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High fat diet-induced hyperglycemia: Prevention by low level expression of a glucose transporter (GLUT4) minigene in transgenic mice

(insulin resistance/type 2 diabetes)

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ABSTRACT **High-fat intake leading to obesity contributes to the development of non-insulin-dependent diabetes mellitus (NIDDM, type 2). Similarly, mice fed a high-fat (safflower oil) diet develop defective glycemic control, hyperglycemia, and obesity. To assess the effect of a modest increase in the expression of GLUT4 (the insulin-responsive glucose transporter) on impaired glycemic control caused by fat feeding, transgenic mice harboring a GLUT4 minigene were fed a high-fat diet. Low-level tissue-specific (heart, skeletal muscle, and adipose tissue) expression of the GLUT4 minigene in transgenic mice prevented the impairment of glycemic control and accompanying hyperglycemia, but not obesity, caused by fat feeding. Thus, a small increase (≤2-fold) in the tissue level of GLUT4 prevents a primary symptom of the diabetic state in a mouse model, suggesting a possible target for intervention in the treatment of NIDDM.**

It is widely accepted that type 2 non-insulin-dependent diabetes mellitus (NIDDM) is caused by a combination of genetic and environmental factors, notably diet and level of physical activity, which can promote progression from normal to impaired glucose tolerance and insulin resistance (1–4). Among environmental factors, the high-fat content of the typical Western diet is considered a major cause of obesity-associated insulin resistance (2, 4). Resistance of glucose uptake to insulin is observed in most individuals with impaired glucose tolerance or NIDDM (5).

GLUT4, the insulin-responsive glucose transporter, which is found only in heart, skeletal muscle, and adipose tissue, plays an important role in whole body glucose homeostasis (6, 7). This transporter is responsible for the acute stimulation of glucose uptake by insulin that occurs only in these tissues (8). The acute regulation of GLUT4 by insulin involves the rapid translocation of the transporter from intracellular vesicles to the plasma membrane (9, 10). In addition to the acute regulation of GLUT4 activity by insulin, expression of the GLUT4 gene is hormonally and metabolically regulated (11–16).

Certain strains of mice when fed a high-fat diet develop impaired glucose tolerance and a condition resembling NIDDM (17, 18). The possibility was considered, therefore, that impaired glucose tolerance, induced by feeding a high-fat diet, might be corrected by increasing the expression of GLUT4 in the appropriate tissues. Thus, transgenic mice harboring a GLUT4 minigene, which is expressed at a low level and in a tissue-specific manner (19, 20), were used to test this hypothesis.

EXPERIMENTAL PROCEDURES

Transgenic Mice and Transcript Detection. Heterozygous transgenic mice harboring a 14-kb GLUT4 minigene were produced as described (19). The GLUT4 minigene contained 7 kb of 5′-flanking and 1 kb of 3′-flanking sequence and all exons and introns of the GLUT4 gene, as well as a small foreign DNA tag. Two-month-old female littermate transgenic or nontransgenic mice, produced by crossing female nontransgenic C57BL/6 × B6AF mice with heterozygous male transgenic mice (harboring 30 copies per genome of a GLUT4 minigene), were distinguished by Southern blotting (20). The 281-bp tag inserted into the 3′ untranslated region of the gene allowed transcript detection without disrupting translation of the minigene mRNA. RNase protection analysis utilized an antisense RNA probe that generates protected fragments of 433 and 399 bp corresponding to the minigene transcript and of 182 bp corresponding to the endogenous GLUT4 transcript (19). RNA transcripts were quantitated with a BAS2000 image analyzer (Fuji).

Mouse Diets. Mice were fed ad libitum either a high-carbohydrate diet containing 4% safflower oil, 23.7% casein, 0.35% DL-methionine, 10% sucrose, 50% starch, 1% vitamin mixture, 7% mineral mixture, and 4% cellulose powder or a high-fat diet containing 32% safflower oil, 33.1% casein, 0.5% DL-methionine, 17.6% sucrose, 1.4% vitamin mixture, 9.8% mineral mixture, and 5.6% cellulose powder. Preliminary feeding trials were conducted and the composition of the diets was adjusted to that described above so that the daily intake of calories and the amounts of dietary components, except fat and carbohydrate, were nearly identical. Care of the mice was performed in accordance with institutional guidelines.

Immunoblotting. Crude membrane fractions from skeletal (gastrocnemius) muscle and white adipose tissue (WAT) were prepared as described (21). Proteins separated by SDS/PAGE were electrophoretically transferred to Immobilon (Millipore) and immunoblotted with antibodies directed against the C-terminal amino acid sequence of GLUT4 and then ^{125}I-labeled protein A as described (21).

Other Tests. Oral glucose tolerance tests were performed after an overnight fast. D-Glucose (1 mg per g of body weight) was administered by stomach tube and blood samples were drawn before and 30, 60, and 120 min later. Blood glucose levels were measured with a Tidex glucose analyzer (Miles). Protein was assayed by the micro-BCA protein assay method (Pierce). Immunoreactive insulin was measured by radioimmunoassay using human insulin as a standard. Triglyceride and total cholesterol were measured by enzyme assays (Kyowa Medics, Tokyo). Statistical comparisons were made with Stu-

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Abbreviations: NIDDM, non-insulin-dependent diabetes mellitus; WAT, white adipose tissue.

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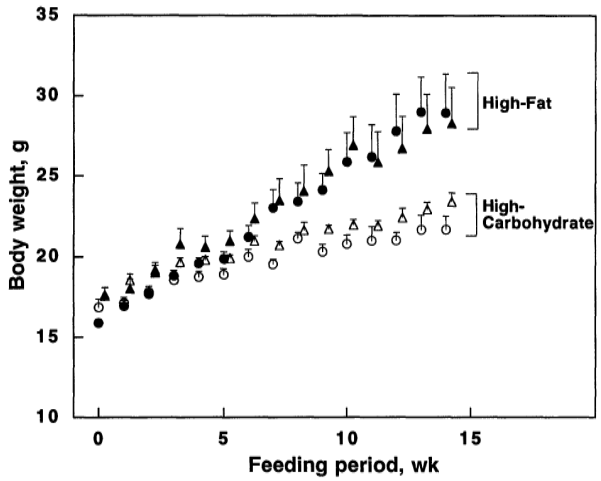

FIG. 1. Comparison of body weight gain by transgenic (GLUT4 minigene) and nontransgenic mice fed a high-fat or high-carbohydrate diet. Two-month-old female littermate transgenic (▲, △) or nontransgenic (●, ○) mice, produced by crossing female nontransgenic C57BL/6 × B6AF mice with heterozygous male transgenic (harboring 30 copies per genome of a GLUT4 minigene) mice, were distinguished by Southern blotting (19). Mice were fed ad libitum either a high-carbohydrate diet or a high-fat diet. Each data point represents mean body weight of five mice.

dent's *t* test; statistical significance is defined as $P < 0.05$. Values are indicated as means ± SE.

RESULTS AND DISCUSSION

Previously, we produced transgenic mice that express an ≈2-fold higher than normal level of GLUT4 in the appropriate tissues (skeletal muscle and brown and white adipose tissue) (20). The GLUT4 minigene contains 7 kb of 5′ and 1 kb of 3′ flanking sequence and all exons and introns of the GLUT4 gene as well as a segment of foreign DNA (281 bp of the chloramphenicol acetyltransferase gene) in the 3′ untranslated region for transcript identification (19). Although the minigene is only weakly expressed despite a copy number of 30 (20), it exhibits normal regulation in other respects—i.e., tissue-specific expression (19, 20), differentiation-induced expression in 3T3-L1 preadipocytes (19), cAMP-induced down-regulation (19), and exercise-induced up-regulation (20). Thus, it was possible to investigate the effect of modest overexpression of GLUT4 on impaired glycemic control caused by feeding a high-fat diet.

Feeding a high-fat (safflower oil) diet to nontransgenic or transgenic mice expressing the GLUT4 minigene resulted in a 27% increase in body weight within 14 weeks compared to mice fed a high-carbohydrate diet (Fig. 1). These results show that expression of the GLUT4 minigene does not affect high-fat diet-induced obesity. It should be noted that mice were allowed free access to food and that the average caloric intake of mice fed both diets was virtually identical—i.e., 10.1 kcal/day for carbohydrate-fed mice and 9.6 kcal/day for fat-fed mice (1 cal = 4.184 J).

Oral glucose tolerance tests were performed after 14 wk of feeding the high-fat or high-carbohydrate diet. Feeding nontransgenic mice the high-fat diet resulted in a large increase (compared to mice fed the high-carbohydrate diet) in the initial fasting plasma glucose level as well as in the levels of plasma glucose following glucose administration (Fig. 2 *Left*). However, transgenic mice (harboring the GLUT4 minigene) fed the high-fat diet exhibited both lower initial plasma glucose levels and dramatically improved glycemic control after glucose administration compared to nontransgenic mice fed the high-fat diet (Fig. 2). Thus, an ≈2-fold increase in the level of GLUT4 in muscle and adipose tissue by mice harboring the minigene (Fig. 3) completely prevented the hyperglycemia and defective glycemic control caused by feeding the high-fat diet. Although not shown here, glucose tolerance tests conducted after 3 and 8 wk of feeding were similar to those illustrated in Fig. 2. The fact that defective glycemic control is evident even after feeding the high-fat diet for only 3 wk indicates that insulin resistance occurs before body weight increase (Fig. 1) is manifested. Moreover, modest overexpression of GLUT4 by transgenic animals after 3 wk of fat feeding prevents the impairment of glycemic control caused by fat feeding.

Despite the increase in fasting plasma glucose level caused by fat feeding and the decrease in plasma glucose level caused by the GLUT4 minigene (Fig. 2), neither of these alterations significantly affected fasting plasma insulin levels (Table 1). However, like the well-documented effect of carbohydrate feeding on plasma triglyceride level (22), triglyceride levels were higher in mice fed the high-carbohydrate diet than in mice fed the high-fat diet (75 ± 11 and 41 ± 4 mg/dl, respectively, for nontransgenic mice and 171 ± 51 and 48 ± 13 mg/dl, respectively, for mice harboring

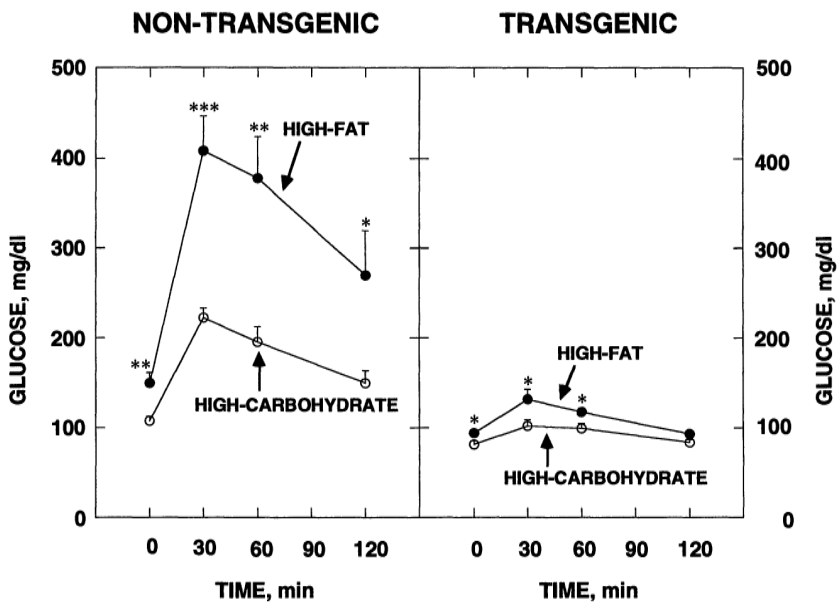

FIG. 2. Effect of fat feeding on glucose tolerance tests with transgenic (GLUT4 minigene) and nontransgenic mice. Female transgenic (GLUT4 minigene) or nontransgenic mice, fed either the high-carbohydrate or the high-fat diet (see legend to Fig. 1) for 14 wk, were fasted overnight and then given D-glucose (1 mg per g of body weight) orally by stomach tube. Plasma glucose levels were determined at the times indicated. Each data point represents mean ± SE of the mean for seven mice. All glucose values for the high-fat-fed nontransgenic group were significantly higher than those for the high-carbohydrate-fed nontransgenic group. Levels of statistical significance are as follows: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ for mice fed the high-carbohydrate vs. the high-fat diet.

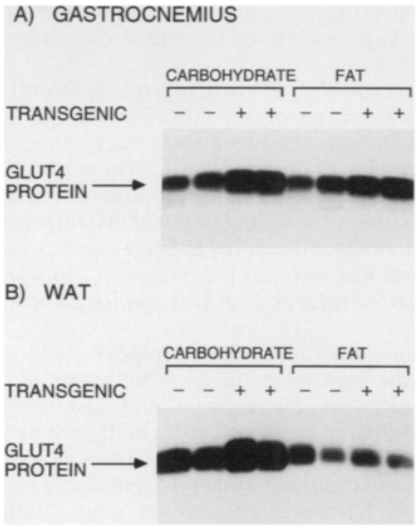

FIG. 3. Effect of fat feeding on expression of GLUT4 in muscle and adipose tissue of transgenic (GLUT4 minigene) and nontransgenic mice. Twenty milligrams of gastrocnemius muscle and 100–200 mg of parametrial WAT from female mice fed either the high-fat or the high-carbohydrate diet for 14 wk were homogenized as described (20). Proteins separated by SDS/PAGE (10% acrylamide) were transferred to membranes and immunoblotted with an antiserum directed against GLUT4 and then with ^{125}I-labeled protein A as described (21). Sixty micrograms of muscle membrane protein and 15 μg of WAT membrane protein were used per lane. Autoradiogram shown is representative of three experiments. Averaged cpm of ^{125}I in the GLUT4 bands from gastrocnemius muscle were 1431 (nontransgenic) and 2885 (transgenic) for the high-carbohydrate diet and 1435 (nontransgenic) and 2775 (transgenic) for the high-fat diet; cpm from WAT were 3275 (nontransgenic) and 5613 (transgenic) for the high-carbohydrate diet and 1330 (nontransgenic) and 1249 (transgenic) for the high-fat diet.

the GLUT4 minigene; Table 1). Neither feeding the high-fat diet nor expression of the transgene affected plasma cholesterol levels.

To ascertain the basis for the hyperglycemia and loss of glycemic control caused by feeding the high-fat diet, the levels of GLUT4 protein in skeletal muscle and WAT were assessed by Western blot analysis. As illustrated in the typical autoradiogram shown in Fig. 3, transgenic mice (harboring the GLUT4 minigene) fed the high-carbohydrate diet expressed a 2-fold higher level of GLUT4 in gastrocnemius muscle and WAT than nontransgenic mice. Although not shown here, mice harboring the transgene also expressed a 2-fold higher level of GLUT4 in other muscles, including quadriceps, triceps,

Table 1. Effect of fat feeding on plasma insulin, triglyceride, and cholesterol levels in transgenic (GLUT4 minigene) and nontransgenic mice

	Insulin, units/ml $\times 10^{-6}$	Triglyceride, mg/dl	Cholesterol, mg/dl
Nontransgenic			
High carbohydrate	6.6 ± 1.6	75 ± 11*	69 ± 3
High fat	5.2 ± 1.2	41 ± 4	66 ± 4
Transgenic			
High carbohydrate	5.6 ± 1.1	171 ± 51*	78 ± 7
High fat	5.9 ± 0.7	48 ± 13	69 ± 6

Transgenic (GLUT4 minigene) and nontransgenic female mice were fed either the high-carbohydrate or the high-fat diet for 14 wk and were then fasted overnight, after which blood samples were drawn from the inferior vena cava. Results are expressed as mean values ± SE for four to six mice.
*$P < 0.05$ by Student's t test for high-carbohydrate vs. high-fat diets.

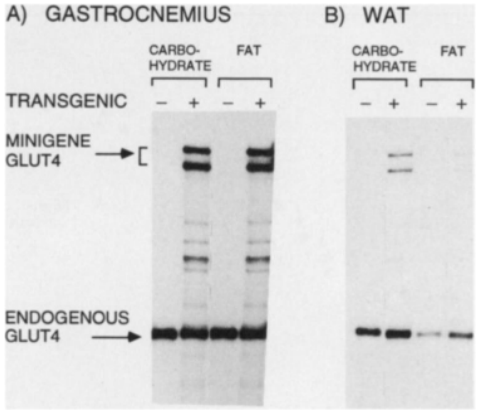

FIG. 4. Effect of fat feeding on expression of GLUT4 minigene and endogenous GLUT4 mRNA from tissues of transgenic and nontransgenic mice. Female transgenic (GLUT4 minigene) mice were fed either the high-carbohydrate or the high-fat diet for 14 wk. Minigene and endogenous GLUT4 mRNA from skeletal (gastrocnemius) muscle and parametrial WAT were measured by RNase protection analysis of RNA obtained from tissues of mice fed ad libitum. Autoradiogram shown is representative of those from three experiments.

diaphragm, and heart. Feeding the high-fat diet to either nontransgenic or transgenic mice had no effect on the GLUT4 level of gastrocnemius muscle but caused a substantial decrease in the GLUT4 level of WAT—i.e., decreases of ≈60% in nontransgenic and ≈80% in transgenic mice (Fig. 3). No changes in GLUT4 level were observed in other muscles (including quadriceps, triceps, and heart) due to fat feeding (results not shown).

To verify that the change in level of GLUT4 protein in adipose tissue caused by fat feeding was due to a change in mRNA level, tissue RNA was subjected to RNase protection analysis. As previously reported (19), it is possible to quantitate both minigene and endogenous GLUT4 mRNA simultaneously by RNase protection assays with an appropriate antisense RNA probe; thus, the minigene message generates 433- and 399-bp protected fragments, whereas the endogenous message generates a 182-bp protected fragment. As shown in Fig. 4, feeding the high-fat diet caused substantial decreases in both the minigene and endogenous GLUT4 messages in adipose tissue but not in gastrocnemius muscle.§ Thus, it can be concluded that the decrease in GLUT4 protein in WAT due to fat feeding is the result of decreased expression of the GLUT4 message and that the cis element(s) responsible for this decline in message level is located within the transfected GLUT4 minigene.

Given that skeletal muscle is the major site of glucose disposal (23) and that fat feeding, which leads to hyperglycemia (Fig. 2) and insulin resistance, has no effect on the GLUT4 level of skeletal muscle, it can be concluded that factors other than GLUT4 are the cause of defective glycemic control. Factors that could give rise to defective glycemic control include impairments of signaling from the insulin receptor, the intrinsic activity of GLUT4, or the process by which intracellular GLUT4 is translocated to the plasma membrane upon stimulation by insulin. In this connection, studies with adult rats have shown that the level of GLUT4 protein in muscle is not decreased by fat feeding (24, 25), which led to the suggestion that a decrease in the intrinsic activity of GLUT4 may be the cause of insulin resistance (25). However, Leturque

§It should be noted that the high-fat diet-mediated decrease of GLUT4 mRNA in adipose tissue was observed under feeding conditions but not fasting. Overnight fasting decreases GLUT4 mRNA level in adipose tissue and masks the high-fat-mediated GLUT4 decrease.

et al. (26) observed that a high-fat diet leads to both decreased GLUT4 protein and GLUT4 mRNA in skeletal muscle of weanling rats. Although adipose tissue is not considered a major site of glucose disposal, a decrease in the level of GLUT4 in adipose tissue may contribute in part to impaired glucose disposal. Shepherd *et al.* (27) recently reported that a 6- to 9-fold overexpression of GLUT4, which was limited to adipose tissue, resulted in a small improvement of glycemic control. In addition to the factors mentioned above that can contribute to insulin resistance in peripheral tissues, a reduced responsiveness of insulin secretion by the pancreatic β cell can lead to impaired glycemic control in animals fed a high-fat diet.

Whatever the cause of insulin resistance and hyperglycemia, the present study shows that a modest ($\leq$2-fold) increase in the level of GLUT4 protein in skeletal muscle, which is not affected by fat feeding, prevents the accompanying hyperglycemia and impaired glycemic control. Assuming that insulin resistance in muscle induced by fat feeding is due to a defective step in signal transmission between the insulin receptor and translocation of GLUT4 to the plasma membrane, it is surprising that a small ($\leq$2-fold) increase in the expression of GLUT4 would reverse the loss of glycemic control and the associated hyperglycemia caused by a high-fat diet. This implies that a significant fraction of the overexpressed glucose transporter must reach the plasma membrane of skeletal muscle cells despite a defect in signal transmission. We suggest, therefore, that alteration of the expression or activity of GLUT4 constitutes a rational target for intervention in the treatment of type 2 diabetes (NIDDM).

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