Glucose transporter isoform-3-null heterozygous mutation causes sexually dimorphic adiposity with insulin resistance

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Ganguly A, Devaskar SU. Glucose transporter isoform-3 null heterozygous mutation causes sexually dimorphic adiposity with insulin resistance. Am J Physiol Endocrinol Metab 294: E1144–E1151, 2008. First published April 29, 2008; doi:10.1152/ajpendo.90251.2008.—We examined male and female glucose transporter isoform-3 (GLUT3; placenta)-null heterozygous+/− mutation-carrying mice and compared them with age- and sex-matched wild-type+/+ littermates. No difference in postnatal (1–2 days, 6–7 days, 12–13 days, 20–21 days), postsuckling (1–2 mo), and adult (3–6 mo) growth pattern was seen except for an increase in body weight of 9–11-mo-old male but not female GLUT3+/− mice. This change in male mutant mice was associated with increased total body fat mass, perirenal and epididymal white adipose tissue weight, and hepatic lipid infiltration. These minimally glucose-intolerant male mutant mice demonstrated no change in caloric intake but a decline in basal metabolic rate and insulin resistance. No perturbation in basal circulating glucose concentrations but an increase in insulin concentrations, triglycerides, and total cholesterol was observed in GLUT3+/− male mice. Tissue analysis in males and females demonstrated diminished GLUT3 protein in GLUT3+/− brain and skeletal muscle with no change in brain and adipose tissue GLUT1 protein concentrations. Furthermore, the male GLUT3+/− mice expressed decreased insulin-responsive GLUT4 in white adipose tissue and skeletal muscle sarcolemma. We conclude that the GLUT3+/− male mice develop adult-onset adiposity with insulin resistance.

FACILITATIVE GLUCOSE TRANSPORTER ISOFORM-3 (GLUT3), the most efficient glucose transporter that mediates basal glucose transport, is expressed predominantly in placenta, testis, and neurons (6, 8, 13). This isoform plays a critical role in mediating transplacental glucose transport that is essential for fueling fetal growth and energy metabolism (3, 12, 14). GLUT3+/− null homozygous mutation was embryonically lethal, whereas heterozygous+/− mutation caused a decline in late-gestation transplacental glucose transport and fetal growth (9). Studies involving nutrient restriction that induce fetal growth restriction demonstrate aberrations in the adult metabolic phenotype. In adult mice subjected to intrauterine caloric restriction, glucose intolerance has been reported mainly in males (11). Previous investigation employing GLUT3+/− mice was limited to the 2-mo-old adult stage and revealed no overt metabolic changes (9). Thus, we hypothesized that the altered fetal nutrition encountered in GLUT3+/− mice may alter the adult phenotype at a later age. To test this hypothesis, we systematically examined the male and female GLUT3+/− mice at various ages spanning 1–2 days to 11 mo and characterized the phenotype.

EXPERIMENTAL PROCEDURES

Animals. The study protocol was approved by the Animal Research Committee of the University of California Los Angeles (UCLA) in accordance with the guidelines set by the National Institutes of Health. Mice were housed in 12:12-h light-dark cycles with ad libitum access to a standard rodent chow (4 kcal/g; Harlan Teklad 7013) and water and maintained in approved mouse-housing areas. The GLUT3+/− C57BL6 mice and their wild-type+/+ C57BL6 littermates as described in detail previously (9) were employed for the study.

Genotyping of mice. Tail DNA genotyping was carried out by the polymerase chain reaction using a forward primer from exon 6 of the GLUT3 gene (5′-cgtagctgtcgaatcggag-3′) and a reverse primer from the neomycin resistance gene (5′-agaggctatgctgtacg-3′). Amplification was carried out in a 50-μl reaction over 30 cycles with an initial temperature of 95°C for 1 min, denaturation at 95°C for 30 s, annealing at 58°C for 30 s, and extension at 72°C for 30 s and finally set at 72°C for 5 min. Amplified products were separated on a 1% agarose gel and the size of the ethidium bromide-stained DNA products confirmed by comparison with standard DNA size markers. Amplification of glyceraldehyde-3-phosphate dehydrogenase DNA served as the internal control to confirm integrity of the extracted tail genomic DNA. The presence of an amplification product signified the GLUT3+/− mice, whereas the absence supported the GLUT3+/+ genotype (Fig. 1).

Body and organ weight. Postnatal and adult body weights were measured using an Ohaus LS 2000 top-loading balance with an accuracy of 1 g (adults at all ages) and the Mettler AB104 precision balance with an accuracy of 0.01 mg (postnatal mice). In addition, organ weights consisting of liver, brain, heart, and different white fat depots such as perirenal and epididymal fat in males and abdominal fat in females were also measured.

Caloric intake. Food intake was assessed accurately by weighing the food at the beginning and end of a 24-h period, taking into account loss due to spillage and evaporation per wild-type or GLUT3−/− male and female mice at 21 days and 9 mo of age.

Body composition. Whole body fat and lean mass were assessed in unanesthetized freely moving male and female 9-mo-old GLUT3+/+ and GLUT3+/− mice using 1H-magnetic resonance spectroscopy (Bruker minispectroscopy analyzer; Echo Medical Systems, Houston, TX).

Glucose and insulin tolerance tests. Following an overnight fast, 9-mo-old GLUT3+/+ or GLUT3+/− male and female mice received intraperitoneally either 0-glucose (1 g/kg body wt) or insulin (0.5 U/kg body wt of pharmaceutical grade; Novo-Nordisk, Clayton, NC). Tail vein blood samples were collected at 0, 15, 30, 45, 60, 75, 105, and 120 min and blood glucose concentrations assessed using the Hemocue system (Mission Viejo, CA).

Plasma glucose, insulin, leptin, and lipid profile measurements. After 9-mo-old mice were euthanized, intracardiac blood samples were collected and plasma separated. Plasma glucose concentrations...
were measured using a Beckman glucose analyzer and plasma insulin and leptin concentrations assessed by ELISA kits (Linco Research, St. Charles, MO; sensitivity: insulin = 0.1 ng/ml, leptin = 0.5 ng/ml). In addition, serum triacylglycerol, cholesterol, high-density lipoprotein (HDL), unesterified cholesterol, and free fatty acids were measured by colorimetric assays, as described previously (7). Plasma glycerol concentrations were determined and used to correct the triacylglycerol values. The HDL cholesterol was derived from the measurement of the supernatant after the precipitation of apoB-containing lipoproteins with heparin and MnCl₂ (7). Each lipid determination was conducted in triplicate. An external control sample with known analyte concentration was run in each plate to ensure accuracy. All lipids were analyzed by the UCLA Lipid and Lipoprotein Laboratory, which is certified by Centers for Disease Control and Prevention and the National Heart, Lung, and Blood Institute Lipid Standardization Program.

Morphology of liver. Livers from 9-mo-old GLUT3⁺/⁺ and GLUT3⁻/⁻ male mice were obtained and fixed with 4% paraformaldehyde in 0.1 M sodium phosphate buffer, pH 7.4. Paraffin sections (6 mm) were then prepared and stained with hematoxylin and eosin. The stained sections were viewed by a Nikon E-600 microscope (Nikon, Melville, NY) and the images obtained using the Metamorph Image analysis software program.

Skeletal muscle preparation. Homogenates and subcellular fractions were prepared from hindlimb skeletal muscle of 9-mo-old GLUT3⁺/⁺ and GLUT3⁻/⁻ male mice. Plasma membrane (PM) and low-density microsomal subfractions were isolated as described previously (17, 18), and the relative purity was determined with enzyme markers (17). The homogenate, PM, and low-density microsomes (LDM) were stored at −70°C until Western blot analysis was performed.

Tissue glucose transporter protein analysis. Various tissues consisting of brain, liver, skeletal muscle, heart (myocardium), and epididymal white adipose tissue were obtained from the 9-mo-old GLUT3⁺/⁺ and GLUT3⁻/⁻ male and female mice and homogenates prepared as described previously (9). Tissue homogenates and fractionated skeletal muscle PM and LDM were sonicated, centrifuged at 10,000 g, and the supernatant subjected to Western blot analysis (9). Primary antibodies consisted of anti-GLUT1 (9), anti-GLUT3 (9), anti-GLUT4 (17, 18), and anti-vinculin (9, 17, 18) IgGs. The protein bands were detected by the chemiluminescence detection system, and the intensity of protein bands was assessed by densitometry using the Scion image computer software program.

Data analysis. Data are shown as means ± SE. Intergenotype differences in pairwise comparisons were detected by Student’s t-test. When the effect of two variables was compared simultaneously, such as age/time and genotype, the two-way analysis of variance was employed and the independent effect of age/time and genotype and the combined effect of age/time X genotype determined. Intergroup differences were established by the post hoc Holm-Sidak method. Significance was assigned for a P value of <0.05.

RESULTS

There were no differences in body weight between GLUT3⁺/⁺ and GLUT3⁻/⁻ genotyped (Fig. 1) male and female mice during the suckling phase (Fig. 2, A and B) and the postweaned period (Fig. 2, C and D), except between 9 and 11 mo when the male body weight was higher in GLUT3⁻/⁻ compared with GLUT3⁺/⁺ mice. Table 1 demonstrates organ weights that show that only the epididymal and perirenal fat stores were heavier in male GLUT3⁺/⁺ compared with the GLUT3⁻/⁻ counterpart, with all other organs being no different in the two genotypes. In female mice, all organ weights including abdominal fat were no different in the two groups.

Body composition measurements demonstrated an increase in the fat mass of male GLUT3⁻/⁻ vs. GLUT3⁺/⁺, with no change in skeletal muscle mass (Fig. 3A). In contrast, no differences were observed in fat and skeletal muscle mass of the female GLUT3⁻/⁻ and GLUT3⁺/⁺ mice (Fig. 3B). Food intake assessed per day and per unit body weight per day revealed no significant differences in males both at 21 days (Fig. 3, C1 and C2) and 9 mo (Fig. 3, D1 and D2). Assessment of basal metabolic rate revealed a decline in the male GLUT3⁻/⁻ vs. the sex-matched GLUT3⁺/⁺, with no change in the female GLUT3⁻/⁻ from that of the GLUT3⁺/⁺ mice (Fig. 3E). In contrast, core body temperature was no different between GLUT3⁻/⁻ (male = 35.93 ± 0.26°C, n = 8; female = 36.2 ± 0.13°C, n = 13) and GLUT3⁺/⁺ (male = 35.8 ± 0.46°C, n = 8; female = 36.02 ± 0.22°C, n = 11) mice.

Table 2 depicts plasma hormonal and glucose concentrations. No differences in male and female glucose concentrations were observed in the two genotypes. An increase in male GLUT3⁺/⁺ circulating insulin concentrations was observed compared with the GLUT3⁻/⁻ genotype. In contrast the female circulating insulin concentrations trended toward a decline in the GLUT3⁻/⁻ vs. the GLUT3⁺/⁺ genotype. Similarly, a trend toward an increase in circulating leptin concentrations was observed in male and female GLUT3⁻/⁻ vs. GLUT3⁺/⁺ mice. Table 3 shows the serum lipid profile. In male GLUT3⁻/⁻ mice, serum triglycerides and total cholesterol were higher than in GLUT3⁺/⁺ mice. In contrast, female GLUT3⁻/⁻ demonstrated no differences compared with GLUT3⁺/⁺ mice except for a trend toward a decline in HDL. Both male and female GLUT3⁻/⁻ exhibited a trend toward higher free fatty acids.

The male GLUT3⁻/⁻ mice demonstrated glucose intolerance at 15 min after (Fig. 4A), and the female GLUT3⁻/⁻ mice were glucose tolerant (Fig. 4B) at 9 mo of age. The male GLUT3⁺/⁺ mice were insulin resistant (Fig. 4C), and the female GLUT3⁻/⁻ mice were insulin sensitive, similar to the sex-matched GLUT3⁺/⁺ mice (Fig. 4D). Morphology of the liver revealed lipid infiltration seen as vacuoles in male GLUT3⁻/⁻ compared with GLUT3⁺/⁺ mice (Fig. 5, A and B).

Tissue expression of GLUT3 confirmed a decline in the brain of male GLUT3⁻/⁻ vs. GLUT3⁺/⁺ mice, with no associated change in GLUT1 protein concentrations (Fig. 6, B and C). Examination of GLUT4 protein concentrations revealed a decline in total amounts only in the GLUT3⁻/⁻ epididymal fat (Fig. 6, A and D), with no change in skeletal muscle and heart (Fig. 6A). Subfractionation of skeletal muscle revealed a decline in plasma PM along with an increase in LDM-associated GLUT4 concentrations in GLUT3⁻/⁻ males vs. the GLUT3⁺/⁺ counterpart (Fig.
In the female GLUT3+/− mice, changes in tissue distribution of GLUT1, GLUT3, and GLUT4 mimicked that of the male counterpart except for no change in adipose tissue GLUT4 protein concentrations (data not shown).

DISCUSSION

The phenotype of GLUT3+/− mice revealed a male preference in developing adult-onset adiposity with insulin resistance. This adiposity appeared predominantly visceral in distribution. Postnatal growth profiles demonstrated no major change during the suckling and postsuckling phase, attesting to comparable nutrition in the mutant and wild-type mice. Thus the only perturbation encountered was restricted to diminished late-gestation transplacental glucose transport that translated as decreased birth weight in GLUT3+/− mice, as reported previously (9). Fetal growth and energy metabolism are fueled largely by transplacental nutrient transport. To maximize fetal availability of glucose, a major substrate that fuels fetal energy metabolism, the mouse hemochorial placenta predominantly expresses at least two facilitative glucose transporter isoforms (6, 9). GLUT1 is found in greater amounts compared with GLUT3 (6, 9). However, GLUT3 demonstrates higher efficiency in transporting glucose and peaks during late gestation to match the exponential fetal growth pattern (6, 9). A 50% reduction in placental GLUT3 with no change in GLUT1 compromised transplacental glucose transport and transiently affected fetal growth during the late-gestation phase (9). As is evident from our present observations, the postnatal phase demonstrated no trace of this compromise, since the pups with ad libitum access to milk recovered and were no different from their wild-type littermates in their postnatal growth pattern.

Table 1. Organ weights in 9-mo-old mice

<table>
<thead>
<tr>
<th>Adipose Tissue</th>
<th>Brain</th>
<th>Liver</th>
<th>Kidney</th>
<th>Sk.M</th>
<th>Lung</th>
<th>Heart</th>
<th>Epid</th>
<th>Perirenal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male GLUT3+/− (n = 6)</td>
<td>0.47±0.02</td>
<td>1.58±0.13</td>
<td>0.54±0.05</td>
<td>1.01±0.11</td>
<td>0.25±0.01</td>
<td>0.2±0.01</td>
<td>0.55±0.1</td>
<td>0.24±0.08</td>
</tr>
<tr>
<td>Male GLUT3−/− (n = 13)</td>
<td>0.45±0.01</td>
<td>1.68±0.16</td>
<td>0.63±0.03</td>
<td>1.24±0.04</td>
<td>0.22±0.01</td>
<td>0.21±0.01</td>
<td>1.07±0.11</td>
<td>0.48±0.05</td>
</tr>
<tr>
<td>Female GLUT3+/− (n = 7)</td>
<td>0.43±0.01</td>
<td>1.28±0.16</td>
<td>0.37±0.03</td>
<td>1.30±0.05</td>
<td>0.20±0.01</td>
<td>0.15±0.01</td>
<td>1.80±0.54</td>
<td></td>
</tr>
<tr>
<td>Female GLUT3−/− (n = 7)</td>
<td>0.46±0.01</td>
<td>1.29±0.15</td>
<td>0.40±0.02</td>
<td>1.14±0.08</td>
<td>0.21±0.01</td>
<td>0.18±0.01</td>
<td>1.86±0.53</td>
<td></td>
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</tbody>
</table>

Data are means ± SE and in g. Sk.M, skeletal muscle; Epid, epididymal fat; GLUT3, glucose transporter isoform-3. *P < 0.05; **P < 0.001 vs. GLUT3+/− mice.
During the early-adult phase at 2 mo of age, no differences in body weight or metabolic profile were evident in both male and female GLUT3<sup>−/−</sup> mutant mice. However, as in previous reports of 15- to 21-mo-old rats exposed in utero to either total calorie or selective protein restriction (7, 10, 18), we observed metabolic perturbations at a later adult stage in GLUT3<sup>−/−</sup> mice. Previously, low maternal protein diet with a diminution in transplacental amino acid transport (20) interfered with fetal pancreatic β-islet cell development (2) and was blamed as the inciting factor for adult metabolic disturbances, even with total maternal caloric restriction (19). However, maternal murine total caloric (11) or selective protein restric-

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**Fig. 3.** Body composition, food intake, and basal metabolic rate. Body composition demonstrating body weight, fat mass, and skeletal (Sk.) muscle mass (A and B) in 9-mo-old males (n = 21–24/genotype; A) and females (n = 14–16/genotype; B), food intake in 21-day- (n = 6/genotype; C1 and C2) and 9-mo-old (n = 6/genotype; D1 and D2) male, and basal metabolic rate (E) in 9-mo-old male (n = 10/genotype) and female (n = 8/genotype) GLUT3<sup>+/−</sup> and GLUT3<sup>−/−</sup> mice. *P < 0.05 is detected by Student’s t-test.
tion (Devaskar SU, unpublished observations) led to male glucose intolerance at 6 mo of age. In contrast, GLUT3+/- mutation that mimicked a selective transplacental glucose-deficient state demonstrated minimal glucose intolerance in the adult progeny. However, this selective fetal glucose deficiency led to adult-onset adiposity with insulin resistance that was more prominent than the glucose intolerance. Thus the long-term impact of fetal glucose deficiency is unique and differs from that of total caloric or selective protein deficiency. Although the latter may involve the β-islets and cause hepatic insulin resistance (10, 2), the former predominantly affected peripheral tissues such as white adipose tissue, skeletal muscle, and liver.

Attempts at limiting fetal glucose supply have previously been made in rats and sheep, but these investigations involved other nutrients as well. Maternal rat undernutrition and bilateral uterine artery ligation were associated with diminished placental GLUT3 and GLUT1 protein concentrations and fetal growth restriction (5, 14, 15). Maternal insulin infusion in pregnant sheep causing maternal and fetal hypoglycemia led to decreased placental GLUT1 but not GLUT3 protein concentrations (4, 5). In both rat and sheep experiments, in addition to glucose, other nutrients such as protein (amino acids), fatty acids, and oxygen supply to the fetus were also compromised (4, 5, 14, 15). Our present investigation with GLUT3+/- mice is the first report of purely limiting the fetal glucose supply. Despite normal placental GLUT1 concentrations and the adaptive increase in transplacental amino acid transport to meet fetal energy requirements (9), the adult male GLUT3+/- mice exhibit adiposity with insulin resistance.

Since brain GLUT3 was diminished in the GLUT3+/- mice, we examined the core body temperature and observed no differences. This was to ensure against thermogenic differences between the two genotypes causing the observed phenotypic changes. However, the adult-onset adiposity in the male GLUT3+/- mice was associated with diminished basal metabolic rate in the absence of hyperphagia. The possibility exists that diminished brain GLUT3-mediated glucose transport may alter peripheral metabolism. Unequivocal exclusion of a role for neuronal GLUT3 deficiency in altering metabolism can be accomplished by the Cre/LoxP strategy in future studies.

The associated insulin resistance could arguably result as a gradual metabolic interference from a diminution in specific glucose transporter isoforms in insulin-responsive tissues. Although GLUT3 and GLUT1 are responsible for basal glucose transport, GLUT4 mainly subserves the insulin-responsive glucose transport function. GLUT3 was observed in brain, placenta, and testis (9), with minimal amounts in skeletal muscle. This decrease in skeletal muscle GLUT3 protein concentrations seen in male GLUT3+/- mutant mice could potentially perturb basal rather than insulin-responsive glucose transport in skeletal muscle. In patients with acanthosis nigricans with severe insulin resistance, a decline in skeletal muscle GLUT3 concentrations was reported previously (16). Should diminished skeletal muscle GLUT3 be responsible for the observed insulin resistance in GLUT3+/- male mice, this phenotype would have surfaced earlier in development. However, at 2 mo of age both male and female GLUT3+/- mice did not exhibit insulin resistance (9). GLUT1 similar to GLUT3 also mediates basal glucose transport and was noted in brain, placenta (9), and white adipose tissue. Unlike the younger adult (9), no difference in tissue GLUT1 concentrations was observed between the wild-type and GLUT3+/- mutant mice at this later adult stage.

The insulin-responsive isoform GLUT4 was noted in myocardium, skeletal muscle, and white adipose tissue of the wild-type mice. In the GLUT3+/- mutant male mice, the diminution in white adipose tissue total GLUT4 and skeletal muscle sarcolemmal GLUT4 protein concentrations was associated with observed insulin resistance. GLUT4 conditional knockout mice demonstrated the insulin-responsive tissue contribution toward insulin resistance, confirming the major role of GLUT4 in establishing insulin sensitivity (1, 21). Skeletal muscle-specific GLUT4 deficiency led to significant insulin resistance attesting to the major role played by skeletal muscle in insulin-responsive glucose transport.

### Table 2. Plasma glucose, insulin, and leptin concentrations in 9-mo-old mice

<table>
<thead>
<tr>
<th>Gender</th>
<th>Glucose, mg/dl</th>
<th>Insulin, ng/ml</th>
<th>Leptin, ng/ml</th>
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<tbody>
<tr>
<td>Male</td>
<td></td>
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<tr>
<td>GLUT3+/-</td>
<td>171.26±13.88 (n = 15)</td>
<td>0.93±0.14 (n = 13)</td>
<td>18.76±1.65 (n = 8)</td>
</tr>
<tr>
<td>GLUT3 +/-</td>
<td>188.37±14.14 (n = 15)</td>
<td>1.42±0.15 (n = 12)</td>
<td>21.31±2.9 (n = 9)</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>GLUT3+/-</td>
<td>165.11±6.75 (n = 9)</td>
<td>0.7±0.18 (n = 7)</td>
<td>11.57±2.11 (n = 6)</td>
</tr>
<tr>
<td>GLUT3 +/-</td>
<td>153.75±7.82 (n = 8)</td>
<td>0.46±0.06 (n = 10)</td>
<td>14.81±4.34 (n = 6)</td>
</tr>
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Data are means ± SE. *P < 0.05 vs. GLUT3+/-.

### Table 3. Plasma lipid profile in 9-mo-old mice

<table>
<thead>
<tr>
<th></th>
<th>TG</th>
<th>T Choles</th>
<th>HDL</th>
<th>UC</th>
<th>FFA</th>
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<tbody>
<tr>
<td>Male (n = 14)</td>
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<tr>
<td>GLUT3+/-</td>
<td>59.28±7.7</td>
<td>110.75±8.09</td>
<td>78.83±8.25</td>
<td>35.05±3.23</td>
<td>15.78±1.5</td>
</tr>
<tr>
<td>GLUT3 +/-</td>
<td>85.26±10.38*</td>
<td>128.68±7.96*</td>
<td>75.76±7.96</td>
<td>34.00±2.49</td>
<td>18.93±1.51</td>
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<tr>
<td>Female (n = 8)</td>
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<td></td>
</tr>
<tr>
<td>GLUT3+/-</td>
<td>34.14±4.20</td>
<td>109.44±9.22</td>
<td>85.75±11.25</td>
<td>26.55±2.14</td>
<td>16.62±0.84</td>
</tr>
<tr>
<td>GLUT3 +/-</td>
<td>31±7.41</td>
<td>102.25±10.80</td>
<td>69.57±14.64</td>
<td>24.12±2.48</td>
<td>20.00±1.15</td>
</tr>
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</table>

Data are means ± SE and in mg/dl. TG, triglycerides; T chole, total cholesterol; UC, unesterified cholesterol; FFA, free fatty acids. *P < 0.05 vs. GLUT3+/-.
In male GLUT3-/- mice, whereas no difference in skeletal muscle total GLUT4 protein concentrations was noted, a diminution in subcellular localization consisting of decreased plasma membrane association was observed. This observation goes hand in hand with hyperinsulinemia that exists in male GLUT3-/- mutant mice. More importantly, adult-onset obesity is characterized by a decline in white adipose tissue with no change in skeletal muscle total GLUT4 concentrations (1, 21). White adipose tissue-specific GLUT4 deficiency using the Cre/LoxP strategy revealed adaptive processes in other tissues. Changes in skeletal muscle and liver had a detrimental effect on whole body physiology causing insulin resistance (1). Similar to the white adipose tissue GLUT4 conditional knockout mice (1), the GLUT3-/- male mice revealed hepatic steatosis with perturbations in circulating triglycerides and total cholesterol. In contrast to GLUT3-/- males, the females are relatively protected at 9–11 mo of age. Subtle trends related to decreased circulating insulin and high-density lipoproteins along with increased leptin and free fatty acids suggest that subsequent emergence of metabolic changes with further aging is a possibility.

Fig. 4. Glucose and insulin tolerance tests. Glucose (A and B) and insulin (C and D) tolerance tests in 9-mo-old male (A and C) and female (B and D) GLUT3+/- and GLUT3+/- mice; n = 7–9 in each sex and genotype. All graphs demonstrate the area under the curve (AUC) in the corresponding right panels. Two-way ANOVA applied to the male glucose tolerance tests revealed the effect of time (F = 8.31, P < 0.001) and genotype (F = 6.42, P = 0.013) but not age X genotype (F = 0.85, P = 0.57) (A), whereas the female glucose tolerance tests revealed the effect of time (F = 40.23, P < 0.001) but not genotype (F = 2.69, P = 0.10) or time X genotype (F = 0.62, P = 0.76) on plasma glucose concentrations (B). The male insulin tolerance tests revealed the effect of time (F = 22.03, P < 0.001), genotype (F = 117.51, P < 0.001), and time X genotype (F = 3.44, P = 0.002) (C), whereas the female insulin tolerance tests revealed the effect of time (F = 18.54, P < 0.001) but not genotype (F = 0.44, P = 0.51) or time X genotype (F = 0.184, P = 0.98) on plasma glucose concentrations (D). Holm-Sidak test revealed in both genotypes, intertime differences between all time points (P < 0.05) in males (A and B) and females (C and D) with intergenotype differences shown (A and C). *P < 0.001 demonstrated by Student’s t-test for intergenotype comparison of the AUC values.
In conclusion, a novel mechanism of GLUT3 haploinsufficiency for adult-onset adiposity and insulin resistance is proposed. Although the role of brain GLUT3 deficiency is unknown at present, placental GLUT3 deficiency may contribute toward selective transplacental glucose-fueled fetal energy deficit (9). This is despite the presence of normal placental GLUT1 and an adaptive increase in fetal amino acid supply (9). Postnataally, this fetal nutrient deficiency is not phenotypically discernible, thereby contributing to the difficulty in predicting subsequent adiposity with insulin resistance. However, this fetal perturbation may set the stage for a sexually dimorphic obese adult. Our present observations support the concept of fetal metabolic programming highlighting the sex- and age-dependent pheno-

Fig. 5. Hepatic histology. Hematoxylin- and eosin-stained section of liver from 9-mo-old male GLUT3**+/+** (A) and GLUT3**+/−** (B) mice. Arrows demonstrate lipid infiltration (n = 6). Scale bar = 200 μm.

Fig. 6. Glucose transporter protein. Western blot analysis demonstrating GLUT4 (A, top), GLUT3 (B, top), and GLUT1 (C, top), along with the internal control, vinculin (A, B, and C, bottom) protein bands in brain, liver, skeletal muscle (Sk.M), heart, and epididymal white adipose tissue (fat) in 9-mo-old old GLUT3**+/+** and GLUT3**+/−** male mice. D: the quantification of epididymal fat GLUT4 protein concentrations is depicted below. Western blots demonstrating GLUT4 protein concentrations in plasma membrane (PM) and low-density microsomes (LDM) of Sk.M (E1) along with quantification in PM (E2) and LDM (E3) is shown. *P < 0.05 is detected by Student’s t-test.
typic expression based on specificity of the fetal nutrient deficiency.

GRANTS

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