Hexosamines have been shown to mediate effects of hyperglycemia and so-called “glucose toxicity” in insulin-sensitive tissues. To determine the effects of hexosamines on insulin synthesis and secretion, transgenic mice were created to overexpress the rate-limiting enzyme for hexosamine synthesis, glutamine:fructose-6-phosphate amidotransferase (GFA), specifically in β-cells. GFA activity in islets of heterozygous transgenic mice was elevated 76% compared with littermate controls. The increased GFA activity led to a 1.4- and 2.1-fold increased pancreatic insulin content in 2- and 10-month-old transgenic mice, respectively (P < 0.005). Fasting insulin levels were 1.6-fold higher than in littermate controls (P < 0.05). Hyperinsulinemia was evident despite a 28% reduction in insulin mRNA levels. The fasting glucose levels in the transgenic mice equaled that of controls aged 2–4 months but exceeded that of the controls aged 6–10 months (means ± SE 6.9 ± 0.2 vs. 5.9 ± 0.2 mmol/l, P < 0.001). By 8 months, the males were overweight and mildly diabetic (fasting glucose 8.8 ± 0.5 mmol/l) despite persistent hyperinsulinemia. Insulin resistance was confirmed in both males and females using the euglycemic-hyperinsulinemic clamp technique; glucose disposal rates decreased by 48% in transgenic mice (P < 0.01). Triglyceride levels did not differ, and free fatty acid levels were lower in the transgenic animals. ATP levels were unchanged in the transgenic islets. We conclude that hexosamine biosynthesis is involved in the regulation of insulin content in β-cells by glucose. Increased hexosamine flux in the β-cell results in hyperinsulinemia, insulin resistance, and (in males) mild type 2 diabetes. Diabetes 49:1492–1499, 2000
RESEARCH DESIGN AND METHODS

Materials. Routine reagents were purchased from Sigma (St. Louis, MO) unless otherwise indicated.

Transgenic animals. Transgene expression is targeted to pancreatic β-cells with the RIP and transcription initiation sites that are contained in a 0.7-kb polymerase chain reaction (PCR)–modified KpnI-HindIII fragment derived from the Rip1-DiPα plasmid (gift of Dr. D. Hanahan, Hormone Research Institute, University of California, San Francisco) (15). This fragment was ligated upstream of the 2.1-kb HindIII-BamHI GFA cDNA (8,16,17) and the 0.4-kb BamHI-XbaI SV40 polyA signal DNA in Bluescript SK +/- phagemid (Stratagene, La Jolla, CA). The resultant RIP-GFA-SV40polyA transgene construct was excised from Bluescript with KpnI and XbaI (Fig. 1A), was purified, and was microinjected into 1-cell mouse embryos that were then surgically reimplanted into pseudopregnant female mice at the University of Alabama at Birmingham Transgenic Facility. All procedures were approved by the Institutional Animal Care and Use Committee.

Heterozygotes for the transgene were bred against C5BL6 mice. Animals used in these studies were the resulting heterozygous transgenic mice and their nontransgenic littermate controls.

DNA and RNA analysis. PCR amplification of mouse tail chromosomal DNA was performed to verify the presence of the transgene in the offspring of transgenic animals. Mouse tail DNA was extracted and amplified using sense and antisense primers, respectively, to anneal to the human GFA cDNA to yield a 639-bp product (17). The thermocycling program was 94°C for 1 min, 55°C for 1 min, and 72°C for 2 min for 30 cycles and then 72°C for 10 min. PCR products were subjected to electrophoresis in a 1% agarose gel.

The presence of transgene-encoded mRNA was confirmed by reverse transcriptase (RT)–PCR followed by restriction digestion at a site unique to the human (transgenic) product. Total RNA was isolated from pancreatic tissue using a Rapid Total RNA Isolation Kit (5′ Prime–3′ Prime, Boulder, CO). RT-PCR was performed with the You-Prime-First Strand Beads RT-PCR Kit (Pharmacia, Piscataway, NJ) using oligonucleotide primers that generate a 712-bp DNA fragment. Because of the high homology between mouse and human GFA, both endogenous and transgenic GFA mRNA are amplified by this procedure. However, only the transgenic human GFA has a site for the restriction enzyme Scal within this fragment. Scal was therefore used to digest the GFA cDNA fragment obtained from RT-PCR to identify the expression of the transgene versus endogenous GFA, with transgenic DNA appearing as 255- and 457-bp fragments after digestion.

Isolation of islets. Islets of Langerhans were isolated from fasted control and transgenic mice by collagenase digestion (18). The islets were then individually handpicked after separation from acinar tissue on a discontinuous Ficoll gradient. A total of 12 islets from each mouse were taken for measurement of insulin content, and the remaining islets from 4 to 5 mice in each transgenic or control group were pooled for the GFA activity assay.

Assay of GFA activity. GFA was assayed as described (19). Briefly, islets pooled from 4 to 5 mice were placed in 100 µl extraction buffer (100 mmol/l KCl, 1 mmol/l EDTA, and 50 mmol/l Na phosphate, pH 7.5). The samples were then sonicated on ice 3 times for 10 s each (50 Sonic Dismembrator, Fisher Scientific, Tustin, CA). After centrifugation at 60,000g for 20 min at 4°C, 50 µl of the supernatant cytoplasmic extract was incubated with 12 mmol/l fructose-6-phosphate, 12 mmol/l glutamine, 40 mmol/l NaH2PO4, 1 mmol/l EDTA, and 1 mmol/l dithiothreitol (final volume 100 µl) for 45 min at 37°C. The reaction was stopped and the protein precipitated by the addition of 50 µl 1 mol/l perchloric acid and by incubation on ice for 10 min. After centrifugation at 16,000g for 10 min, 145 µl supernatant was extracted with 258 µl tri-N-olylamine:1,1,2-trichloro-trifluoroethane (1:4). GlcN-6-P generated in the reaction was detected by derivatization of the 110-µl aqueous phase with 2 vol O-phthaldialdehyde (OPA) reagent (4 mg OPA in 50 µl ethanol added to 5 ml 0.1 mol/l sodium borate, pH 9.7, and 10 µl 2-mercaptoethanol) for 1 min. Samples were then neutralized with 120 µl 0.1 mol/l sodium phosphate (pH 7.4), filtered, and separated over a reverse-phase C18 column (25 cm x 4.6 mm; Phase Separations, Norwalk, CT). Absorbance of the sample eluent was analyzed fluorometrically, and the peak area was integrated. OPA-derivatized GlcN-6-P standards were run separately to determine retention time and to generate a standard curve to correlate area to activity. Activity was calculated as units per microgram of protein where 1 U represents the generation of 1 pmol GlcN-6-P/min.

Insulin content in islets. A total of 12 islets isolated from each mouse were placed into 100 µl phosphate-buffered saline (PBS) and then sonicated.
3 times for 10 s. Insulin concentrations were measured by radioimmunoassay (RIA) using a rat insulin RIA Kit (Linco Research, St. Louis, MO). Insulin, nonesterified fatty acid, and triglyceride levels in serum. Insulin levels were determined by RIA on blood samples collected from control and transgenic mice. Triglyceride levels were measured using a diagnostic reagent kit (Sigma), and free fatty acid (FFA) levels were measured using the Farb-Test Colorimetric Kit (Boehringer Mannheim, Mannheim, Germany).

Intraperitoneal glucose tolerance test. After an 18 h fast, glucose (1 mg/kg body wt) was administered intraperitoneally to nontransgenic animals. Tail vein blood was sampled for glucose determination (Miles Elite Glucometer, Elkhart, IN) before and 15, 30, 60, 90, and 120 min after glucose administration. To determine insulin responsiveness 5 min after a glucose challenge, the dose was increased to 3 mg/kg body weight.

Determination of glucose disposal rates. To measure glucose disposal rate (GDR), all experiments were performed in weight-matched nontransgenic transgenic and littermate control mice using the euglycemic-hyperinsulinemic clamp technique previously described (8). Catheters were implanted into the right internal jugular vein. The animals were allowed to recover from surgery for 1 day and were then fasted overnight before the experiment. Animals were infused with recombinant human insulin (Humulin; Lilly, Indianapolis, IN) at a rate of 20 mU · kg⁻¹ · min⁻¹ while 50% dextrose was infused by a variable infusion pump (Harvard Apparatus, South Natick, MA). Whole blood samples (3 µl) were collected every 5–10 min from tail bleeds and were measured by glucometer.

Analysis of mRNA levels by Northern blot and ribonuclease protection assay. Total RNA was isolated from the pancreases with the Rapid Total RNA Isolation Kit. RNA samples (20 µg) were electrophoresed in a 1% agarose-formaldehyde gel and were transferred to a nylon membrane. The membrane was probed with a 380-bp insulin I cDNA probe (a gift from Dr. Graeme I. Bell, Chicago, IL) for 1 h at 68°C in ExpressHyb hybridization solution (CLONTECH Laboratories, Palo Alto, CA). The membrane was washed and exposed to film at –80°C for 5–7 h. For the ribonuclease protection assay, a cDNA fragment encoding insulin I cDNA was inserted into the HindIII site of pBluescript, and a biotin-16-UTP-labeled antisense cRNA was transcribed from the T7 polymerase promoter using a MaxIsCript In Vitro Transcription Kit (Ambion, Austin, TX). A 380-bp [UTP]-labeled cRNA was annealed with 40 µg of total RNA by using the RPA III Ribonuclease Protection Assay Kit (Ambion). Products were electrophoresed in a 5% polyacrylamide gel containing 8 mol/l urea and were transferred to a nylon membrane. Protected insulin I mRNA was detected with a BrightStar BioDetect Kit (Ambion) and was quantitated by densitometry.

ATP content of islets. Groups of 90–300 islets (total yields from individual mice) were isolated, and ATP content was measured with a bioluminescent assay (Sigma).

Statistical analysis. Linear regression was used to determine the degree of association between the difference in the area under the glucose curve (AUC) (ratio of transgenic to control mice) and age, and analysis of variance (ANOVA) was applied to test whether the relationship was significant. All other comparisons were made between transgenic mice and their age-matched littermate controls by Student’s t test. Data are means ± SE.

RESULTS

Determination of transgene expression in mouse tissue. The heterozygotic founder was bred with a nontransgenic C57BL/6j mate. Approximately half of these offspring carried the integrated transgene in their genome as demonstrated by the presence of a transgene-specific 740-bp PCR product after amplification of genomic DNA (Fig. 1B, lanes 2, 3, and 4). All experiments were performed using the heterozygous offspring of a transgenic mouse crossed with a C57BL/6j background. Resulting litters were ~50% heterozygous transgenic; nontransgenic littermates were used as control animals. Transcription of the transgene was verified by RT-PCR. Total RNA from the pancreases of transgenic and control mice was reverse transcribed and amplified using oligonucleotide primers resulting in the generation of a 712-bp DNA fragment. Because of the high homology between human and rodent GFA, these primers can generate a product from either mouse or human (transgenic) mRNA. However, within this fragment, only the human GFA has a unique site for the restriction enzyme Scal. The GFA cDNA fragment obtained from RT-PCR was therefore digested with Scal. The presence of the human transgene product was verified by the appearance of 255- and 457-bp digestion products in the transgene-carrying (but not control) littermates, which indicates transcription of the transgene in these mice (Fig. 1C, lanes 2 and 5). RT-PCR product was not detected in muscle or liver (data not shown).

Increased GFA activity in the transgenic mice. Overexpression of GFA activity in the heterozygous transgenic mouse was confirmed using an in vitro assay for GFA. Cytoplasmic extracts of pooled islets from 4 to 5 transgenic or control mice were incubated with fructose-6-phosphate and glutamine, and Glcn-6-P production was quantified. GFA activity in islets was elevated 76% in transgenic mice compared with nontransgenic littermates (Fig. 2, P < 0.01 by paired t test in 3 separate batches of control and transgenic islets assayed in parallel). Because of the sensitivity of the assay and the difficulty in obtaining sufficient quantities of islet tissue from the mice, we were unable to measure the levels of UDP-N-acetylglucosamine, which is the principal end product of the hexosamine pathway.

ATP content in islets. Overexpression of GFA had no effect on the ATP content of the islets. Control islets had 33 ± 7.3 pmol/islet, and transgenic islets had 31 ± 6.1 pmol/islet (4 separate control and 6 transgenic preparations each assayed in duplicate).

Pancreatic insulin content. We next investigated the effect of increased hexosamine flux on insulin content in the transgenic animals. As seen in Fig. 3, both 2-month-old and 10-month-old transgenic animals had higher total insulin content per pancreas than their control littermates (P < 0.05 at each age). Electron microscope examination of the islet cells revealed no change in the size, distribution, or number of secretory granules in the β-cells (data not shown).

We performed Northern blot analysis for the insulin message in mRNA purified from whole pancreas and normalized those results to the mRNA levels for actin (Fig. 4). The level of insulin message was surprisingly decreased by 36% in the transgenic animals despite their hyperinsulinemia (n = 9/group, P < 0.02). This result was confirmed by ribonuclease protection analysis of the insulin message, again normalized...
to that of actin. In this case, the insulin message in the transgenic animals was decreased by 28% compared with controls (n = 2/group, data not shown).

**Metabolic parameters in transgenic and control animals.**

The effects of the increased GFA expression and increased pancreatic insulin content on serum levels of insulin, glucose, FFAs, triglycerides, and body weight are shown in Table 1. In animals not segregated by sex or age, significantly higher glucose and insulin levels were evident in the transgenic animals compared with controls. Insulin levels were 64% higher in the transgenic animals in the fasting state. Insulin hypersecretion was also evident after an intraperitoneal glucose challenge. At 5 min after receiving 3 mg/g of glucose i.p., insulin levels were 65% higher in transgenic animals compared with littermate controls despite an equivalent glucose excursion (P < 0.05). We also noted relative hyperinsulinemia 60 min after intraperitoneal injection of the lower concentration of glucose (1 mg/g) used in the routine glucose tolerance tests reported below (2.3-fold increase in 2-month-old transgenic mice compared with littermate controls) (n = 3–4/group, P < 0.05) (data not shown). Serum triglycerides were not elevated and FFA levels were lower in the transgenic animals.

No difference was evident in weight between the younger transgenic and control animals. Female control and transgenic mice remained similar in weight, but the transgenic males became heavier than controls over time (P < 0.05 at 10 months).

**Glucose tolerance as a function of age in the transgenic and control animals.**

The data in Table 1 suggest that the transgenic animals were not only hyperinsulinemic but also insulin resistant. To analyze the evolution of this situation in

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**TABLE 1**

<table>
<thead>
<tr>
<th>Metabolic parameters</th>
<th>Control</th>
<th>Transgenic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting blood glucose (mmol/l)</td>
<td>6.0 ± 0.1</td>
<td>6.6 ± 0.1*</td>
</tr>
<tr>
<td>Fasting plasma insulin (pmol/l)</td>
<td>24.2 ± 3.3</td>
<td>39.7 ± 4.8*</td>
</tr>
<tr>
<td>Insulin 5 min after IPGTT (pmol/l)</td>
<td>81.8 ± 21.3</td>
<td>135.0 ± 15.9*</td>
</tr>
<tr>
<td>Glucose 5 min after IPGTT (mmol/l)</td>
<td>13.2 ± 1.1</td>
<td>13.7 ± 1.1</td>
</tr>
<tr>
<td>Fasting serum triglycerides (mmol/l)</td>
<td>0.65 ± 0.03</td>
<td>0.64 ± 0.02</td>
</tr>
<tr>
<td>Fasting serum FFAs (mmol/l)</td>
<td>0.98 ± 0.06</td>
<td>0.88 ± 0.05*</td>
</tr>
<tr>
<td>Weight (g)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3–6 months old</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>24.3 ± 0.6</td>
<td>25.6 ± 0.9</td>
</tr>
<tr>
<td>Female</td>
<td>19.5 ± 0.8</td>
<td>20.1 ± 0.6</td>
</tr>
<tr>
<td>8–10 months old</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>25.9 ± 0.3</td>
<td>28.7 ± 1.0*</td>
</tr>
<tr>
<td>Female</td>
<td>23.0 ± 1.7</td>
<td>22.9 ± 0.7</td>
</tr>
</tbody>
</table>

Data are means ± SE. *P < 0.05 compared with control; †IPGTT used in these studies was performed after the injection of 3 mg/g glucose to elicit a rapid measurable insulin response (11 control and 10 transgenic mice). Note that subsequent IPGTTs were performed at a lower glucose dose (1 mg/g). Blood tests were performed after an overnight fast. n = at least 10/group.
transgenic animals, and because of the dependence of insulin sensitivity on age and sex, we next followed the degree of glucose tolerance in the mice over time. An intraperitoneal glucose tolerance test (IPGTT) was performed at 2- to 3-month intervals in a cohort of transgenic and littermate control animals. As seen in Fig. 5A, the younger transgenic mice tended to have lower glucose excursions than their control littermates after glucose administration (when comparing AUCs, $P = 0.20$ for females and $P = 0.26$ for males). In the young females, the 2-h glucose level was significantly lower in the transgenic mice ($P = 0.03$). With age, however, this situation reversed. In the 6- to 10-month-old mice (Fig. 5B), the females exhibited a slight trend ($P = 0.32$) toward higher glucose excursions during the IPGTT, whereas AUCs and all individual glucose levels during IPGTT for the transgenic males were significantly higher than that of their control littermates ($P < 0.05$).

Further analysis of the IPGTT data is shown in Fig. 6. One can see that the glucose levels after an overnight fast before the injection of glucose were identical in the younger mice but were significantly greater in the older transgenic mice. In fact, the fasting glucose levels of the older male transgenic mice were in the diabetic range ($8.8 \pm 0.5$ vs. $5.8 \pm 0.4$ mmol/l for transgenic mice vs. control males, respectively, $P < 0.001$). Older transgenic females also tended toward higher glucose levels than their sex-matched littermate controls ($6.6 \pm 0.9$ vs.

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**FIG. 5.** Blood glucose levels during the IPGTT in younger (A) and older (B) control and transgenic animals. Data are means ± SE for 27 and 34 younger control and transgenic mice, respectively, and 30 and 60 older control and transgenic mice, respectively.

**FIG. 6.** Fasting glucose levels (A) and AUC (B) after IPGTT as a function of aging. Data are from the IPGTTs illustrated in Fig. 7. At each age and for each sex, the AUCs during the IPGTT were averaged and normalized to that of the control mice. For the fasting glucose data ($n = 27$ and 34 for the younger control and transgenic mice, respectively, and $n = 30$ and 60 for the older control and transgenic mice, respectively). For the AUC data, $n = 6-30$ for each control and transgenic group at each age.
Overexpression of GFA in the liver also results in a phenotype typically similar to that observed in type 2 diabetes. Various in vitro and in vivo systems have shown that GFA overexpression targeted to striated muscle results in significant insulin resistance that is phenotypically similar to that observed in type 2 diabetes. This has been confirmed experimentally in several other laboratories in various animal models and in humans.

The mechanism for the increased insulin secretion in the current model is not yet known. Levels of mRNA for insulin are decreased when normalized for actin mRNA in the entire pancreas, although actual insulin synthesis rates have not been determined. Downregulation of insulin mRNA is not inconsistent with hyperinsulinemia; in the Zucker diabetic rat, for example, insulin secretion can be 8-10 times as high in control animals even in the face of only half the rate of insulin resistance. Indeed, in contrast with the current results, insulin resistance in β-cells is manifested by relative insulinopenia.

Two groups have previously demonstrated defects of insulin secretion in animals treated with glucosamine that are similar to those observed in type 2 diabetes. However, treatment of cells with glucosamine can have unanticipated side effects, and glucokinase in the β-cell is inhibited by glucosamine, so we thought that using the transgenic approach to study the contribution of hexosamines to glucose homeostasis would be more critical. In overexpressing GFA by ~2-fold, the hexosamine pathway that normally contributes 4% to glucose metabolism was decreased by ~20%, a change that should not affect overall intracellular energy balance. Indeed, in our transgenic models, key metabolic intermediates such as glucose-6-phosphate were not affected by GFA overexpression, and because GFA is rate limiting in hexosamine generation, elevations of even glucosamine-6-phosphate are minimal.

The mechanism for the increased insulin secretion in the current model is not yet known. Levels of mRNA for insulin are decreased when normalized for actin mRNA in the entire pancreas, although actual insulin synthesis rates have not been determined. Downregulation of insulin mRNA is not inconsistent with hyperinsulinemia; in the Zucker diabetic rat, for example, insulin secretion can be 8-10 times as high in control animals even in the face of only half the rate of insulin biosynthesis. The observed effect of hexosamines on insulin mRNA levels is consistent with an effect of hexosamines on gene transcription, which is an attractive
hypothesis given the known effects of hexosamines on transcription of other growth factors (34,35) and current hypotheses of hexosamine-induced effects (36).

Downregulation of the insulin gene has been observed in glucose toxic models (37–39), and our results are yet a further parallel between increased hexosamine flux and glucose toxicity. The mechanisms of downregulation of insulin mRNA in diabetic models are not known, although the current data do suggest at the least that elevated serum FFA levels are not responsible for the downregulation.

The hyperinsulinemia in the transgenic animals is seen both in the fasting and fed states and occurs with near normoglycemia in the fasted younger animals, which is consistent with well-compensated insulin resistance. However, in these same younger animals, the AUC curve after IPGTT actually tends to be lower than in controls, and in young female transgenic mice, a significantly lower glucose level is evident at 2 h of the IPGTT (Fig. 5). The changes observed in the AUC with aging (Fig. 6) also support a shift is evident at 2 h of the IPGTT. The changes observed in the AUC with aging (Fig. 6) also support a shift.

The hyperinsulinemia and blood glucose level. Thus, the hexosamine pathway could play some role in acute glucose sensing for insulin secretion. Alternatively, the results may reflect enhanced release of insulin secondary to higher amounts of insulin in each secretory granule or accelerated secretion of newly synthesized insulin as has been observed in the Zucker fatty rat (33). Electron microscope analysis of the islets of the transgenic animals has not revealed differences in the number, size, or distribution of insulin granules in the β-cells (data not shown). Studies of basal and stimulated insulin secretion in isolated islets will be needed to shed further light on the dynamics of insulin secretion in these animals.

As the transgenic mice age, they become more clearly insulin resistant. Because GFA was specifically targeted to the β-cell and was not expressed in muscle or liver, the primary causative change in these animals must be at the level of the β-cell. In other words, the changes in insulin content and insulin levels cannot be only a response to peripheral insulin resistance because that resistance must have evolved as a secondary result of the alterations in insulin secretion. Although only the males gain excess weight and develop overt type 2 diabetes, the euglycemic-hyperinsulinemic clamp studies demonstrate similar levels of insulin resistance in both male and female animals. Furthermore, the normal weights of the female animals indicate that the insulin resistance is not simply a reflection of excess weight. That insulin can induce insulin resistance has been previously demonstrated in a wide variety of model systems (12–14,40,41). Whether hyperinsulinemia in human type 2 diabetes is a cause of insulin resistance or a compensatory effect of insulin resistance is still unknown. Although the majority opinion is probably that the latter is the case (42), some researchers have suggested that hyperinsulinemia may in fact precede insulin resistance (43,44). Exogenous hyperinsulinemia can also induce other aspects of the human insulin resistance syndrome including hypertension, obesity, and hypertriglyceridemia (38,45,46). In the present study, the transgenic animals overexpressing GFA were not hyperlipidemic, and in fact, FFA levels were lower than in the control mice. However, the observed levels of hyperinsulinemia in our animals were not as pronounced as in the studies cited above.

We should point out that these effects are seen in a single founder line of mice, and we cannot rule out the possibility that some of the effects may be nonspecific (e.g., secondary to transgene insertion or disruption of normal insulin transcription). We view this as unlikely because 1) the effects are consistent with the role of hexosamines in nutrient sensing as demonstrated in numerous other models, 2) artifacts secondary to use of the insulin promoter have not been previously noted despite the widespread use of the insulin promoter to target transgenes to the β-cell, and 3) the nature of the effects (increased insulin levels despite decreased mRNA) do not suggest a nonspecific inhibitory effect.

A single report showed that glucosamine treatment caused decreased ATP levels in cultured cells, and therefore some have speculated that hexosamine effects may be trivial or artifactual (47). This is not the case. ATP levels were not altered in the islets overexpressing GFA, nor were ATP levels decreased in the skeletal muscle or liver of mice overexpressing GFA in those tissues (9) (D.A.M., unpublished data). The same is true of animals infused with glucosamine (48).

The most impressive evidence for the applicability of the in vivo hexosamine models to diabetes is, however, the consistency of phenotypes observed with increased flux in several different tissues: muscle, liver, fat, and now the β-cell. In each tissue, the effects strikingly mimic the human insulin resistance syndrome. Further phenotypic characterization of the mice, the dynamics of their insulin secretion, and the molecular mechanism of the pathway by which the effects of hexosamines are signaled should further illuminate this relationship.

ACKNOWLEDGMENTS

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