GLUT-1 or GLUT-4 transgenes in obese mice improve glucose tolerance but do not prevent insulin resistance

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Marshall, Bess Adkins, Polly A. Hansen, Nancy J. Ensor, M. Allison Ogden, and Mike Mueckler. GLUT-1 or GLUT-4 transgenes in obese mice improve glucose tolerance but do not prevent insulin resistance. Am. J. Physiol. 276 (Endocrinol. Metab. 39): E390–E400, 1999.—Insulin-stimulated glucose uptake is defective in patients with type 2 diabetes. To determine whether transgenic glucose transporter overexpression in muscle can prevent diabetes induced by a high-fat, high-sugar diet, singly (GLUT-1, GLUT-4) and doubly (GLUT-1 and -4) transgenic mice were placed on a high-fat, high-sugar diet or a standard chow diet. On the high-fat, high-sugar diet, wild-type but not transgenic mice developed fasting hyperglycemia and glucose intolerance (peak glucose of 337 ± 19 vs. 185–209 mg/dl in the same groups on the high-fat, high-sugar diet and 293 ± 13 vs. 166–194 mg/dl on standard chow). Hyperinsulinemic clamps showed that transporter overexpression elevated insulin-stimulated glucose utilization on standard chow (49 ± 4 mg·kg⁻¹·min⁻¹ in wild-type vs. 61 ± 4, 67 ± 5, and 63 ± 6 mg·kg⁻¹·min⁻¹ in GLUT-1, GLUT-4, and GLUT-1 and -4 transgenic mice given 20 μU·kg⁻¹·min⁻¹ insulin, and 54 ± 7, 85 ± 4, and 98 ± 11 in wild-type, GLUT-1, and GLUT-4 mice given 60–80 μU·kg⁻¹·min⁻¹ insulin). On the high-fat, high-sugar diet, wild-type and GLUT-1 transgenic mice displayed marked insulin resistance, but GLUT-4 and GLUT-1 and -4 mice were somewhat protected (glucose utilization during hyperinsulinemic clamp of 28.5 ± 3.4 vs. 42.4 ± 5.9, 51.2 ± 8.1, and 55.9 ± 4.9 mg·kg⁻¹·min⁻¹ in wild type, GLUT-1, GLUT-4, GLUT-1, and -4 mice). These data demonstrate that overexpression of GLUT-1 and/or GLUT-4 enhances whole body glucose utilization and prevents the development of fasting hyperglycemia and glucose intolerance induced by a high-fat, high-sugar diet. GLUT-4 overexpression improves the insulin resistance induced by the diet. We conclude that upregulation of glucose transporters in skeletal muscle may be an effective therapeutic approach to the treatment of human type 2 diabetes.

diabetes mellitus; obesity; transgenic mice; glucose clamp technique; glucose transport

THE PERIPHERAL INSULIN-SENSITIVE TISSUES express two facilitative glucose transporter isoforms, GLUT-1 (3, 38) and GLUT-4 (3, 7, 11, 24, 25). GLUT-1 is involved in basal glucose transport, whereas GLUT-4, the predominant transporter, accounts for the increase in glucose transport seen after insulin stimulation or contraction (2, 6, 10, 15, 21, 28, 47). Glucose transport is rate limiting for glucose metabolism in skeletal muscle and fat under most physiological conditions (43, 58), and a defect in glucose transport is responsible for the insulin resistance in type 2 diabetes mellitus (50).

Several groups have demonstrated that increasing the GLUT-4 content of skeletal muscle and/or fat by transgenic methods causes relative hypoglycemia, improves glucose tolerance, and increases insulin responsiveness in the whole animal or in isolated muscle (4, 9, 19, 31, 32, 42, 44, 51, 55, 56). In contrast, overexpression of GLUT-1 increases basal glucose transport markedly but causes impairment of insulin stimulation of transport (17, 43), perhaps due to elevation in skeletal muscle or adipocyte glucoseamine and its metabolites producing an impairment in the glucose transport response to insulin (5, 12, 45, 46, 49).

GLUT-4 overexpression prevents the development of hyperglycemia in genetic models of obesity and in obesity induced by a high-fat diet (13, 22). In mice with genetic obesity (db/db leptin receptor-deficient mice), overexpression of GLUT-4 in insulin-sensitive tissues delays the onset of hyperglycemia and β-cell failure but not of obesity (13). In mice on a high-fat diet, GLUT-4 overexpression prevents the development of fasting hyperglycemia but not of obesity (22). However, overexpression of GLUT-4 in white fat alone does not prevent the development of hyperglycemia or glucose intolerance on a high-fat diet (14).

The available evidence thus indicates that overexpression of GLUT-4 in muscle can ameliorate the hyperglycemia associated with some animal models of diabetes. Therefore, glucose transporter overexpression or upregulation in muscle may be an effective means of treating human diabetes. However, several fundamental questions remain unanswered concerning the interaction between glucose transporter overexpression and diabetes in these animal models. 1) Does GLUT-4 overexpression in muscle counteract diabetic hyperglycemia by preventing the development of insulin resistance and enhancing whole body glucose disposal or by an indirect mechanism? 2) Can GLUT-1 overexpression in muscle also prevent or ameliorate the development of diabetic hyperglycemia, and, if so, does it also act by a direct mechanism involving enhanced glucose uptake and disposal in muscle? 3) Is the overexpression of both GLUT-1 and GLUT-4 in muscle more effective in counteracting diabetic hyperglycemia than the overexpression of either isoform alone, and if so, are there any deleterious side effects of combined overexpression? 4) Does a diabetogenic diet influence the expression of the GLUT-1 or GLUT-4 transgenes in skeletal muscle? 5) Does overexpression of GLUT-1 in muscle increase only the basal rate of glucose disposal, and GLUT-4 only the insulin-stimulated rate of glucose disposal, as would be...
expected on the basis of the current model of GLUT-1 and GLUT-4 function in muscle?

To address these questions, we placed transgenic mice, overexpressing either GLUT-1, GLUT-4, or both transporter isoforms, on a high-fat, high-sugar diet shown by Surwit et al. (53) to induce obesity and fasting hyperglycemia. We chose this particular regimen because it is perhaps the closest model to the common form of obesity-associated human type 2 diabetes mellitus available in the mouse. We then assessed the ability of the transporter isoforms to influence the development of obesity, glucose intolerance, fasting hyperglycemia, insulin resistance, glucose transport, and glucose-transporter content of muscle.

METHODS

Animals

Transgenic mouse lines that overexpress GLUT-1 in skeletal muscle or GLUT-4 in skeletal muscle, fat, and heart, described previously (33, 36), were bred with a female heterozygous for the GLUT-1 transgene and a male heterozygous for the GLUT-4 transgene. Litters from these crosses contained mice carrying one, two, or neither of the transgenes. Sixteen such litters were placed at the time of weaning on high-fat, high simple-carbohydrate diet consisting primarily of lard, sucrose, and casein: 20% protein, 36.5% fat, 36.6% carbohydrate (67% mono- and disaccharides), 10% moisture, 3.7% ash, and 0.1% fiber (BioServe diet no. F3282, Frenchtown, NJ) described by Surwit et al. (53). The remaining mice were fed Purina 5001 laboratory rodent diet: 23% protein, 5.5% fat, 49% carbohydrate (65% as starch), 5.3% fiber, and 6.9% ash. The mice were housed in an approved, temperature-controlled facility on a 6 AM-6 PM light-dark cycle and had access to water ad libitum. All protocols were approved in advance by the Washington University Animal Studies Committee.

Experimental Protocols

Hyperinsulinemic clamp. Clamp experiments were carried out as previously described (35) with the following modifications. After placement of the infusion catheter, an infusion of HPLC-purified [3-3H]glucose (Du Pont-NEN, Boston, MA) at 0.04 µCi/min was begun for measurement of the rate of appearance of glucose, hepatic glucose production, and total body glucose utilization. The infusion was continued during a 1-h control period, and 20 μl of blood were taken from the tail for determination of glucose specific activity at 45 and 60 min. After 60 min, an infusion of insulin (regular human, Eli Lilly, Indianapolis, IN) was begun and continued for ≥90 min for each experimental period.

In the mice fed the high-fat, high-carbohydrate diet, the insulin infusion rate was 20 μU·kg⁻¹·min⁻¹. Dextrose (25%) was begun, and the infusion rate was varied during the first clamp period to maintain the blood glucose at the level of appearance during the last 15 min of the control period. This was done in an attempt to isolate the effect of hyperinsulinemia, because the blood glucose during the basal period differed significantly between the genotypes. During the second “euglycemic” clamp period, the blood glucose was adjusted to maintain the blood glucose at ~170 mg/dl, the average blood glucose in a freely feeding, wild-type, conscious mouse of these strains in our hands (35).

In one group of mice on the standard diet (20 μU·kg⁻¹·min⁻¹ insulin infusion rate group), the same protocol was used in one-half of the mice, and in the other one-half, the first clamp period was left out. Data from the euglycemic (170 mg/dl) clamp period did not differ whether the first clamp was included or not, so the data are combined. In the second group of mice on the standard diet (60–80 μU·kg⁻¹·min⁻¹ insulin infusion rate group), only a euglycemic clamp period was carried out.

In all the clamp experiments, the continuous infusion of [3-3H]glucose tracer was continued during the insulin infusion periods and, in addition, the tracer was added to the 25% dextrose infusion to approximate the glucose specific activity in the blood at the end of the control period. This approximation was based on measurement of specific activity during identical conditions in the same type of mice in previous experiments. Blood samples for determination of specific activity were taken 15 min before and at the end of the experimental period. The glucose infusion rate was not changed for ≥20 min before the first determination of specific activity. Both the blood glucose and the glucose specific activity were in steady state during these 15-min sampling periods. Blood for hormone and metabolite measurement was obtained by cardiac puncture at the conclusion of the experiment. Tissues (skeletal muscle and epididymal fat) were harvested and snap-frozen in liquid nitrogen at the conclusion of the experiment to confirm genotyping.

Glucose tolerance tests. Mice were fasted for 5 h and then weighed. The tip of the tail was clipped, and blood glucose was measured. Then the mice were injected intraperitoneally with 10% dextrose (1 mg/g body wt), and blood glucose was measured subsequently, at times shown, from the tail without a recutting of the tail.

Measurement of glucose transport activity. Wild-type and GLUT-4 transgenic littermates were fed the Surwit diet for 4 wk. On the day of study, the animals were anesthetized with pentobarbital sodium. Soleus muscle was excised bilaterally for determination of transport activity. Muscles were incubated for 30 min at 35°C in 2 ml of oxygenated Krebs-Henseleit buffer (KHB) supplemented with 8 mM glucose, 32 mM mannitol, and 0.1% BSA in the absence or presence of insulin (2 μU/ml). The gas phase throughout the incubation was 95% O₂-5% CO₂.

After the initial incubation period, all muscles were transferred to KHB containing 40 mM mannitol, 0.1% BSA, and insulin at the concentration present in the previous incubation medium for 10 min to remove glucose from the extracellular space before measurement of glucose transport activity. The gas phase in the flasks was 95% O₂-5% CO₂, and the temperature was 29°C.

2-Deoxy-o-glucose (2-DG) transport was measured as described previously (18, 57). After the rinse, muscles were incubated for 20 min in 1.5 ml KHB containing 1 mM 2-deoxy-o-[1,2-3H]glucose (1.50 µCi/ml), 39 mM [U-14C]mannitol (0.2 µCi/ml), and 0.1% BSA. Insulin was added if it was present in the previous incubations. Muscles were processed by boiling, and the extracellular space and intracellular 2-DG concentration (µmol·ml intracellular water⁻¹·20 min⁻¹) were determined as described previously (18, 57).

Analytical procedures. Blood glucose was measured with 5 μl of whole blood in the Hemocue blood glucose meter (Mission Viejo, CA). Plasma insulin, free fatty acids, blood lactate, and β-hydroxybutyrate were measured as previously described (35).

Specific activity of glucose in whole blood was determined by aqueous scintillation counting of 20 μl of blood deproteinized with barium hydroxide (0.3 N) and zinc sulfate (0.3 N) (Sigma, St. Louis, MO). The supernatant resulting from the deproteinization was dried at 70°C to remove tritiated water before resuspension and counting. The rate of appearance of the glucose tracer was calculated from the average blood glucose concentration over the 15-min period and the specific activity of glucose measured at 45 and 60 min of the control period.
glucose (R_a), which equals the rate of total body glucose utilization (R_d) when the blood glucose is in steady state, was calculated by dividing the infusion rate of [3-3H]glucose by the specific activity at the same time. Hepatic glucose production was calculated by subtracting the cold glucose infusion rate from the rate of appearance of glucose.

Percent body fat was determined by solubilizing the entire carcass in ethanolic potassium hydroxide (35% 3 M KOH-65% ethanol) overnight at 70°C (150–250 ml as required to solubilize all visible lipid). Triglyceride content of the resulting solution was measured with a semienzymatic, quantitative triglyceride assay kit (Sigma).

Statistical analysis. Data were analyzed with analysis of variance or unpaired t-test and Fisher’s post hoc test with StatView 4.51 software (Abacus Concepts, Berkeley, CA).

RESULTS

Overexpression of Glucose Transporters Alters Blood Metabolite and Hormone Levels in Chow-Fed Mice

Overexpression of GLUT-1 or GLUT-4 lowered the blood glucose concentration by 13–24% in males or females in either the fed or fasted state, consistent with previous reports. Overexpression of both transporters in the same animal lowered the blood glucose by an additional 4–9%. The blood β-hydroxybutyrate and lactate concentrations were or tended to be higher in all groups of transgenic mice in both the fed and fasted states, consistent with previously published data from these lines of mice (data not shown) (35, 36, 44, 55).

Effect of the Surwit High-Fat, High-Sugar Diet on Body Mass, Blood Glucose, and Mortality in Wild-Type and Transgenic Mice

Feeding the Surwit diet from the time of weaning produced obesity in all four groups of mice relative to age-matched mice maintained on a standard chow diet (Fig. 1A), but the GLUT-1 and -4 transgenic mice were the least obese, and the GLUT-4 transgenic mice were the most obese. The percent body fat in mice fed the Surwit diet was markedly higher than in age-matched mice on standard chow (Fig. 1B), but no consistent effect of the transgenes on percent body fat was observed. The blood glucose levels in the mice fell initially after the start of the Surwit diet and then began to rise gradually in the wild-type mice after day 90. The blood glucose levels continued to fall in all three transgenic groups (Fig. 2). Interestingly, a significant number of the GLUT-1 and -4 doubly transgenic mice died soon after beginning the diet (in one cohort, 10 of 15 of the GLUT-1- and -4 mice of both sexes died before 100 days compared with none of 23 wild-type, 12 GLUT-1, and 24 GLUT-4 mice). The cause of the deaths is unclear. The GLUT-1 and -4 mice that died tended to be smaller. The five males that died weighed 15.4 ± 2.1 g on the first day of the Surwit diet, compared with the five male GLUT-1- and -4 mice that survived for 180 days that weighed 19.8 ± 1.1 g on the first day of the Surwit diet. In a subgroup of seven GLUT-1- and -4 transgenic mice, blood glucose in the freely feeding state was measured 5 days after the start of the Surwit diet. The blood glucose had fallen from 116 ± 8 mg/dl before the diet to 101 ± 10 mg/dl. It is possible that the deaths were due to the GLUT-1- and -4 transgenic mice becoming excessively hypoglycemic after starting the high-fat, high-sugar diet. We have observed that the doubly transgenic mice are more susceptible to death after being subjected to overnight fasting and subsequent bleeding procedures. However, they appear to live a normal life span when left unperturbed on a normal chow diet.

Overexpression of Glucose Transporters Enhances Glucose Tolerance in Chow-Fed Mice

Overexpression of either GLUT-1 or GLUT-4 in male (Fig. 3A) or female (data not shown) mice lowered the peak glucose excursion and hastened the time to recovery of normoglycemia during intraperitoneal glucose tolerance testing. Overexpressing both transporters
in the same animal did not further improve glucose tolerance, although it did lower the initial fasting blood glucose concentration.

The Surwit Diet Impairs Glucose Tolerance in Wild-Type But Not in Transgenic Mice

Glucose tolerance in the wild-type mice deteriorated significantly when mice were fed the Surwit diet compared with mice of the same age on a chow diet (Fig. 3B). The fasting blood glucose was elevated in the wild-type male mice, at 183 ± 8 mg/dl (n = 19), compared with 149 ± 7 mg/dl (n = 17) in the chow-fed mice, and the blood glucose at 1 h was 349 ± 20 mg/dl compared with 263 ± 12 mg/dl in chow-fed male mice (Fig. 4). Data in female mice were similar but with lower blood glucose concentrations overall (data not shown). In contrast to the wild-type mice, the fasting and 1-h blood glucose concentrations in the three groups of transgenic mice on the Surwit diet were not different from those of chow-fed control mice for either males or females.

The Surwit Diet Induces Peripheral Insulin Resistance in Wild-Type Mice and GLUT-1-Overexpressing Mice But Not in GLUT-4-Overexpressing Mice

Hyperinsulinemic-euglycemic clamps were carried out to assess insulin responsiveness on a standard diet or the Surwit diet.

Basal blood glucose concentrations. On the chow diet, the average blood glucose concentration during the last 15 min of the control period was ~25% lower in the GLUT-1 and GLUT-4 mice and ~30% lower in the mice bearing both transgenes, compared with the wild-type mice (Fig. 4A). These differences were even more marked in the mice on the Surwit diet, in which the GLUT-1, GLUT-4, and doubly transgenic mice had blood glucose concentrations 27, 40, and 45% lower than wild-type mice, respectively. The differences between the mice on the two different diets were primarily due to an elevation in the blood glucose of wild-type mice on the Surwit diet.

Insulin concentrations. Insulin at the end of the hyperinsulinemic clamp periods tended to be higher in the mice on the Surwit diet than in those on the standard chow diet despite identical infusion rates, but the differences were statistically significant only in the wild-type mice (Table 1). Because of the discrepancy in the insulin concentrations, additional clamp experiments were performed with higher insulin infusion rates to provide a group in which insulin concentrations, rather than insulin infusion rates, were more closely matched. An exact match was difficult to achieve (Table 1), but a group with insulin levels approximately twice those in the Surwit diet-fed mice is shown.
Clamped blood glucose concentrations. During the last 15 min of the first hyperinsulinemic clamp period, the blood glucose concentrations were matched to those seen in the basal period in an attempt to separate the effect of hyperinsulinemia alone from the combination of hyperinsulinemia and a change in blood glucose concentration. During the second clamp period, blood glucose concentrations were normalized to the blood concentration. During the second clamp period, blood glucose concentrations were matched to those seen in the basal period and the two clamp periods.

Basal glucose utilization rates. The basal rate of tracer-determined glucose utilization tended to be elevated in the GLUT-1 and GLUT-4 mice, in agreement with previous reports (44, 55, 56). Hepatic glucose production was not entirely suppressed from that seen in the basal period in any of the four genotypic groups on either diet (data not shown), which is consistent with data in larger animals during anesthesia (8, 23, 54).

Table 1. Insulin concentrations during hyperinsulinemic clamps using insulin infusion rates given in 5-h-fasted mice on standard chow or Surwit diet

<table>
<thead>
<tr>
<th>Insulin Rate</th>
<th>Chow Fed, Clamped with 20 mU·kg⁻¹·min⁻¹</th>
<th>Chow Fed, Clamped with 60–80 mU·kg⁻¹·min⁻¹</th>
<th>Surwit Diet Fed, Clamped with 20 mU·kg⁻¹·min⁻¹</th>
<th>Surwit Diet Fed, Clamped with 60–80 mU·kg⁻¹·min⁻¹</th>
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<tbody>
<tr>
<td>Wild type</td>
<td>365 ± 48 (6)</td>
<td>3,747 ± 515* (3)</td>
<td>1,182 ± 344† (5)</td>
<td>3,747 ± 515* (3)</td>
</tr>
<tr>
<td>GLUT-1</td>
<td>313 ± 39 (8)</td>
<td>2,273 ± 45† (2)</td>
<td>1,180 ± 696* (3)</td>
<td>2,273 ± 45† (2)</td>
</tr>
<tr>
<td>GLUT-4</td>
<td>352 ± 57† (7)</td>
<td>2,406 ± 306* (3)</td>
<td>1,019 ± 481† (3)</td>
<td>2,406 ± 306* (3)</td>
</tr>
<tr>
<td>GLUT-1 and -4</td>
<td>213 ± 43 (2)</td>
<td>—</td>
<td>1,408 ± 726 (5)</td>
<td>—</td>
</tr>
</tbody>
</table>

Data shown are means ± SE and are measured in µU/ml. Nos. in parens, no. of mice per group. *Differs from chow-fed 20 mU·kg⁻¹·min⁻¹ insulin rate group of same genotype (P < 0.05). †Differs from chow-fed 60–80 mU·kg⁻¹·min⁻¹ insulin rate group of same genotype (P < 0.05).
In the second clamp period, when blood glucose concentrations were equalized in the four genotypic groups, overexpression of either GLUT-1 or GLUT-4, or both, increased the rate of glucose utilization on either diet (Fig. 4B). On the standard chow diet, the wild-type mice showed equivalent rates of glucose utilization: 49.0 ± 4.3 and 53.5 ± 7.4 mg·kg⁻¹·min⁻¹ at insulin concentrations of 365 µU/ml and ten times that, respectively (see Table 1), indicating that either insulin level is maximally stimulating in wild-type mice. In contrast, the GLUT-1- and the GLUT-4-overexpressing mice have significantly higher rates of glucose utilization when studied with higher insulin infusion rates (in mice have significantly higher rates of glucose utilization below that of chow-fed wild-type mice. Therefore, overexpression of transporters appears to raise the insulin dose-response curve in mice.

The Surwit diet induced marked insulin resistance in the wild-type mice, in which the rate of total body glucose utilization was ~45% lower in the Surwit diet-fed group compared with either chow-fed group. The GLUT-1-overexpressing mice had a 30% impairment in the rate of total body glucose utilization on the Surwit diet compared with the 20 mU·kg⁻¹·min⁻¹ insulin infusion rate chow-fed group and a 50% impairment compared with the 60–80 mU·kg⁻¹·min⁻¹ insulin infusion rate group, resulting in a rate of glucose utilization below that of chow-fed wild-type mice. However, overexpression of GLUT-4 raised the rate of glucose utilization in the GLUT-4-overexpressing mice on the Surwit diet was higher than that in wild-type chow-fed mice (although lower than in chow-fed GLUT-4-overexpressing mice).

Interestingly, in both the chow-fed and the Surwit diet-fed mice, the increment in the rate of glucose utilization with insulin was similar in the wild-type and GLUT-1 mice (31.3 and 35.3 mg·kg⁻¹·min⁻¹ for wild-type and GLUT-1 chow-fed mice and 12.3 and 18.6 mg·kg⁻¹·min⁻¹ for wild-type and GLUT-1 Surwit diet-fed mice, respectively, and higher in the GLUT-4 and GLUT-1 and -4 mice; 49.0 and 41.3 mg·kg⁻¹·min⁻¹ for GLUT-4 and GLUT-1 and -4 chow-fed mice and 37.4 and 30.3 mg·kg⁻¹·min⁻¹ for GLUT-4 and GLUT-1 and -4 Surwit diet-fed mice, respectively, with data from the second clamp period and 20 mU·kg⁻¹·min⁻¹ insulin infusion rate). These data agree with the hypothesis that GLUT-1 is involved primarily in basal muscle glucose transport, whereas GLUT-4 is responsible for the increment in glucose uptake in response to insulin.

Metabolites. The blood lactate concentration tended to be higher in the transgenic mice than in wild-type mice on chow (1.4 ± 0.2, 3.7 ± 0.5, 4.6 ± 1.7, and 2.9 ± 0.7 mM in wild type, GLUT-1, GLUT-4, and GLUT-1 and -4, respectively, with GLUT-4 significantly higher than wild type). The Surwit diet tended to raise the lactate concentration compared with mice on the chow diet, but this was significant only in the wild type and GLUT-1 and -4 mice (3.5 ± 0.4, 5.9 ± 0.1, 3.6 ± 0.6, and 5.6 ± 0.7 mM in wild type, GLUT-1, GLUT-4, and GLUT-1 and -4, respectively). The blood β-hydroxybutyrate concentration was suppressed in all groups of mice at the end of the clamp (range of 120–410 µM).

The Surwit Diet Did Not Decrease Glucose Transporter Expression in Muscle

The high-fat diet did not induce insulin resistance by decreasing the level of expression of the endogenous or transgenically expressed GLUT-1 or GLUT-4 protein in quadriceps muscle (Fig. 5). In fact, expression of endogenous GLUT-4 in the GLUT-1 transgenic and expression of total GLUT-4 in the GLUT-1 and -4 doubly transgenic mice were higher in mice on the Surwit diet than in age-matched mice on standard chow. Expression of GLUT-1, either endogenous or from the GLUT-1 transgene, was not altered by the Surwit diet.
diet in muscle. In subcutaneous fat, expression of GLUT-4, both endogenous and transgenically expressed, was ~50% lower in all three groups of transgenic mice on the Surwit diet compared with fat from age-matched mice on standard chow (data not shown).

Glucose Transport in Soleus Muscle from Mice on the Surwit Diet Is Impaired in Wild-Type but Not GLUT-4 Transgenic Mice

Wild-type mice fed the Surwit diet for only 4 wk showed a 35% impairment in 2-DG uptake compared with littermates fed standard chow (Fig. 6A). In contrast, GLUT-4 transgenic littermate mice show no impairment (Fig. 6B). After 4 wk on the Surwit diet, there was no change in endogenous or transgenically expressed GLUT-4 content of the muscle, but body mass, percent total body lipids, and plasma insulin had already risen significantly (Marshall and Hansen, unpublished data).

DISCUSSION

These studies demonstrate that overexpression of either GLUT-1 or GLUT-4 in muscle can prevent the development of hyperglycemia and glucose intolerance in response to a high-fat, high-sugar diet described by Surwit et al. (53). This diet has been previously shown to cause obesity and hyperglycemia in mice (53), and the hyperinsulinemic clamp experiments described herein demonstrate that the diet induces marked insulin resistance in wild-type mice. These studies also demonstrate that overexpression of GLUT-4 prevented the development of impaired glucose transport in skeletal muscle and, in part, prevented the development of insulin resistance on this diet by enhancing insulin-stimulated whole body glucose disposal. GLUT-1 overexpression tended to increase the basal rate of whole body glucose utilization and prevented the development of hyperglycemia and glucose intolerance in mice on the Surwit diet. The Surwit diet did not decrease expression of either the GLUT-1 or the GLUT-4 transgene or of endogenous GLUT-1 or GLUT-4 in muscle. Surprisingly, GLUT-4 expression in muscle was increased on the Surwit diet in those mice carrying the GLUT-1 transgene. The mice overexpressing both transporters did not develop glucose intolerance or hyperglycemia on the Surwit diet. Unfortunately, overexpression of GLUT-1 and GLUT-4 in combination clearly increased mortality in mice fed the high-fat, high-sugar diet, possibly due to devastating hypoglycemia soon after the diet was started. This observation suggests that care must be exercised with regard to the level of therapeutic overexpression of glucose transporters in muscle.

Overexpression of GLUT-1 raised the basal rate of glucose utilization without altering the increment in utilization caused by insulin infusion. Overexpression of GLUT-4 had a small effect on basal glucose utilization, but its primary effect was to increase the increment in glucose utilization observed in response to insulin. Overexpression of GLUT-4 had a small effect on basal glucose utilization, but its primary effect was to increase the increment in glucose utilization observed in response to insulin. Overexpression of GLUT-4 altered the in vivo insulin dose-response curve as well, allowing glucose utilization rates to continue to rise with increasing insulin dose beyond that seen in wild-type mice.

Overexpression of either GLUT-1 or GLUT-4 did not prevent the development of obesity on the high-fat, high-sugar diet. Others have reported that overexpression of GLUT-4 does not prevent obesity on a high-fat diet or in the genetically obese db/db mouse (13, 22). Overexpression of GLUT-1 and GLUT-4 together reduced the degree of weight gain that occurred on the
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Surwit diet but at the expense of unexplained mortality. The mechanism of the glucose intolerance and insulin resistance associated with obesity is not known, but these data indicate that as early as 4 wk after the start of the Surwit diet, an impairment in 2-DG uptake in muscle is present. Insulin resistance appears soon after a high-fat diet is begun in adipose, muscle, and hepatic tissue in vivo in humans, rats, and mice (14, 29, 34, 52). GLUT-4 protein in fat is decreased in rats (26, 41) or mice (14) on a high-fat diet but not in muscle (30, 39, 48), as was seen in this study. However, preventing the decline in GLUT-4 expression in fat in mice by transgenic overexpression of GLUT-4 specifically in fat does not prevent the development of glucose intolerance (14). Various groups have reported that a high-fat diet induces defects in muscle insulin receptor binding (16), postreceptor defects (16, 39) including a defect in insulin stimulation of GLUT-4 activation (48) or translocation (59), a defect in activation of phosphatidylinositol 3-kinase by insulin, and perhaps an additional defect further downstream in the insulin signaling pathway (59). The data presented here show that if GLUT-4 transporter content is increased transgenically, then insulin-stimulated glucose utilization in an obese mouse can be normal compared with that in a wild-type nonobese mouse. However, compared with GLUT-1- or GLUT-4-overexpressing nonobese mice, the obese transporter-overexpressing mice are insulin resistant. This could be either because the Surwit diet impairs all transporters proportionately, whether endogenous or transgenically expressed, or because the diet impairs another step or steps besides transport. The normal insulin response found in the GLUT-4-overexpressing isolated muscle from mice fed the Surwit diet for 4 wk indicates that if any defects develop in transport in the transgenic mice, they must occur later than 4 wk.

The data in the chow-fed GLUT-4-overexpressing mice are qualitatively similar to those published previously (9, 22, 31, 35, 44, 51, 56). There are some variations in the absolute measures of blood glucose, circulating metabolites, plasma insulin levels, and rate of glucose utilization that are attributable to differences in duration of fasting, age of the animals, and anesthesia. Four papers in the literature, including two from this group, have measured insulin responsiveness with clamp techniques (9, 35, 44, 56). The first two did not measure hepatic glucose production during hyperinsulinemic clamp but based the determination of insulin responsiveness on the glucose infusion rate required to maintain euglycemia (9, 35). Therefore, these most likely underestimated the rate of glucose utilization during insulin infusion because insulin, even at the high dose given in the present studies, did not markedly suppress hepatic glucose utilization after pentobarbital anesthesia in mice. The studies differed in the length of fast, as the mice in the present study were fasted 5 h, and in the previous studies they were fasted overnight or not at all (9, 35, 44, 56). One would expect a longer fast to produce a lower rate of hepatic glucose production, a lower rate of total glucose utilization, and relative insulin resistance, as was seen in a subsequent study in conscious mice of this GLUT-4-overexpressing line (44). The mice in our earlier study were considerably younger (2 mo vs. 4–12 mo) than those in the present study and so may have been more insulin sensitive in general (35). Tsao et al. (56) have shown that insulin sensitivity, as assessed informally by comparing blood glucose and plasma insulin, declines markedly in the mouse between 2 and 4–6 mo in at least one strain (56). The data gathered in the present study did not show a difference in blood glucose or plasma insulin from age 3 mo to 18 mo but did not examine mice as young as 2 mo of age. The one set of clamp studies conducted on conscious GLUT-4-overexpressing mice showed higher rates of tracer-determined total glucose utilization, as would be expected in nonanesthetized animals (44).

Whereas a number of groups have studied mice overexpressing GLUT-4, only a single previous paper from this group examined insulin responsiveness in the whole mouse overexpressing the GLUT-1 isoform, and that study found insulin resistance in the GLUT-1 transgenic mice (35). This previous study, as noted above, did not measure tracer-determined glucose utilization but used the glucose infusion rate to assess insulin responsiveness, with the assumption of a low hepatic glucose production rate with insulin. A high rate of hepatic glucose production was present in the anesthetized GLUT-1-overexpressing mice in the presence or absence of insulin, which would make the error in the GLUT-1 mice in those former studies larger than in the GLUT-4 mice or wild-type mice and make the GLUT-1 mice appear more insulin resistant. In addition, the younger age of the mice in the former studies may have made the wild-type mice more insulin sensitive, and the longer fast may have made the GLUT-1 mice more insulin resistant, thus accentuating the difference in apparent insulin responsiveness between the wild-type and transgenic mice. The GLUT-1 mice have elevated hexosamine concentrations in muscle (5), which have been shown to cause insulin resistance (1, 20, 37, 46, 49). It is possible that the insulin resistance that develops normally with age is appearing more rapidly in the GLUT-1 mice because of this hexosamine accumulation, so that when young GLUT-1 mice are compared with equally young wild-type mice as in the earlier clamp and in vitro studies, the GLUT-1 mice show relative insulin resistance, whereas if they are compared with older wild-type mice, as in this study, little difference is seen because the wild-type mice have “caught up” in developing age-related insulin resistance.

In isolated muscle from the GLUT-1 transgenic mice, glucose transport (assessed with uptake of 2-DG or 3-O-methylglucose) is not responsive to insulin (17, 43) or to high-fat feeding (19a). In the whole animal, however, the clamp data indicate a significant insulin response. The reason for this paradox is not entirely clear. Photolabeling studies in muscle from these GLUT-1-overexpressing mice show that GLUT-4 does translo-
icate in response to insulin, suggesting that the failure of insulin to increase transport of glucose analogs in this muscle is due to effects on the intrinsic activity of GLUT-4 (19b), which may be in some way exacerbated in isolated muscle compared with the whole animal. The presence of free glucose intracellularly indicates that in the GLUT-1-overexpressing muscle, transport is no longer rate limiting for glucose metabolism (43). The hyperinsulinemic clamp measures glucose metabolism not only at the transport step but at steps both proximal, such as blood flow and glucose delivery to the cell surface, and distal, such as phosphorylation and glyogen synthesis. The results of the clamp data in the GLUT-1-overexpressing mice therefore are consistent with a normal response of these other steps to insulin and may suggest that hexokinase, which apparently becomes rate limiting for glucose metabolism in the GLUT-1-overexpressing mice, may be responsive to insulin in this setting.

In conclusion, overexpression of either GLUT-1 or GLUT-4 in muscle, fat, and heart, or a combination of GLUT-1 and GLUT-4 produces relative hypoglycemia and increases glucose tolerance in mature, Chow-fed mice. Transporter overexpression increases total insulin-stimulated glucose utilization, but only overexpression of GLUT-4 markedly increases the increment in glucose utilization due to insulin. In addition, overexpression of transporters prevents the development of fasting hyperglycemia, glucose intolerance, and, in the case of GLUT-4, insulin resistance caused by a high-fat, high-sugar (Surwit) diet. These studies suggest that increasing either GLUT-1 or GLUT-4 activity by pharmacological or genetic means may be effective in relieving or preventing the complications of obesity and type 2 diabetes.

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