Pancreatic β-Cell Lipotoxicity Induced by Overexpression of Hormone-Sensitive Lipase

Maria Sörhede Winzell,[1] Håkan Svensson,[1] Sven Enerbäck,[2] Kim Ravnskjaer,[3] Susanne Mandrup,[3] Victoria Esser,[4] Peter Arner,[5] Marie-Clotilde Alves-Guerra,[6] Bruno Miroux,[6] Frank Sundler,[7] Bo Ahrén,[8] and Cecilia Holm[1]

Lipid perturbations associated with triglyceride overstorage in β-cells impair insulin secretion, a process termed lipotoxicity. To assess the role of hormone-sensitive lipase, which is expressed and enzymatically active in β-cells, in the development of lipotoxicity, we generated transgenic mice overexpressing hormone-sensitive lipase specifically in β-cells. Transgenic mice developed glucose intolerance and severely blunted glucose-stimulated insulin secretion when challenged with a high-fat diet. As expected, both lipase activity and forskolin-stimulated lipolysis was increased in transgenic compared with wild-type islets. This was reflected in significantly lower triglycerides levels in transgenic compared with wild-type islets in mice receiving the high-fat diet, whereas no difference in islet triglycerides was found between the two genotypes under low-fat diet conditions. Our results highlight the importance of mobilization of the islet triglyceride pool in the development of β-cell lipotoxicity. We propose that hormone-sensitive lipase is involved in mediating β-cell lipotoxicity by providing ligands for peroxisome proliferator–activated receptors and other lipid-activated transcription factors, which in turn alter the expression of critical genes. One such gene might be uncoupling protein-2, which was found to be upregulated in transgenic islets, a change that was accompanied by decreased ATP levels. *Diabetes* 52:2057–2065, 2003

From the [1]Department of Cell and Molecular Biology, Lund University, Lund, Sweden; the [2]Department of Molecular Biology, Göteborg University, Göteborg, Sweden; the [3]Department of Biochemistry and Molecular Biology, University of Southern Denmark, Odense, Denmark; the [4]Department of Internal Medicine, University of Texas Southwestern Medical Center, Dallas, Texas; the [5]Department of Medicine, Huddinge University Hospital Karolinska Institute, Stockholm, Sweden; [6]CNRS UPR 9078, Meudon, France; the [7]Department of Physiological Sciences, Lund University, Lund, Sweden; and the [8]Department of Medicine, Lund University, Lund Sweden.

Address correspondence and reprint requests to Dr. Maria Sörhede Winzell, Department of Cell and Molecular Biology, Section for Molecular Signaling, Lund University, Biomedical Center (BMC), C11, SE-221 84 Lund, Sweden. E-mail: maria.sorhede_winzell@medkem.lu.se.

The established relation between obesity and type 2 diabetes has brought much attention to the pathophysiological role of lipids for the development of type 2 diabetes (1–3). Besides the link between lipids and the major complication of diabetes, i.e., macroangiopathic disease (4), the lipid abnormalities may also be a key factor for the development of both insulin resistance and impaired glucose-stimulated insulin secretion (GSIS), the two hallmarks of type 2 diabetes (5). Thus, inappropriate accumulation of triglycerides in nonadipose cells may be an initial event, which in turn may abrogate GSIS in β-cells and decrease insulin sensitivity (6), processes referred to as lipotoxicity. In support of the lipotoxicity theory, a strong correlation between accumulation of triglycerides in myocytes and insulin resistance has been observed (5,7), and infusion of lipids interferes with glucose disposal in skeletal muscle (8). With regard to β-cells, their failure in the Zucker diabetic fatty (ZDF) rat is preceded by a rise in plasma nonesterified fatty acid and accumulation of triglycerides in islets (9). Reduction of β-cell lipid accumulation before the development of frank diabetes improves β-cell function (10), suggesting a causal relation between triglyceride overstorage and β-cell failure. The mechanisms underlying lipotoxicity are yet poorly understood.

Despite the accumulating data suggesting the importance of lipids for the development of the β-cell secretory defects that characterize type 2 diabetes, little is known about how fatty acids are mobilized from the intracellular pool(s) of triglycerides in β-cells and how this process is regulated. We recently showed that hormone-sensitive lipase (HSL), which is mostly known as the hormonally regulated acylglycerol lipase in adipocytes (11), is expressed and enzymatically active in β-cells (12). To gain further insight into the pathophysiology of lipotoxicity, including the role of HSL in this process, we have generated transgenic mice with β-cell–specific overexpression of HSL. These mice exhibit impaired GSIS when challenged with a high-fat diet, suggesting that HSL may be involved in β-cell lipotoxicity by providing lipid ligands for transcription factors. The gene expression changes that follow may be responsible for the development of β-cell dysfunction. We have identified one such change, which may fully or partly explain the observed phenotype, i.e., the upregulation of the uncoupling protein 2 (UCP2) gene, which recently was found to negatively regulate GSIS (13).
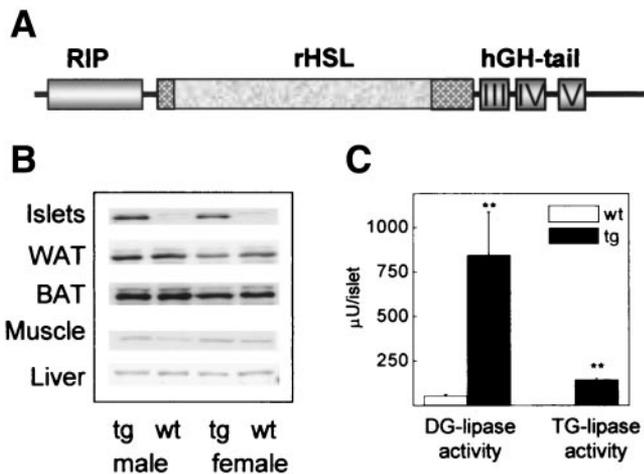
FIG. 1. Specific overexpression of HSL in pancreatic β-cells. *A*: Schematic representation of a vector for transgenic overexpression of HSL in β-cells. Details are given in the text. *B*: Analysis of HSL expression in islets and selected tissues from transgenic (tg) and wild-type (wt) female and male mice using Western blot analysis. Equal amounts of total protein of homogenate from each tissue were loaded on the gel. *C*: Diglyceride (DG) and triglyceride (TG) lipase activity measured in homogenates of islets from wild-type (wt) and transgenic (tg) mice. Increased triglyceride lipase activity correlated with increased release of glycerol, an end product of lipolysis. Values are means ± SE of three different experiments; **$P < 0.01$ vs. wild-type islets. BAT, brown adipose tissue; WAT, white adipose tissue.

RESEARCH DESIGN AND METHODS

DNA construct and generation of transgenic mice. A pBluescript vector containing the rat adipocyte HSL cDNA (nucleotides 610–3,225) downstream of the scavenger receptor gene promoter was used as starting plasmid (kind gift from Drs. Karen Reue and Jean-Louis Escary, VA Lipid Research Laboratory, Los Angeles, CA). After excision of the scavenger receptor gene promoter, the rat insulin II gene promoter, obtained from a pBR322/RIP vector (kind gift from Dr. Henrik Semb, Göteborg University, Göteborg, Sweden), was subcloned into the vector, resulting in a 4.5-kb fragment, harboring the RIP-rHSL construct with a human growth hormone tail (Fig. 1*A*). This fragment was injected into the male pronucleus of fertilized eggs from superovulated (C57BL/6J × CBA/J) females that had been mated to males with the same genetic background. Genotyping was performed by PCR from tail biopsies (14), using primers specific for the rat insulin promotor (5′-GAAACTGCAGCTTCAGCCCCTCTGGC-3′) and HSL (5′-CAGTGCCTGTTCCC GAACACCTGCAAAGAC-3′), respectively.

Animals were housed in temperature-controlled facilities on a 12-h light-dark cycle. The mice had free access to food and water and were fed either a high-fat diet with 58% fat by energy or a low-fat diet with 11% fat by energy (Research Diets, New Brunswick, NJ). Blood samples were taken from the orbital capillary plexus, and plasma insulin, glucagon, and leptin were determined radioimmochemically (Linco Research). Plasma glucose was determined with the glucose oxidase method (Sigma) and free fatty acids (FFAs) were measured photometrically (NEFA C; Wako Chemicals). The study was approved by the Animal Ethics Committee at Lund University.

Western blot analysis of islets and other tissues. Islets were isolated by collagenase digestion as described (15) and then lysed in SDS-PAGE sample buffer. Other tissues were homogenized in 10 volumes of homogenization buffer (0.25 mol/l sucrose, 1 mmol/l dithioerythritol, 1 mmol/l EDTA, 10 μg/ml leupeptin, 10 μg/ml antipain, 1 μg/ml pepstatin A) using a polytron. Total protein was measured (BCA protein, Pierce), and 100 μg of protein were fractionated by SDS-PAGE and electroblotted onto nitrocellulose membranes (Hybond-c extra, Amersham Pharmacia Biotech). Primary antibodies used were affinity-purified rabbit anti-rat HSL rabbit anti-mouse GLUT-2 (Chemicon), affinity-purified rabbit anti-human UCP2 (antibody 2–605) (16), rabbit anti-rat liver CPT-I (17), rabbit anti-rat acyl-CoA oxidase (a kind gift from Dr. Stefan Alexson, Karolinska Institute, Stockholm, Sweden), and rabbit anti-rat PDX-1/IPF1 (kind gift from Drs. Palle Serup and Ole D. Madsen, Novo Nordisk, Bagsvaerd, Denmark). The secondary antibody was a horseradish peroxidase–conjugated anti-rabbit IgG prepared in goat (Amersham Pharmacia Biotech) or donkey (Pierce). The blots were developed by enhanced chemiluminescene (SuperSignal, Pierce), and detection was performed using a CCD camera (LAS 1000; Fuji).

RNA isolation and Northern blot analysis. Total RNA was isolated from islets (18), electrophoresed, and transferred to nylon membranes (Hybond-N+). The blots were probed with a ^{32}P-labeled mouse UCP2 cDNA using ExpressHyb (Clontech). Membranes were analyzed and quantified by digital imaging (Fujix Bas 2000; Fuji).

Quantitative PCR. Pooled total RNA isolated from islets was DNase-treated, and three independent reverse transcription reactions were performed using Superscript II reverse transcriptase (Invitrogen) following the manufacturer's instructions. The mRNA expression levels were determined using real-time fluorescent detection and the following primer combinations: 5′-GTACCAC-TACGGAGTTCACGCAT-f-3′ and 5′-CGCCGAAAGAAGCCCTTAC-r-3′ for peroxisome proliferator–activated receptor (PPAR)-α, 5′-CAGATAATTGGCACC TACGCC-f-3′ and 5′-AAGATGAGTTCCGTGGCCC-r-3′ for acylCoA oxidase, 5′-TCTGGATGGCTATGGTCAAG-f-3′ and 5′-TACCACTGCAATTTGGGTCC-f-3′ for CPT-I, 5′-AGCTGCTAGTGGAGCACCAAG-f-3′ and 5′-TCGCCATTTCT GCGAGC-r-3′ for medium-chain acyl dehydrogenase (MCAD), and 5′-CCTAC AGCGCCAGATGAGCT-f-3′ and 5′-GAGTCGTAGAGGCCAATGCG-r-3′ for UCP2. The mRNA expression levels were determined twice in each first-strand synthesis reaction and normalized to the expression levels of 18S rRNA as described by the vendor (Applied Biosystems).

Glucose tolerance tests and insulin release. Glucose tolerance tests were performed in anesthetized mice after intravenous administration of D-glucose (1 g/kg) or arginine (0.25 g/kg). Blood samples were taken at 0, 1, 5, 20, and 50 min.

Insulin secretion, insulin content, and ATP in isolated islets. Insulin secretion from isolated islets was measured as described (15). Total insulin content in islets was measured after extraction of insulin with acidic ethanol (0.2 mol/l HCl in 75% ethanol) (Linco).

For determination of ATP levels, batches of 10 islets were incubated in RPMI-1640 medium containing 16.7 mmol/l glucose at 37°C for 5 min. The ATP concentration was measured using a luciferase-based assay (ATP monitoring kit; Thermo Labsystems).

Triglyceride measurements in islets. Acylglycerols were extracted from isolated islets (19). After hydrolyzation, the amount of released glycerol was measured using a bioluminescence assay (20).

Enzyme activity assays and measurement of lipolysis. Homogenates, prepared as for Western blot analysis, were assayed for diglyceride and triglyceride lipase activity, as described (21) using mono-oleoyl-2-*O*-monooleylglycerol, a diacylglycerol analog, and triolein, respectively, as substrates. One unit of enzyme activity corresponds to 1 μmol of fatty acid released per minute at 37°C.

Lipolysis was measured, in groups of 50 islets incubated with 11 mmol/l glucose and 10 μmol/l forskolin for 30 min at 37°C, as release of glycerol using a bioluminescence assay (20).

Glucose and palmitate oxidation in islets. Glucose oxidation was determined as production of $^{14}CO_2$ from [U-^{14}C]glucose (22), and palmitate oxidation was determined as production of $^{14}CO_2$ from [1-^{14}C]palmitate (23).

Immunocytochemistry. Pancreatic tissue was prepared and used for indirect immunofluorescence (24) using affinity-purified rabbit anti-rat HSL, anti-insulin and anti-glucagon (Euro-Diagnostica), and rabbit anti-mouse GLUT2 (Chemicon) as primary antibodies and a secondary antibody coupled to fluorescein isothiocyanate (Euro-Diagnostica).

Statistical analyses. All results are expressed as means ± SE. Differences between individual data were compared with unpaired, two-tailed *t* test, where the critical probability to determine significance was $P < 0.05$.

RESULTS

Generation of transgenic mice overexpressing HSL in pancreatic β-cells. A vector containing the rat insulin gene promoter and a rat HSL cDNA (Fig. 1*A*) was microinjected into zygotes. Seven male and nine female founder mice were obtained. After breeding the male founders to C57BL/6J female mice, six generated offspring. After analysis of the HSL expression in islets by Western blot, one founder line showing ~50-fold overexpression compared with matched littermates (Fig. 1*B*) was chosen for the studies described in this paper. Following the complete phenotypic characterization of this line, an additional line with the same degree of overexpression was subjected to a limited characterization in order to confirm critical aspects of the phenotype (see last paragraph of RESULTS).

To confirm that the overexpression was confined to

β-cells, the expression of HSL was analyzed by Western blot in several tissues, i.e., white and brown adipose tissue, skeletal muscle, liver, spleen, kidney, stomach, intestine, lung, heart, brain, and genitals. No differences in the expression pattern, for tissues other than islets, were observed between transgenic and wild-type mice (selected tissues are shown in Fig. 1*B*).
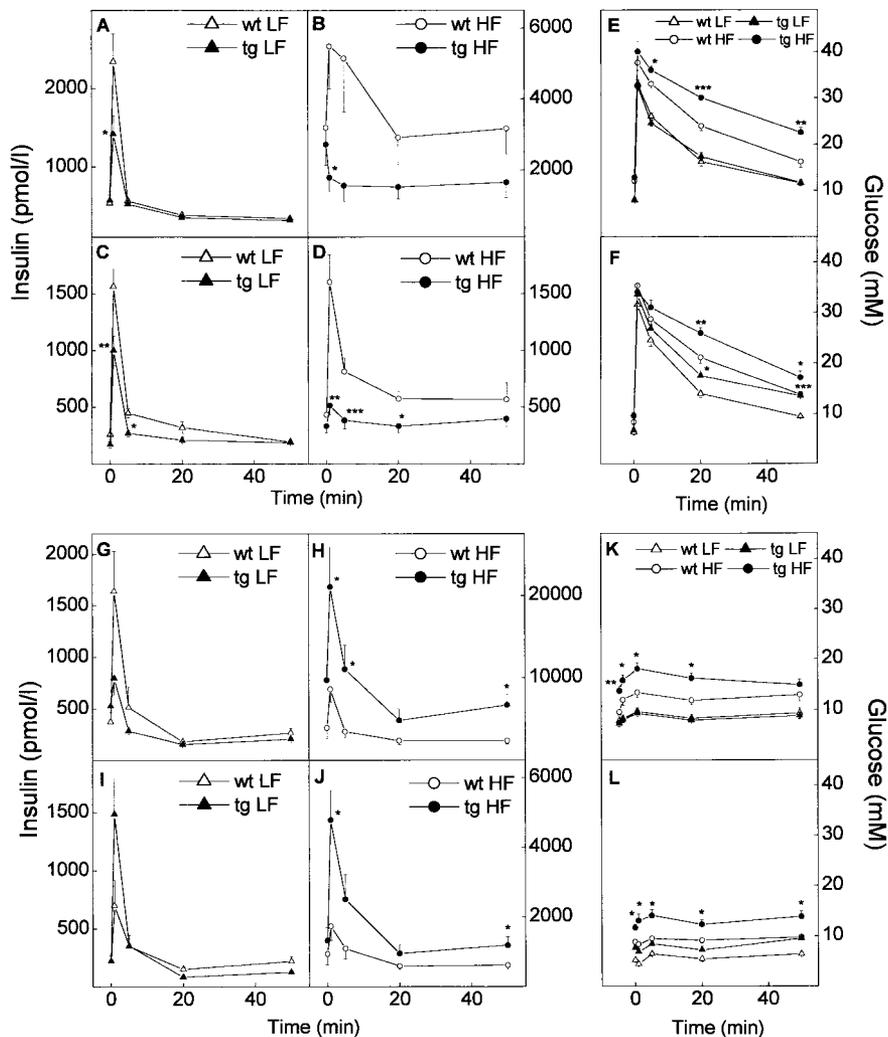
Diglyceride lipase activity was found to be elevated in islets of transgenic mice (Fig. 1*C*). Upon stimulation of isolated transgenic islets with the cAMP-elevating agent forskolin, triglyceride lipase activity (Fig. 1*C*) and the release of glycerol (an end product of lipolysis) were increased. Transgenic islets stimulated with forskolin secreted 88 ± 36 pmol glycerol $\cdot$ islet$^{-1} \cdot$ 30 min^{-1}, while there was no detectable glycerol in medium samples from incubations with wild-type islets.

Transgenic mice grow and develop normally with no gross abnormalities in plasma parameters. Body weight and plasma levels of glucagon, leptin, and free fatty acid (FFA) were not different between transgenic and wild-type mice fed a low-fat diet (Table 1 and data not shown). Insulin levels were significantly elevated in young transgenic male mice (7 weeks) and were accompanied by reduced blood glucose. On the contrary, in older male transgenic mice (11–20 weeks), insulin levels were lower (transgenic 121 ± 14 pmol/l [$n = 25$] and wild-type 270 ± 39 pmol/l [$n = 20$]; $P < 0.001$), with no difference in blood glucose between the groups (transgenic 7.6 ± 0.3 mmol/l and wild-type 7.7 ± 0.3 mmol/l). There were no significant differences in insulin or glucose for female mice at any of the time points.

For high-fat–fed male mice, there were no differences in body weight between transgenic and wild-type mice, while for female mice the increase in body weight was greater for transgenic than for wild-type mice (Table 1). In transgenic male mice, basal insulin levels were lower than in wild-type mice, while no differences in glucose levels were observed (Table 1). In female transgenic mice, insulin levels were not different from high-fat–fed control mice. As for the low-fat–fed animals, there were no differences between the transgenic and wild-type high-fat–fed mice with regard to glucagon, leptin, and FFA (Table 1 and data not shown). Thus, male transgenic mice in both diet groups appeared to exhibit increased insulin sensitivity. This may reflect a possible feedback between β-cells and insulin sensitivity, which deserves further studies.

Blunted insulin secretion in mice with β-cell–specific overexpression of HSL. Intravenous glucose tolerance tests (IVGTTs) performed on mice fed a low- or high-fat diet showed a significant reduction in acute insulin release in both male and female transgenic mice (Fig. 2*A–D*). In female transgenic mice at both feeding conditions and in high-fat–fed transgenic males, this was accompanied by impaired glucose elimination, while in low-fat–fed transgenic males, glucose elimination was normal (Fig. 2*E–F*). Both male and female transgenic mice were found to respond adequately to the arginine challenge (Fig. 2*G–J*), indicating that the exocytotic machinery is not affected in the transgenic mice (Fig. 2*K–L*). The insulin response to arginine was increased in high-fat–fed transgenic mice due to the elevated blood glucose.

Insulin secretion from isolated islets was analyzed after

TABLE 1
Body weight and basal values of different plasma parameters in wild-type (Wt) and transgenic (Tg) mice with overexpression of HSL in β-cells

| | 7–8 Weeks of age | | | | 16 weeks of age (3 weeks of high-fat diet) | | | | 22 weeks of age (9 weeks of high-fat diet) | | | |
| | Female | | Male | | Female | | Male | | Female | | Male | |
	Wt	Tg	Wt	Tg	Wt	Tg	Wt	Tg	Wt	Tg	Wt	Tg
n	12	13	16	16	6	5	8	7	6	5	8	5
Body weight (g)	18.2 ± 0.4	18.2 ± 0.4	23.0 ± 0.7	23.6 ± 0.5	25.5 ± 1.6	27.8 ± 1.6	37.6 ± 1.2	36.4 ± 2	31.0 ± 2.2	38.5 ± 2.1*	37.6 ± 1.2	36.4 ± 2.0
Insulin (pmol/l)	72 ± 13	75 ± 10	157 ± 14	237 ± 22†	333 ± 62	259 ± 69	997 ± 57	510 ± 74†	335 ± 69	219 ± 65	4540 ± 1120	3146 ± 1323
Glucagon (pmol/l)	39 ± 8	52 ± 8	42 ± 8	52 ± 6	102 ± 11	90 ± 11	72 ± 10	70 ± 4	98 ± 12	82 ± 12	89 ± 5	76 ± 5
Glucose (mmol/l)	6.4 ± 0.4	6.6 ± 0.4	6.7 ± 0.2	5.8 ± 0.2†	7.2 ± 0.5	6.6 ± 0.5	10.3 ± 0.3	9.7 ± 0.7	6.4 ± 0.5	7.8 ± 0.6	11.4 ± 0.9	10.9 ± 1.6
FFA (mmol/l)	1.09 ± 0.05	0.88 ± 0.04	1.28 ± 0.04	1.18 ± 0.09	1.13 ± 0.06	1.06 ± 0.09	1.02 ± 0.04	1.34 ± 0.09	0.64 ± 0.05	0.75 ± 0.03	0.69 ± 0.04	0.75 ± 0.06
Leptin (nmol/l)	1.8 ± 0.1	1.7 ± 0.1	1.6 ± 0.1	1.5 ± 0.1	20 ± 5	11 ± 3	27 ± 4	33 ± 7	25 ± 5	28 ± 7	41 ± 2	36 ± 6

Data are means ± SE. Blood samples were taken at 8, 16, 22, and 26 weeks of age from mice in each feeding group. The table shows data from diet-fed mice at 8 weeks of age and after feeding high-fat diet for 3 and 9 weeks, respectively. Differences between individual data were compared with unpaired, two-tailed t-test. *$P < 0.05$, †$P < 0.01$.

FIG. 2. Glucose and insulin homeostasis in HSL transgenic (tg) and wild-type (wt) mice. All tolerance tests were performed in anesthetized age-matched male and female mice, and plasma glucose and insulin levels were analyzed at the indicated time points. _A_, _F_: IVGTTs performed in low-fat–fed transgenic and wild-type mice (males _n_ = 8 wild-type and _n_ = 9 transgenic; females _n_ = 6 wild-type and _n_ = 8 transgenic) and in high-fat–fed mice (males _n_ = 8 wild-type and _n_ = 6 transgenic; females _n_ = 6 wild-type and _n_ = 5 transgenic). Insulin response to glucose in male mice (_A_, _B_) and in female mice (_C_, _D_) with corresponding blood glucose levels (_E_ and _F_, respectively) from two separate tests performed at 11 and 17 weeks of age. _G_, _L_: Intravenous injection of arginine in mice fed low-fat or high-fat diets at 31 and 34 weeks of age. Insulin response in males (_G_, _H_) and in females (_I_, _J_) to arginine with corresponding blood glucose levels (_K_ and _L_, respectively). All results are expressed as means ± SE. Differences between transgenic and wild-type mice were analyzed using unpaired, two-tailed _t_ test (*_P_ < 0.05, **_P_ < 0.01, ***_P_ < 0.001).

stimulation with glucose at different concentrations. Islets from transgenic female mice exhibited a significantly lower insulin response to high glucose concentrations compared with islets from wild-type mice at both feeding conditions (Fig. 3_A_). In islets from male mice, the blunted response to glucose was less pronounced during low-fat feeding (Fig. 3_B_) and reached statistical significance only in the high-fat–fed group.

Accumulation of islet triglycerides is reduced in HSL transgenic mice. There was no significant difference in triglyceride content between transgenic and wild-type islets from mice fed the low-fat diet (Fig. 3_C_). High-fat feeding induced a more than threefold increase in islet triglyceride content in the wild-type group; this increase was significantly lower in the transgenic group.

Glucose and fatty acid oxidation in islets. There were no differences in glucose oxidation among the four experimental groups at 2.8 mmol/l glucose (Fig. 3_D_). At 16.7 mmol/l glucose, glucose oxidation was slightly reduced in islets from transgenic animals of the low-fat–fed group compared with islets of wild-type mice of the same group. This effect was much more pronounced and statistically significant in islets from mice fed the high-fat diet. With regard to palmitate oxidation, this was slightly increased at low glucose in high-fat–fed wild-type islets and in transgenic islets of both feeding groups (Fig. 3_E_). There

was no difference in palmitate oxidation at high glucose between transgenic and wild-type mice fed either low- or high-fat diet.

UCP2 is upregulated in HSL-overexpressing islets. The expression levels of proteins of particular relevance were investigated by Western blot analyses. In view of the decreased glucose oxidation observed in islets of transgenic animals, we measured the levels of GLUT2 and glucokinase. Both of these were found to be upregulated in islets of transgenic mice compared with wild-type islets and accompanied by an increased expression of pancreatic/duodenal homeobox-1 (PDX-1), a known transactivator of these genes (25,26) (Fig. 4_A_).

The expression levels of carnitine palmitoyltransferase I (CPT I), which transports long- and medium-chain acyl-CoA into the mitochondria, was found to be upregulated in wild-type mice fed the high-fat diet compared with the low-fat diet, while high-fat–fed transgenic mice had a similar expression of CPT I in islets as low-fat–fed wild-type and transgenic mice (Fig. 4_A_).

It was recently demonstrated that UCP2 negatively regulates insulin secretion (13,27). Upregulation of UCP2 by fatty acids was furthermore suggested to play an important role in obesity-induced β-cell dysfunction (13). We thus examined UCP2 protein levels in the islets of HSL transgenic mice and found these to be higher than in islets
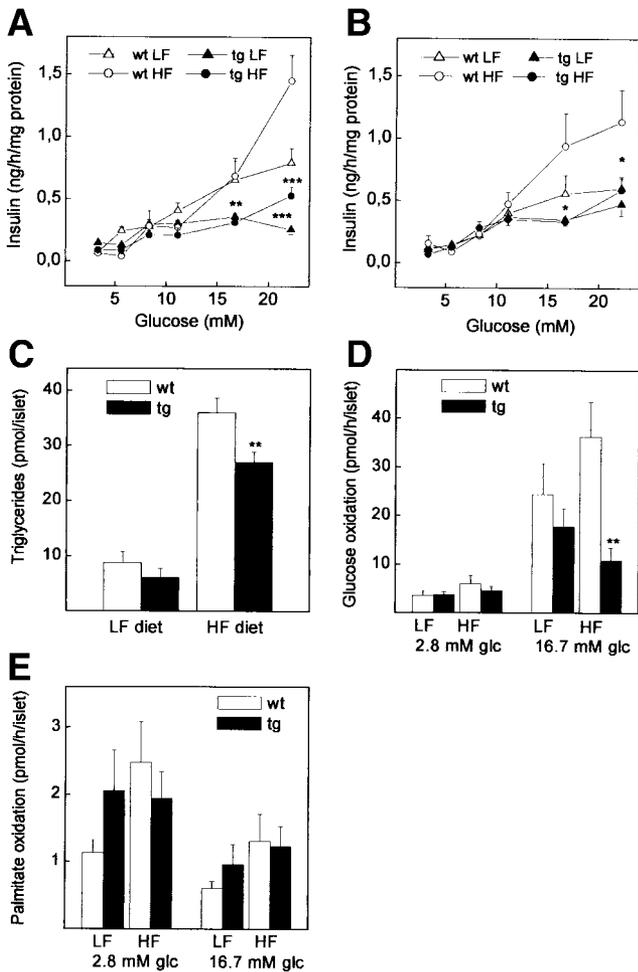
FIG. 3. Insulin secretion studied in islets isolated from HSL transgenic (tg) and wild-type (wt) mice, aged 34 weeks and fed either a low- or high-fat diet for 21 weeks in female (*A*) and male (*B*) mice. The results are from three independent experiments. All values are means ± SE, where differences between transgenic and wild-type mice were analyzed using unpaired, two-tailed *t* test (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$). *C*: Triglyceride content in islets isolated from wild-type (wt) and transgenic (tg) mice fed low-fat or high-fat diet. The results are from three independent experiments where each group was analyzed in duplicate or triplicate. Glucose oxidation (*D*) and palmitate oxidation (*F*) in islets from wild-type and transgenic mice at 2.8 and 16.7 mmol/l glucose, respectively. The results are from three independent experiments. All values are means ± SE, where differences between transgenic and wild-type mice were analyzed using unpaired, two-tailed *t* test (**$P < 0.01$).

from wild-type mice (Fig. 4*A*). Recent studies point to the involvement of PPARs in the upregulation of UCP2 by fatty acids in β-cells (28–30), and acyl CoA oxidase, a known target for PPARα regulation (31), was shown to be upregulated in islets from transgenic mice of both feeding groups compared with the respective wild-type controls. Upregulation of PPARα-responsive genes in transgenic islets was further confirmed at the mRNA level using quantitative real-time PCR. Acyl-CoA oxidase, MCAD, and UCP2 were all found to be upregulated in transgenic islets from both diet groups compared with wild-type islets (Fig. 4*B*). The increased UCP2 mRNA levels in transgenic islets were further verified in Northern blot experiments (Fig. 4*C*). To confirm that the upregulation of UCP2 has a functional relevance, we measured the levels of cellular ATP and found these to be reduced in transgenic islets compared

with wild-type islets under both feeding conditions (Fig. 4*D*).

Immunocytochemical characterization of islets. No gross abnormalities in general islet architecture were observed in HSL transgenic mice. As expected, HSL immunofluorescence was markedly increased in islets of transgenic mice, with a staining pattern compatible with overexpression in β-cells (Fig. 5*B* and *D*). There was no staining of exocrine pancreas. Staining for insulin (Fig. 5*E*–*H*) and glucagon (Fig. 5*I*–*L*) revealed no differences between transgenic and wild-type islets. Moreover, insulin content was similar in freshly isolated islets from both low-fat–fed (wild-type 104 ± 17 ng/islet and transgenic 115 ± 11 ng/islet) and high-fat–fed (wild-type 90 ± 10 ng/islet and transgenic 93 ± 7 ng/islet) mice. Immunofluorescence for GLUT2 was similar in wild-type and transgenic islets from low-fat–fed mice, and the pattern of staining was indistinguishable in transgenic and wild-type islets, displaying the typical plasma membrane localization of the transporter (Fig. 5*M*–*P*). In islets from high-fat–fed mice there was a disrupted, sometimes cytoplasmic, distribution of GLUT2, leaving only few islet cells with a normal plasma membrane localization, which is an early sign of cellular dysfunction following triglyceride accumulation described in islets of ZDF rats (32). This redistribution of GLUT2 was observed in both transgenic and wild-type islets.

Limited phenotypic characterization of an additional transgenic line. To rule out the possibility that the
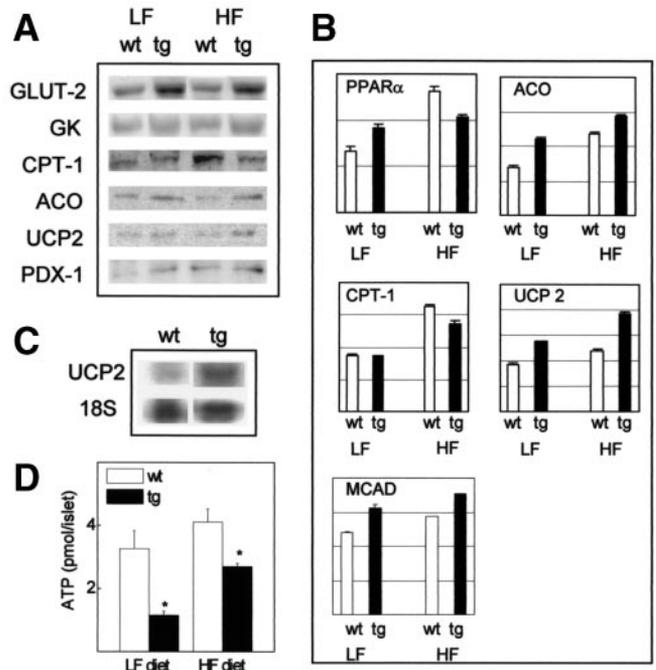
FIG. 4. Expression pattern in islets of transgenic and wild-type mice. *A*: Western blot analysis of islets isolated from wild-type (wt) and transgenic (tg) mice fed a low- or high-fat diet. The blots were developed with antibodies against GLUT2, glucokinase (GK), acyl-CoA oxidase (ACO), CPT I, UCP2, and PDX-1. *B*: Quantitative real-time PCR data on PPARα-responsive genes in transgenic and wild-type islets. *C*: Northern blot analysis of UCP2 expression in islets from high-fat–fed wild-type and transgenic mice. *D*: ATP levels in isolated islets. The results are from two independent experiments where each group was analyzed in triplicate. Values are means ± SE. Differences between transgenic and wild-type mice were analyzed using unpaired, two-tailed *t* test (*$P < 0.05$, **$P < 0.01$).
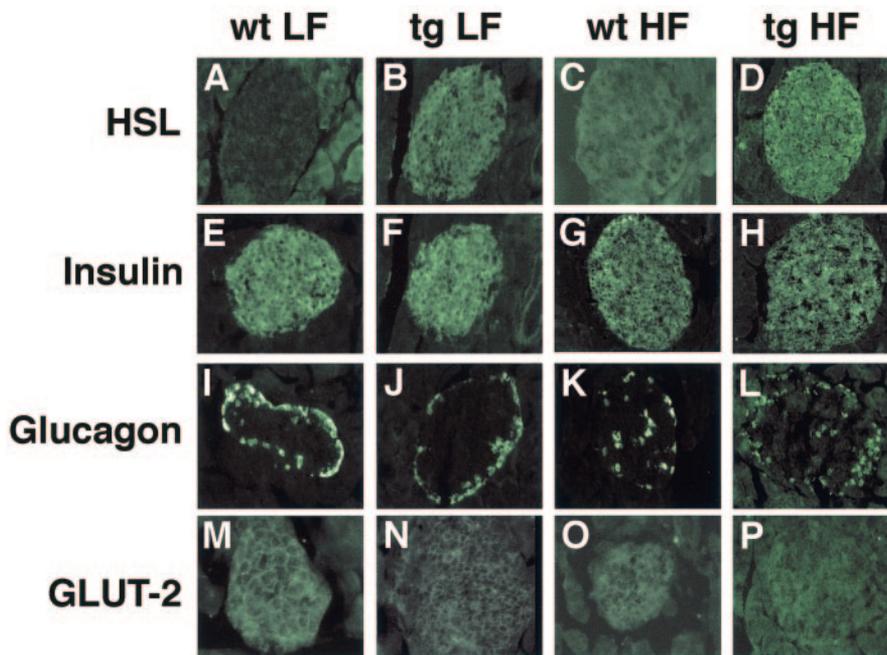
FIG. 5. Immunofluorescence in pancreatic sections from transgenic (tg) and wild-type (wt) male mice fed a low- or high-fat diet, using antibodies toward rat HSL (*A–D*), insulin (*E–H*), glucagon (*I–L*), and GLUT2 (*M–P*). (Magnification 16×).

phenotype described above is due to the disruption of an endogenous gene at the site of insertion or transgenic expression at some other site than β-cells, critical aspects of the phenotype were confirmed in an additional transgenic line with the same degree of overexpression (i.e., 50-fold). Also, mice of this second line developed severely blunted GSIS, when challenged with a high-fat diet, as evident from both IVGTT (Fig. 6*A*) and batch incubations of isolated islets (Fig. 6*B*). Furthermore, UCP2 was found to be upregulated in transgenic islets compared with wild-type islets (Fig. 6*C*). Thus, these studies confirm that the observed phenotype is the result of overexpression of HSL specifically in β-cells.

DISCUSSION

The present study demonstrates that increased expression of HSL in β-cells results in impaired GSIS. Transgenic mice overexpressing HSL specifically in β-cells exhibit glucose intolerance and abrogated GSIS. The phenotype was mild in mice fed a low-fat diet but further accentuated in mice fed a high-fat diet, despite the fact that overexpression of HSL partly prevented the accumulation of islet triglycerides that occurs upon fat feeding. Thus, the rate of influx of fatty acids into the islet triglyceride pool and the capacity to mobilize this pool are both important determinants of islet lipotoxicity. In fact, the recently demonstrated inverse correlation between cytotoxicity and triglyceride accumulation in β-cells led the authors to propose that the ability of normal β-cells to form and accumulate cytoplasmic triglycerides might serve a cytoprotective mechanism against FFA-induced islet dysfunction and apoptosis (33). The phenotype of our mice strongly supports this notion. Further support comes from a separate study (34) in which we show that the expression of islet HSL is downregulated during prolonged high-fat feeding of C57BL/6J mice. Thus, downregulation of HSL and possibly other, as yet unidentified, lipases in β-cells might be part of a defense mechanism against development of lipid-induced islet dysfunction.

HSL is the only known mammalian lipase for which hormonal regulation, mediated by reversible protein phosphorylation, has been firmly established (11). Thus, elevations in cAMP levels, resulting from glucose metabolism
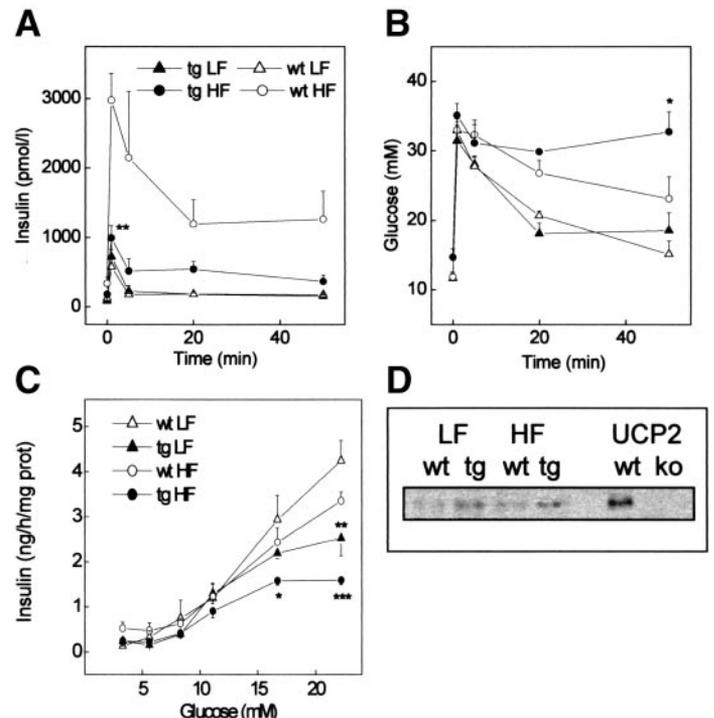

FIG. 6. Phenotypic characterization of an additional HSL transgenic mouse line. IVGTT was performed on low- or high-fat–fed female mice (wild-type $n = 6$ and transgenic $n = 6$ in each feeding group), and plasma levels of insulin (*A*) and glucose (*B*) were measured. *C*: Batch incubation of islets isolated from high- and low-fat–fed female mice. Insulin secretion was analyzed after incubation of the islets in different glucose concentrations. *D*: Western blot analysis of UCP2 expression in isolated islets. A mitochondrial homogenate of lung obtained from UCP2 knockout mice and wild-type littermates were used to aid the identification the UCP2 protein. Values are means ± SE. Differences between transgenic and wild-type mice were analyzed using unpaired, two-tailed *t* test (**$P < 0.01$, ***$P < 0.001$).
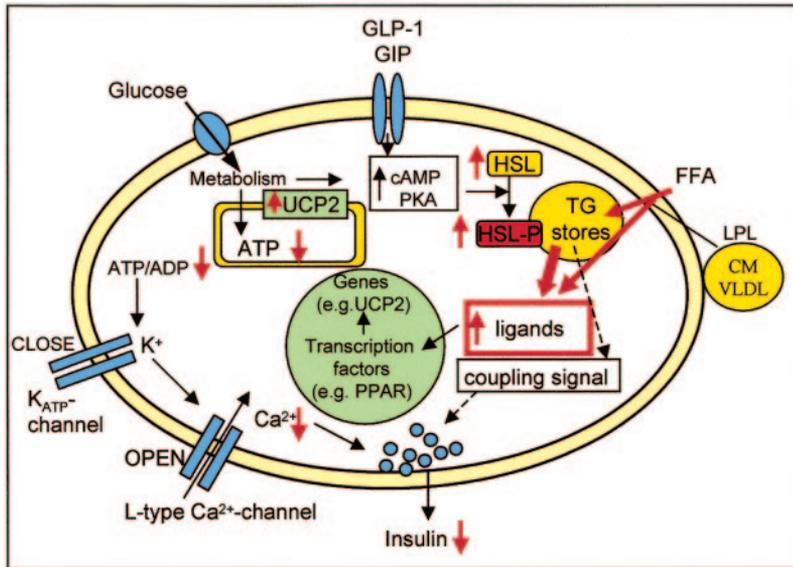
FIG. 7. Proposed model for the role of HSL and products of lipolysis in the lipotoxic pathway. Glucose enters the β-cell via GLUT2 and is metabolized in the glycolytic pathway/tricarboxylic acid cycle, where phosphorylation of glucose by glucokinase represents a rate-limiting step. Glucose metabolism results in increased ATP-to-ADP ratio, which closes the ATP-sensitive potassium channels leading to depolarization and opening of the voltage-gated calcium channels. The resulting increase in cytoplasmic calcium promotes insulin secretion. A lipid-derived signal has been found to be involved in stimulus-secretion coupling, and HSL may under normal conditions be involved in generation of this signal. In this situation, increased cAMP levels, caused by glucose metabolism and signaling via glucagon-like peptide 1 (GLP-1) and gastric inhibitory polypeptide (GIP) receptors, results in activation of HSL and thus lipolysis. Diet-induced type 2 diabetes is accompanied by overstorage of triglycerides in β-cells and other nonadipose cells, caused by increased influx of FFA derived from plasma lipoproteins (VLDL and chylomicrons [CM]), through the action of LPL, and plasma FFA. Overexpression of HSL, failure to downregulate HSL during prolonged high-fat feeding, or other situations of inadequate expression and/or activation of HSL increases fatty acid flux, a situation which is further aggravated by simultaneous increases in the triglyceride pool. At a certain threshold level of fatty acid flux, the lipotoxic pathway is activated, leading to alterations in the expression of genes critical to insulin secretion, e.g., UCP2, via provision of ligands for ligand-activated transcription factors, such as PPARs, and other mechanisms. Alterations associated with diet-induced type 2 diabetes and activation of the lipotoxic pathways are indicated by red arrows.

and binding of GLP-1 and other cAMP-elevating incretins to their respective receptors, may activate HSL via phosphorylation by protein kinase A, thus resulting in increased hydrolysis of stored acylglycerols. In the proposed model (Fig. 7), the generation of fatty acids and other lipolytic products are under normal conditions tightly controlled and hence dynamic and may play a role in providing the lipid signal that appears to be necessary for GSIS (35). Support for a role of HSL in GSIS has been provided by studies of islets of HSL knockout mice, which displayed increased basal insulin secretion and blunted GSIS (36). When the size of the triglyceride pool increases substantially, as during high-fat feeding, the supply of lipolytic products as a result of basal, i.e., cAMP-independent, lipolysis becomes significant. Even further increases in the mobilization of fatty acids may be caused by increased expression of HSL, as is the case in the HSL transgenic mice, and/or by dysregulation of the enzyme. At a certain point, the chronic increase in fatty acid flux, caused by triglyceride overstorage and/or increased HSL action, exceeds a threshold level, a point at which the β-cell enters the lipotoxic pathway (Fig. 7).

A possible mechanism behind lipotoxicity is changes in the expression of critical genes, induced by fatty acids, acyl CoA, or other fatty acid metabolites (3,37). In a hypothesis-driven attempt to identify such changes, the expression of UCP2 was found to be increased in transgenic compared with wild-type islets under both feeding conditions. Recent data have clearly established UCP2 as a negative regulator of insulin secretion. Impaired GSIS has been demonstrated in rat islets overexpressing UCP2 (27), and in UCP2 null mice, GSIS was found to be increased (13). According to the proposed mechanism, UCP2 activation decreases mitochondrial membrane potential, resulting in reduced ATP levels and increased activity of ATP-sensitive K^+ channels, which in turn impairs GSIS (27). It has been proposed that UCP2-dependent mechanisms play an important role in obesity-

induced β-cell dysfunction, and there is much to suggest that fatty acids may be responsible for the upregulation of UCP2 observed in this disease (13). Upregulation of UCP2 by fatty acids has been demonstrated in cultured β-cells (38–40) as well as in isolated rat islets (29). Moreover, UCP2 has been shown to be upregulated in *ob/ob* mice as well as in rodents fed a high-fat diet (13,27). PPARα (41,42) as well as PPARγ (29) are expressed in pancreatic islets, and both have been implicated in mediating the fatty acid–induced upregulation of UCP2 (28–30). Sterol regulatory element–binding protein is another transcription factor that has been implicated in the regulation of UCP2 by fatty acids (40,43), presumably operating downstream of PPAR (40). In islets of transgenic HSL mice, decreased ATP levels accompanied the increased UCP2 levels. The UCP2 protein levels were increased by the high-fat diet in the HSL transgenic mice. Several PPARα-responsive genes, as well as PPARα itself, were upregulated in islets from HSL transgenic mice compared with the respective wild-type controls. Thus, we hypothesize that a PPARα-mediated upregulation of UCP2 at least partly explains the impaired GSIS observed in mice with β-cell–specific overexpression of HSL. Furthermore, we propose that fatty acids, mobilized from the triglyceride stores through the action of HSL, directly or after derivatization to some other lipid component, are responsible for the upregulation of UCP2 (Fig. 7). It is possible that additional alterations in gene expression, induced by the increased flux of ligands to lipid-activated transcription factors or by alternative routes for fatty acid–induced changes in gene expression (37), also contribute to the phenotype.

The expression of both GLUT2 and glucokinase were found to be upregulated in islets of transgenic mice compared with wild-type islets. The reason for this, as well as the mechanism(s) involved, is not known. It seems unlikely that fatty acids are responsible for the upregulation because exposure of islets to palmitate has been shown to decrease the expression of both GLUT2 and

glucokinase via downregulation of the transcription factor PDX-1 (26), a known transactivator of both GLUT2 (25) and glucokinase (44). Since we found PDX-1 levels to be elevated in transgenic islets, it is possible that the effects on GLUT2 and glucokinase observed in the HSL transgenic mice are mediated via PDX-1. Despite the increase in GLUT2 and glucokinase expression, we found glucose oxidation to be significantly decreased in islets of high-fat–fed transgenic mice. There was an increase in palmitate oxidation in high-fat–fed wild-type islets, which was less pronounced in transgenic islets. With regard to transportation of fatty acids into the mitochondria, CPT I was found to be upregulated in islets from high-fat–fed wild-type mice, while CPT I expression was not upregulated in HSL transgenic islets. It is thus possible that the mechanism behind the observed reduction in GSIS in HSL transgenic mice is due to reduced ATP levels, caused not only by increased expression of UCP2 but also by reduced glucose oxidation. Increased generation of intracellular long-chain acyl-CoA, due to increased activity of HSL, may result in allosteric activation or inactivation of metabolic enzymes leading to deteriorated β-cell function.

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