GLUT4 Gene Regulation and Manipulation

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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)-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 insulinoenic, 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). This suggests the predominant 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 isofrom (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

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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 promoters 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 subject 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 5.5 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 narrow these 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).
Prevention of Insulin Resistance in GLUT4 Mice: A Model for Type 2 Diabetes

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). 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). 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. GLUT4 ablation also results in an extreme depletion of fat mass. General visceral fat pads 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). 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 wild type controls. Soleus muscle of GLUT4 null males displays 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 soleus 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 uptake.,
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
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 glycanogen content of female GLUT4 null soleus muscle
was tightly associated with maintenance of ATP and phosphocre-
ate 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 be-
cause of increased utilization of fatty acids (36). The reduced fed
serum free fatty acids in GLUT4 null mice are consistent with
this hypothesis.

MLC-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 glu-
ose disposal, insulin sensitivity, energy homeostasis, and the com-
position of 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.

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