718-430-2852; Fax: 718-430-8676; E-mail: charron@aecom.yu.edu.

[1] The abbreviations used are: STZ, streptozotocin; kb, kilobases; CAT, chloramphenicol acetyltransferase; EDL, extensor digitorum longus; MLC, myosin light chain.

[2] A. L. Olson, unpublished data.

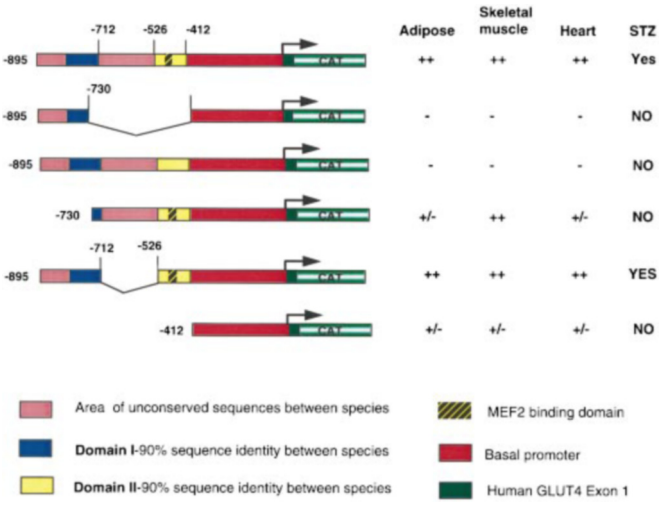

FIG. 1. **Summary of functional analysis of the human *GLUT4* promoter in transgenic mice.** A schematic depiction of transgenic constructs is shown on the *left*. The *colored bars* indicate various structural domains of the *GLUT4* gene. The *bent arrow* refers to the transcription initiation site. Expression of transgenic mRNA in adipose tissue (brown and white), hindquarter skeletal muscle, and heart is indicated to the *right*. A designation of ++ indicates full expression, − indicates no expression, and +/− indicates a low level of expression relative to the endogenous *GLUT4*. STZ indicates whether the construct is regulated in STZ-induced diabetes.

consistent with a metabolic rather than hormonal regulation of *GLUT4* gene expression.

Transcriptional Regulation of the GLUT4 Promoter

To understand how the factors described above exert their influence on *GLUT4* gene expression, it is necessary to identify the molecular elements (cis-DNA sequences and trans-acting factors) responsible for the coordinate transcriptional control of gene expression. In the case of *GLUT4*, a complex pattern of gene expression is observed in various physiologic states that are difficult to mimic *in vitro* using traditional methods of studying the function of gene promotors in model cell culture systems. Without a clear understanding of the molecular basis for *GLUT4* gene expression, it is difficult to develop a suitable *in vitro* model to understand how the *GLUT4* gene is regulated *in vivo*. To circumvent these difficulties, promoter analysis using human *GLUT4* reporter genes was performed in transgenic mice. This system allows analysis of transcriptional activity of the *GLUT4* promoter in a natural physiologic context, which is essential for a gene that is subjected to a complex mixture of tissue-specific and nutritional/metabolic factors.

To date, 12 different transgenic constructs have been analyzed for appropriate tissue-specific and hormone-dependent *GLUT4* gene regulation (Fig. 1). The first established transgenic line was engineered to express a human *GLUT4* minigene consisting of the entire coding region of the gene and 5.3 kb of 5′-flanking DNA (8). Expression of this construct demonstrated that the human gene, in a mouse background, was subject to the same regulation as the mouse *GLUT4* gene. Furthermore, the complex pattern of human *GLUT4* transcription initiation site selection was also observed in these transgenic mice. A second line of transgenic mice carrying a construct in which expression of the chloramphenicol acetyltransferase (CAT) reporter gene was driven by 2.4 kb of 5′-flanking DNA demonstrated that this region of DNA was sufficient to confer not only tissue-specific expression but also regulated expression of the *GLUT4* gene in chronic fasted and STZ-induced diabetic mice (8, 17).

Because all the apparent regulatory cis-DNA elements were located within 2.4 kb of the transcription initiation site, studies have been concentrated on this area of the human *GLUT4* promoter to define the functional elements that drive transcription. The initial approach to studying this promoter was to narrowly define the cis-acting DNA sequences required to support a full program of gene expression by generating CAT fusion genes driven by various fragments of the 5′-*GLUT4* regulatory region. Using this approach the structure of the *GLUT4* promoter and its regulatory regions has begun to be defined (Fig. 1).

A series of deletions in the 5′-end defined the first 900 base pairs upstream of the major transcription initiation site of the human *GLUT4* gene as putative regulatory DNA (18). A comparison of sequences of this regulatory region in the human gene with the analogous regions of the rat and mouse *GLUT4* gene revealed the existence of two areas with greater than 90% sequence identity referred to as domains I and II (19). A 5′ deletion that removes both of these domains left a basal promoter which was ubiquitously expressed at a very low level in all tissues, including those that do not normally express the *GLUT4* gene (20). A construct in which most of domain I was deleted leaving domain II intact was able to support a high level of CAT activity in skeletal muscle, but a low level of CAT expression was observed in heart and adipose tissue and tissues that do not normally express the *GLUT4* gene (20). In this construct, domain II was not sufficient to support regulated expression of transgenic mRNA in STZ deficiency in skeletal muscle or any other tissue. Although domain II was not sufficient to support full transgenic expression in heart and adipose tissue, deletion of this region ablated transgene expression in these tissues. Thus domain II is necessary, but not sufficient, for full promoter function of the *GLUT4* gene (18). Inspection of domain II revealed the existence of a perfectly conserved binding domain for the MEF2 family of transcription factors. A loss of function of this MEF2 binding domain had the same effect as deletion of the entire conserved region, demonstrating that this binding domain was the functional element within these sequences (18).

A DNA binding site functions by binding a specific protein complex. The MEF2 DNA binding site is known to bind isoforms of the MEF2 family (MEF2A, MEF2B, MEF2C, and MEF2D) of DNA binding proteins belonging to a larger family of MADS-box domain transcription factors (21). Although these transcription factors have been studied largely in the context of myogenesis, the expression of these proteins extends beyond muscle tissues. Using isoform-specific antibodies in electrophoretic mobility shift assays, MEF2A and MEF2D isoforms were shown to bind the *GLUT4* MEF2 binding domain in skeletal muscle, heart, and adipose tissue (18).[2] These studies were the first to establish a role for MEF2 binding activity in gene expression in adipose tissue. Such a role is not unexpected as adipose tissue and skeletal muscle arise from embryonic mesoderm.

The identification and characterization of domain I in the human *GLUT4* promoter are currently under way. Interestingly, analysis of the mouse *GLUT4* promoter in 3T3-L1 adipocytes revealed an element responsible for insulin-mediated down-regulation of *GLUT4* gene expression in those cells (22). This insulin-responsive element in mouse coincides with domain I in the human gene. Although insulin effects *in vivo* and *in vitro* are dissimilar, it remains possible that the insulin-responsive element defined in 3T3-L1 cells may be functional in mediating the effects of insulin deficiency *in vivo*. Establishing functional region(s) of this domain will pave the way for solving the complex regulation of the *GLUT4* gene. Understanding the molecular basis for expression of the *GLUT4* gene will be useful for targeting the expression of this gene in a manner appropriate for treatment of insulin-resistant glucose transport.

GLUT4+/− Mice Exhibit Age-related Insulin Resistance and Diabetic Pathologies

Knockout mice with one null allele of *GLUT4* (*GLUT4*+/−) have been generated that exhibit reduced GLUT4 expression (23). Though all mice have the same mutation, namely one disrupted allele of *GLUT4*, a spectrum of phenotypes is observed with age. Specifically, when considering serum-fed glucose and insulin levels, *GLUT4*+/− mice could be divided into three distinct groups: normal glycemia with normal insulin levels (N/N), normal glycemia with high insulin levels (N/H), or hyperglycemic with high insulin levels (H/H). The diabetes present in *GLUT4*+/− mice is not coincident with alterations in body weight or fat pad weight. Curiously, adipocytes from diabetic H/H *GLUT4*+/− mice are significantly enlarged. Secretion of cytokines such as tumor necrosis factor α and leptin that influence insulin sensitivity and energy balance is altered in obese adipocytes (24), and this may contribute to the diabetic phenotype of the H/H mice. Additionally, neither pancreatic insufficiency, dyslipidemia, nor hepatic insulin resist-

ance is associated with the *GLUT4*+/− phenotype. Diabetic H/H *GLUT4*+/− mice display a 40% increase in peak arterial blood pressure but a normal response to inotropic challenge, demonstrating that ventricular performance was not compromised. However, histopathological analysis revealed diabetic cardiomyopathies including cardiomyocyte hypertrophy, focal cell necrosis, interstitial fibrosis, and vascular sclerosis. Oddly, despite the lack of dyslipidemia in H/H *GLUT4*+/− mice, micro- and macro-steatosis similar to that seen in diabetics was noted throughout the liver.

Beginning at 2 months of age all male *GLUT4*+/− mice have reduced adipose and muscle GLUT4 expression (75 and 25–46%, respectively) (23). Decreased adipocyte GLUT4 content in *GLUT4*+/− mice is the first measurable cellular defect to occur, the significance of which is yet to be realized. *In vitro* insulin-stimulated glucose uptake into soleus and extensor digitorum longus (EDL) muscles of prediabetic N/H *GLUT4*+/− mice is significantly diminished (23). Using euglycemic/hyperinsulinemic clamps it was demonstrated that the *in vivo* peripheral insulin resistance of prediabetic (N/H) *GLUT4*+/− mice was as severe as in uncontrolled type II diabetes (25). Additionally, the rate of insulin-stimulated glycogen synthesis was significantly impaired directly because of reduced muscle GLUT4-mediated glucose uptake and not defective activation of glycogen synthase. The selective primary reduction in GLUT4 protein is the most direct proof to date of the rate-limiting role of glucose transport in whole body and skeletal muscle insulin-stimulated glucose uptake. This supports the idea that spontaneous variations in *GLUT4* gene expression in human muscle may be a major determinant of peripheral insulin sensitivity. *In vivo* tracer studies also demonstrated that prediabetic N/H *GLUT4*+/− mice exhibit a modest reduction in postabsorptive hepatic glucose production and normal intrahepatic distribution of glucose fluxes through glucose cycling, gluconeogenesis, and glycogenolysis (25). These data confirm the relatively minor role GLUT4 plays in basal glucose homeostasis.

Prevention of Insulin Resistance in GLUT4+/− Expressing a Muscle-specific GLUT4 Transgene

Myosin light chain 1-GLUT4 (*MLC-GLUT4*) transgenic mice that specifically overexpress GLUT4 in fast twitch muscle (26) were mated into the genetic background of the *GLUT4*+/− mutation to assess the therapeutic merit of muscle *GLUT4* gene therapy in type II diabetes (23).[3] *MLC-GLUT4* transgenic mice have increased insulin sensitivity without hypoglycemia (26). GLUT4 content and insulin-stimulated glucose uptake were normalized in fast twitch muscles of *MLC-GLUT4*+/− mice. Fed plasma glucose and insulin levels were normal throughout the lives of *MLC-GLUT4*+/− mice, and cardiac histopathologies were minimal. *In vivo* tracer studies demonstrated that whole body glucose utilization, glycolysis, and glycogen synthesis were normal in *MLC-GLUT4*+/− mice, confirming the central role of muscle GLUT4 in peripheral insulin sensitivity and the genesis of diabetic complications. Although these studies strongly suggest muscle *GLUT4* gene therapy could be effective in treating type II diabetes or other diseases of peripheral insulin resistance, they do not prove that point. To specifically test this hypothesis, design of an inducible, muscle-specific *GLUT4* transgene (*iGLUT4*) is required (27). The *iGLUT4* transgenically expressed in *GLUT4*+/− mice could be activated at various points in the disease (*e.g.* N/H prediabetic; H/H diabetic) to determine whether muscle *GLUT4* gene therapy can halt or reverse any or all diabetic complications.

GLUT4 Null Mice Maintain Normal Glycemia without GLUT4

In comparison with *GLUT4*+/− mice it is both exciting and perplexing that mice which lack the insulin-sensitive glucose transporter (*GLUT4* null) are not diabetic but do exhibit abnormalities in glucose and lipid metabolism (1, 28–30). Surprisingly, blood glucose levels in *GLUT4* null mice are normal under fasted and fed conditions. Although *GLUT4* null mice have normal glucose tolerance, they do exhibit hyperinsulinemia in the fed state and impaired insulin tolerance, suggesting insulin resistance. Careful analysis of serum metabolites of *GLUT4* null mice reveals a significant reduction in fed lactate and free fatty acid levels. Additionally, 9-fold reductions in fasting ketones are noted. *GLUT4* null mice are 15–20% growth-retarded and have severely reduced adipose tissue depots and extreme cardiac hypertrophy. Northern and Western blot analyses verified that *GLUT4* null mice could compensate for the lack of GLUT4 and maintain normal circulating glucose levels by a mechanism that did not involve overexpression of a known facilitative or Na^+-dependent glucose transporter isoform in skeletal muscle (28, 31).[4] Curiously, *GLUT2* expression in *GLUT4* null liver is significantly increased. The increase in *GLUT2* expression suggests that the liver is capable of increased hepatic glucose uptake. This excess glucose subsequently could be converted to fatty acids or glycogen.

Though *GLUT4* null hearts display characteristics of hypertrophy caused by hypertension, they have normal blood pressure (1). The *GLUT4* null heart represents a unique model of hypertrophy that may be used to study the consequences of altered substrate utilization in both normal and pathophysiological conditions. Consistent with this notion, GLUT1 expression is increased in *GLUT4* null hearts, and serum free fatty acids are reduced, as are peripheral adipose tissue depots. The *GLUT4* null heart may in fact be glycolytically primed, which could present an advantage under ischemic conditions. Indeed, preliminary studies demonstrate that *GLUT4* null hearts resist loss of function following ischemia/reperfusion.[5] *GLUT4* ablation also results in an extreme depletion of fat mass. Gonadal fat pad weights are reduced 10-fold, and fat cells are approximately 50% smaller in size. This reduction in cell size may affect the secretion of the metabolic modifiers mentioned earlier (23). Interestingly, endurance exercise training results in reduced fat mass, smaller fat cell size, cardiac hypertrophy without hypertension, and reduced fed serum free fatty acids (32). The striking similarity between *GLUT4* null mice and endurance exercise-trained athletes may suggest that similar adaptive responses are elicited by these two forms of cellular stress.

Characterization of a Novel Glucose Transport Activity in GLUT4 Null Soleus Muscle

The ability of two *GLUT4* null muscle types to take up glucose in the presence of maximally stimulating concentrations of insulin was measured *in vitro* (29–31). Perhaps as expected, fast twitch EDL muscles of *GLUT4* null mice failed to take up more glucose in response to insulin. Surprisingly, a 2-fold increase in insulin-stimulated glucose uptake was noted in female *GLUT4* null slow twitch soleus muscles compared with a 3-fold increase in wild type controls. Soleus muscle of *GLUT4* null males displayed a 2-fold increase in basal glucose uptake with no further increase following insulin stimulation. The molecular basis for the sexually dimorphic response to *GLUT4* ablation in soleus muscle may be linked to the superior insulin sensitivity of female mice. These results indicate that highly oxidative soleus muscle can adapt to ablation of *GLUT4* and take up a large amount of glucose, whereas glycolytic EDL muscle cannot. The specificity of glucose uptake was demonstrated by incubating muscles in the presence of 50 μM cytochalasin B, a fungal metabolite that inhibits facilitated D-glucose transport (29). Basal and insulin-stimulated glucose uptake in soleus and EDL muscles from *GLUT4* null and control mice was reduced by cytochalasin B to the same extent. This result, combined with the failure to detect increased expression of any known GLUT, led to the hypothesis that a novel glucose transport system is responsible for glucose uptake into highly oxidative muscle, which contributes to euglycemia in *GLUT4* null mice (1, 28–31).

Recently glucose uptake was measured in *GLUT4* null muscles under normoxic and hypoxic conditions (33). Hypoxia has been shown to stimulate glucose transport in skeletal muscle via a pathway that is independent from that of insulin by recruitment of GLUT4 to the plasma membrane (34). In both soleus and EDL from *GLUT4* null mice, hypoxia treatment failed to stimulate glucose uptake to levels above basal normoxic conditions (33). This result proved GLUT4 is essential for hypoxia-induced increase in glucose

[3] Tsao, T. S., Stenbit, A. E., Factor, S. M., Chen, W., Rossetti, L., and Charron, M. J. (1999) *Diabetes*, in press.

[4] A. E. Stenbit and M. J. Charron, unpublished observation.

[5] A. E. Stenbit, D. L. Geenen, and M. J. Charron, unpublished observations.

uptake, and the compensatory glucose transport activity in *GLUT4* null soleus does not respond to stimulation by hypoxia *in vitro*. As hypoxia is a useful model for exercise, the above data suggest that GLUT4 is essential for exercise-stimulated increases in muscle glucose uptake.

Basal and insulin-stimulated insulin receptor tyrosine kinase activity was shown to be normal in muscles of male *GLUT4* null mice (31). Furthermore, insulin receptor autophosphorylation was also shown to be unchanged in null muscle. Thus, *GLUT4* ablation does not alter the activation of insulin receptor tyrosine kinase activity in skeletal muscle. Glycogen synthase, like GLUT4, is a major downstream target of insulin receptor action (35). Basal glycogen synthesis was increased in *GLUT4* null soleus muscle; however, insulin stimulation resulted in a parallel decrease in glucose uptake and glycogen synthesis (31). Although *GLUT4* null soleus muscles are able to maintain nearly normal steady state levels of glycogen, acute insulin-stimulated glucose uptake and incorporation into glycogen appear to be GLUT4-dependent. The increased glycogen content of female *GLUT4* null soleus muscle was tightly associated with maintenance of ATP and phosphocreatine levels similar to controls in response to hypoxic stress (33). Though *GLUT4* null muscles take up significantly less glucose, they maintain normal high energy phosphate stores possibly because of increased utilization of fatty acids (36). The reduced fed serum free fatty acids in *GLUT4* null mice are consistent with this hypothesis.

MLT-GLUT4 Null Mice (Muscle Only GLUT4 Expression)

Mice expressing GLUT4 only in fast twitch skeletal muscle were generated to assess the role of muscle GLUT4 in whole body glucose disposal, insulin sensitivity, energy homeostasis, and the complex phenotype of the *GLUT4* null model. The muscle-specific *MLC-GLUT4* transgene (26) was mated into the genetic background of GLUT4 null mice to generate a population of *MLC-GLUT4* null mice (37). *MLC-GLUT4* null mice have restored GLUT4 expression in glycolytic muscle such as EDL but not in oxidative muscles like soleus. Fed serum lactate levels of *MLC-GLUT4* null mice compared with *GLUT4* nulls are restored to normal. Unlike *GLUT4* null mice, *MLC-GLUT4* null mice do not exhibit fed hyperinsulinemia, suggestive of improved insulin sensitivity. Insulin tolerance tests demonstrate that *MLC-GLUT4* nulls clear glucose as well as wild type control mice, unlike *GLUT4* null mice, which are significantly insulin-resistant. This result was corroborated by a significant increase in insulin-stimulated glucose transport into EDL muscles. Furthermore, *MLC-GLUT4* null soleus muscle retained the compensatory glucose transport activity measured in *GLUT4* null soleus. When combined, these findings indicate that expression of the compensatory glucose transporter is not dependent upon hyperinsulinemia.

Like *GLUT4* null mice, *MLC-GLUT4* null mice weigh approximately 20% less than controls and exhibit significantly decreased fed serum free fatty acid levels (37). Further, *MLC-GLUT4* null mice also have significantly reduced inguinal fat pads and adipose cells compared with controls. These data demonstrate that complementation of fast twitch muscle with GLUT4 does not correct defects in adipose tissue mass or lipid metabolism because of *GLUT4* ablation. When combined, these data indicate that muscle GLUT4 is a major regulator of whole body glucose metabolism and that defects in glucose metabolism and adipose tissue mass and lipid metabolism in *GLUT4* null mice arise independently.

Conclusion

To date *GLUT4*+/− mice represent the only gene knockout model where the phenotype of the heterozygote is more severe than that noted in the homozygote (with respect to the diabetes). Additionally, this is the first mouse model that demonstrates that a heterozygous knockout of a single known diabetogene can yield the plethora of pathologies that characterize type II diabetes with increasing age. In contrast to *GLUT4*+/− mice, which express normal amounts of GLUT4 in their insulin-responsive tissue until 2 months of age, *GLUT4* null mice never express GLUT4 and do not develop a diabetic phenotype. The mechanisms that develop in *GLUT4* null mice to help maintain euglycemia may not be apparent in *GLUT4*+/− mice because the function of the compensatory glucose transport system may be masked by the remaining functionally dominant GLUT4. Perhaps, endurance exercise training that elicits characteristics seen in *GLUT4* null mice (*i.e.* reduced adipose tissue, cardiac hypertrophy, reduced serum free fatty acids) might lead to expression of the glucose transport system seen in *GLUT4* null soleus muscle. This expression could be responsible in part for the therapeutic effects of exercise by improving whole body insulin sensitivity in diabetics (32). Understanding the genetic and molecular basis of the adaptive responses to *GLUT4* ablation responsible for the maintenance of euglycemia will undoubtedly lead to novel therapies for diseases of insulin resistance including type II diabetes.

Acknowledgments—We thank Drs. R. Burcelin, J. Li, A. E. Stenbit, and T.-S. Tsao for invaluable contributions to these studies and Drs. J. Olefsky, M. Czech, and J. Pessin for critical comments.

REFERENCES

1. Katz, E. B., Burcelin, R., Tsao, T. S., Stenbit, A. E., and Charron, M. J. (1996) *J. Mol. Med.* **74,** 639–652
2. Kahn, B. B. (1996) *Diabetes* **45,** 1644–1654
3. Richardson, J. M., Balon, T. W., Treadway, J. L., and Pessin, J. E. (1991) *J. Biol. Chem.* **266,** 12690–12694
4. Charron, M. J., and Kahn, B. B. (1990) *J. Biol. Chem.* **265,** 7994–8000
5. Gerrits, P. M., Olson, A. L., and Pessin, J. E. (1993) *J. Biol. Chem.* **268,** 640–644
6. Neufer, P. D., Carey, J. O., and Dohm, G. L. (1993) *J. Biol. Chem.* **268,** 13824–13829
7. Treadway, J. L., Hargrove, D. M., Nardone, N. A., McPherson, R. K., Russo, J. F., Milici, A. J., Stukenbrok, H. A., Gibbs, E. M., Stevenson, R. W., and Pessin, J. E. (1994) *J. Biol. Chem.* **269,** 29956–29961
8. Olson, A. L., Liu, M.-L., Moye-Rowley, W. S., Buse, J. B., Bell, G. I., and Pessin, J. E. (1993) *J. Biol. Chem.* **268,** 9839–9846
9. Garcia de Herreros, A., and Birnbaum, M. J. (1989) *J. Biol. Chem.* **264,** 9885–9890
10. Calderhead, D. M., Kitagawa, K., Tanner, L., Holman, G. D., and Lienhard, G. E. (1990) *J. Biol. Chem.* **265,** 13801–13808
11. Weiland, M., Schurmann, A., Schmidt, W. E., and Joost, H. G. (1990) *Biochem. J.* **1990,** 331–336
12. Cusin, I., Terrettaz, J., Rohner-Jeanrenaud, F., Zarjevski, N., Assimacopoulos-Jeannet, F., and Jeanrenaud, B. (1990) *Endocrinology* **127,** 3246–3248
13. Postic, C., Leturque, A., Rencurel, F., Printz, R. L., Forest, C., Granner, D. K., and Girard, J. (1993) *Diabetes* **42,** 922–929
14. Tordjman, K. M., Leingang, K. A., James, D. E., and Mueckler, M. M. (1989) *Proc. Natl. Acad. Sci. U. S. A.* **86,** 7761–7765
15. Flores-Riveros, J. R., McLenithan, J. C., Ezaki, O., and Lane, M. D. (1993) *Proc. Natl. Acad. Sci. U. S. A.* **90,** 512–516
16. Tordjman, K. M., Leingang, K. A., and Mueckler, M. (1990) *Biochem. J.* **271,** 201–207
17. Liu, M. L., Olson, A. L., Moye-Rowley, W. S., Buse, J. B., Bell, G. I., and Pessin, J. E. (1992) *J. Biol. Chem.* **267,** 11673–11676
18. Thai, M. V., Guruswamy, S., Cao, K. T., Pessin, J. E., and Olson, A. L. (1998) *J. Biol. Chem.* **273,** 14285–14292
19. Olson, A. L., Edgington, N. P., Moye-Rowley, W. S., and Pessin, J. E. (1995) *Endocrinology* **136,** 1962–1968
20. Olson, A. L., and Pessin, J. E. (1995) *J. Biol. Chem.* **270,** 23491–23495
21. Shore, P., and Sharrocks, A. D. (1995) *Eur. J. Biochem.* **229,** 1–13
22. Cooke, D. W., and Lane, M. D. (1998) *J. Biol. Chem.* **273,** 6210–6217
23. Stenbit, A. E., Tsao, T.-S., Li, J., Burcelin, R., Geenen, D. L., Factor, S. M., Houseknecht, K. L., Katz, E. B., and Charron, M. J. (1997) *Nat. Med.* **3,** 1096–1101
24. Prins, J. B., and O'Rahilly, S. (1997) *Clin. Sci. (Colch)* **92,** 3–11
25. Rossetti, L., Stenbit, A. E., Chen, W., Hu, M., Barzilai, N., Katz, E. B., and Charron, M. J. (1997) *J. Clin. Invest.* **100,** 1831–1839
26. Tsao, T.-S., Burcelin, R., Katz, E. B., Huang, L., and Charron, M. J. (1996) *Diabetes* **45,** 28–36
27. Wang, Y., DeMayo, F. J., Tsai, S. Y., and O'Malley, B. W. (1997) *Nat. Biotechnol.* **15,** 239–243
28. Katz, E. B., Stenbit, A. E., Hatton, K., DePinho, R., and Charron, M. J. (1995) *Nature* **377,** 151–155
29. Charron, M. J., Katz, E. B., and Zierath, J. R. (1997) *Biochem. Soc. Trans.* **25,** 963–968
30. Charron, M. J., and Katz, E. B. (1998) *Mol. Cell. Biochem.* **182,** 143–152
31. Stenbit, A. E., Burcelin, R., Katz, E. B., Tsao, T. S., Gautier, N., Charron, M. J., and Le Marchand-Brustel, Y. (1996) *J. Clin. Invest.* **98,** 629–634
32. Vranic, M., Wasserman, D., and Bukowiecki, L. (1990) in *Diabetes Mellitus: Theory and Practice* (Rifkin, H., and Porte, D., eds) pp. 198–219, Elsevier Science Publishing Co., Inc., New York
33. Zierath, J. R., Tsao, T.-S., Stenbit, A. E., Ryder, J. W., Galuska, D., and Charron, M. J. (1998) *J. Biol. Chem.* **273,** 20910–20915
34. Cartee, G. D., Douen, A. G., Ramlal, T., Klip, A., and Holloszy, J. O. (1991) *J. Appl. Physiol.* **70,** 1593–1600
35. White, M. F., and Kahn, C. R. (1994) *J. Biol. Chem.* **269,** 1–4
36. Randle, P. J., Garland, B. B., Hales, C. N., and Newsholme, E. A. (1963) *Lancet* **i,** 785–789
37. Tsao, T.-S., Stenbit, A. E., Li, J., Houseknecht, K. L., Zierath, J. R., Katz, E. B., and Charron, M. J. (1997) *J. Clin. Invest.* **100,** 671–677