Hormone-Sensitive Lipase Has a Role in Lipid Signaling for Insulin Secretion but Is Nonessential for the Incretin Action of Glucagon-Like Peptide 1

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We previously reported decreased glucose-stimulated insulin secretion (GSIS) in hormone-sensitive lipase−null mice ($HSL^{-/-}$), both in vivo and in vitro. The focus of the current study was to gain further insight into the signaling role and regulation of lipolysis in islet tissue. The effect of glucagon-like peptide 1 (GLP-1) on GSIS was also studied, as GLP-1 could augment GSIS via protein kinase A activation of HSL and lipolysis. Freshly isolated islets from fasted and fed male $HSL^{-/-}$ and wild-type ($HSL^{+/+}$) mice were studied at ages 4 and 7 months. Neutral cholesteryl ester hydrolase activity was markedly reduced in islets from both 4- and 7-month-old male $HSL^{-/-}$ mice, whereas a marked deficiency in triglyceride lipase activity became evident only in the older mice. The deficiencies in lipase activities were associated with higher islet triglyceride content and reduced lipolysis at basal glucose levels. Lipolysis was stimulated by high glucose in islets of both wild-type and HSL-null mice. Severe deficiencies in GSIS were found, but only in islets from 7-month-old, fasted, male $HSL^{-/-}$ mice. GSIS was less affected in 4-month-old fasted male $HSL^{-/-}$ mice and not reduced in female mice. Exogenous delivery of free fatty acids (FFAs) rescued GSIS, supporting the view that the lack of endogenous FFA supply for lipid-signaling processes in $HSL^{-/-}$ mice was responsible for the loss of GSIS. GLP-1 also rescued GSIS in $HSL^{-/-}$ mice, indicating that signaling via HSL is not a major pathway for its incretin effect. Thus, the secretory phenotype of HSL-null mice is gender dependent, increases with age, and is influenced by the nutritional state. Under most circumstances, the major determinant of lipolytic flux in the β-cell involves an enzyme(s) other than HSL that is acutely activated by glucose. Our results support the view that the availability of endogenous FFA through HSL and an additional enzyme(s) is involved in providing lipid moieties for β-cell signaling for secretion in response to glucose. *Diabetes* 53:1733–1742, 2004

The free fatty acid (FFA) supply to the pancreatic β-cell is of importance to both its normal function and its failure in type 2 diabetes (1,2). Fatty acid deprivation causes a loss of glucose-stimulated insulin secretion (GSIS), a process rapidly reversed by replacement with exogenous FFAs (3). In contrast, elevated levels of FFAs supply augment GSIS (4); however, if the excess is chronic, particularly in association with elevated glucose (5), it can induce β-cell apoptosis (1,2,5,6). Although the mechanisms involved in FFA modulation of insulin secretion and β-cell toxicity are not well understood, it is increasingly apparent that the intracellular metabolism of FFAs, resulting in the synthesis of lipid-signaling molecules such as diacylglycerols (DAGs) (1,7) and the accumulation of toxic lipid species such as ceramide (8,9), is involved.

The fatty acid supply to the β-cell can be from exogenous sources such as plasma FFAs and lipoproteins, or endogenous sources such as intracellular triglyceride (TG) stores. The role of islet TG stores in β-cell signaling is unclear. The TG stores may act to buffer intracellular lipid molecules, being a source of FFAs via hydrolysis when the exogenous supply is low and a site to which excess FFAs can be diverted to prevent lipid toxicity. The enhanced capacity to accumulate TGs within islets has been associated with reduced islet FFA−induced cytotoxicity (10). Paradoxically, TG accumulation has also been associated with β-cell failure and apoptosis (6,11,12), but it is unclear whether the excess islet TG stores per se are toxic or whether the potential protective mechanism of FFA esterification to TGs (13) is overwhelmed in such circumstances.

TG hydrolysis (lipolysis) within the β-cell could also be highly regulated and directly involved in insulinotropic stimulus-secretion coupling. Severe depletion of islet TGs (14) or inhibition of lipolysis by the potent inhibitor 3,5-dimethylpyrazole (15) or orlistat (16) causes reduction of GSIS. In addition, the potent hormone, glucagon-like peptide 1 (GLP-1), has been shown to stimulate lipolysis in clonal pancreatic β-cells (HIT) (16). Furthermore, its incretin effect can be inhibited in rat islets (17) by the lipase inhibitor orlistat. The proposed mechanism of GLP-1−induced lipolysis is via activation of hormone-sensitive

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Received for publication 15 January 2004 and accepted in revised form 12 April 2004.

DAG, diacylglycerol; DTT, dithiothreitol; FFA, free fatty acid; GLP-1, glucagon-like peptide 1; GSIS, glucose-stimulated insulin secretion; HSL, hormone-sensitive lipase; IBMX, 3-isobutyl-1-methylxanthine; IPGTT, intraperitoneal glucose tolerance test; KRBH, Krebs-Ringer bicarbonate buffer with HEPES; LC-CoA, long-chain acyl-CoA; NCEH, neutral cholesteryl ester hydrolase; PKA, cAMP-dependent protein kinase; TG, triglyceride; TGL, triglyceride lipase.

lipase (HSL) (16). HSL is a hormonally regulated intracellular acylglycerol lipase with broad substrate specificity and highest expression in adipose and steroidogenic tissues (18,19); it is also expressed in β-cells (20). It catalyzes the hydrolysis of TG, DAG, monoacylglycerides, cholesteryl esters, lipoidal esters of steroid hormones, and retinyl esters (18,19). In adipose tissue it is activated by hormones such as catecholamines, ACTH, and glucagon via cAMP-dependent protein kinase A (PKA) and inhibited by insulin (18,19,21). It has been postulated that GLP-1, a cAMP agonist, activates β-cell HSL via PKA phosphorylation, as occurs in adipocytes (18).

We previously reported defective GSIS in HSL-knockout ($HSL^{-/-}$) mice (22), in both isolated islets and in vivo experiments (23). Islets from $HSL^{-/-}$ mice also had increased TG stores consistent with a role for HSL in β-cell lipolysis (23). The aim of this study was to evaluate the role of β-cell lipolysis in the coupling mechanisms of insulin secretion in response to various classes of secretagogues (glucose, FFAs, a depolarizing concentration of KCl, and GLP-1) and to better define the role of HSL in these processes.

RESEARCH DESIGN AND METHODS

HSL-null ($HSL^{-/-}$) BALB/c mice were created by gene targeting, as previously described (22). These mice have no detectable immunoreactive HSL or cholesteryl esterase activity in adipose tissue, consistent with a total deficiency of HSL (22). Mice described in the current study were derived by breeding F1 SV129/BALB/c hybrid mice onto a C57BL/6 background for five generations (95% C57BL/6). They were genotyped by PCR. Unless otherwise stated, 4- and 7-month-old male homozygous $HSL^{-/-}$ and wild-type $HSL^{+/+}$ littermate mice were used. The presence and absence of HSL in islets of $HSL^{+/+}$ and $HSL^{-/-}$ mice, respectively, were confirmed by RT-PCR (data not shown). Briefly, total RNA was extracted from 150 islets by guanidinium thiocyanate/phenol/chloroform extraction using 20 μg of yeast tRNA as a carrier for islet RNA. First-strand cDNA was generated from the total amount of islet RNA in 150 μl (final volume) of a buffer containing the oligonucleotide $Pd(N)_6$. Then 3 μl of the reverse transcription mixture was amplified in a final volume of 50 μl using the following parameters and primers: 94°C for 45 s, 62°C for 45 s, and 72°C for 1.30 min for 40 cycles; 5′-CTGCGTCTCCAGGAG GATTG-3′ (sense) and 5′-AGCGGTGAGTCCTTCATCAC-3′ (antisense). Animals were housed under controlled temperature (21°C) and light conditions (12-h light/dark cycle) with free access to water and standard diet (11% fat by energy). All procedures were approved by the Institutional Committee for the Protection of Animals at the Centre Hospitalier de l'Université de Montréal.

Blood and plasma parameters. Blood was collected between 8:00 and 10:00 A.M. in both fed and 16 h–fasted mice. Tail vein blood (~60 μl) collected into heparinized tubes from awake mice was used for blood glucose and plasma insulin measurements, and cardiac puncture blood (400 μl) from anesthetized mice anticoagulated with EDTA was used for plasma TG and FFA determinations. Blood glucose was determined by an Ascensia Elite XL glucometer (Bayer, Toronto, Ontario, Canada). Plasma insulin was measured by radioimmunoassay using a rat insulin standard (Linco Research, St. Charles, MO). Plasma TG levels were determined using a commercial kit (GPO Trinder; Sigma-Aldrich, St. Louis, MO). Plasma FFAs were assessed using the NEFA C kit (Wako Chemicals, Neuss, Germany).

Intraperitoneal glucose tolerance tests. Intraperitoneal glucose tolerance tests (IPGTTs) were performed in conscious mice in the morning after a 16-h fast. Warmed (37°C) 18% glucose (1.2 g glucose/kg body weight) was administered intraperitoneally. Tail blood samples (~60 μl) were collected into heparinized tubes at 0, 15, 30, 60, and 120 min for measurement of blood glucose and plasma insulin levels.

Islet isolation. Mice were anesthetized with sodium pentobarbital (Somnotol; MTC Pharmaceuticals, Hamilton, Ontario, Canada) and killed by exsanguination, and used for islet isolation by collagenase digestion of the total pancreas (24). After being digested and washed, the islets were hand picked under a stereomicroscope. Before initiating insulin secretion and lipolysis experiments or collecting islets for TG and lipase activity measurements, islets were cultured for 2 h in regular RPMI-1640 medium containing 2.8 mmol/l glucose supplemented with 10% FCS, 10 mmol/l HEPES (pH 7.4), 1 mmol/l sodium pyruvate, 100 units/ml penicillin, and 100 μg/ml streptomycin at 37°C in a humidified atmosphere containing 5% CO_2.

Islet insulin secretion and insulin content measurements. Freshly isolated islets, cultured for 2 h as described above, were distributed in batches of 10 into 12-well plates, with five replicates for each condition per genotype. They were washed and preincubated for 30 min at 37°C in 1 ml Krebs-Ringer bicarbonate buffer containing 10 mmol/l HEPES (KRBH; pH 7.4), 0.07% defatted BSA (Sigma), and 2.8 mmol/l glucose. The islets were then incubated for 1 h at 37°C in 1 ml of KRBH containing 2.8, 8.3, or 16.7 mmol/l glucose and 0.5% BSA, in the absence or presence of 0.4 mmol/l palmitate or 10 nmol/l human GLP-1 fragment 6–36 amide (Bachem Bioscience, King of Prussia, PA) or 10 μmol/l triacsin C (Sigma). At the end of the incubation, media were withdrawn for insulin determination. Total insulin content of the islets was measured after acid-ethanol (0.2 mmol/l HCl in 75% ethanol) extraction. Insulin was measured by radioimmunoassay, as described above for plasma.

Islet DNA, protein, and triglyceride content determinations. Batches of 10 freshly isolated islets were used to determine DNA by a fluorimetric method using the SYBR Green I nucleic acid dye (Molecular Probes, Eugene, OR) and to determine protein content by the spectrometric bicinchoninic acid method (Pierce, Rockford, IL). DNA salmon sperm and BSA were used as the standard for DNA and protein contents, respectively. Islet TG content was measured in batches of 100 freshly isolated islets, as previously described (23).

Lipolysis measurement in isolated islets. Batches of 100 freshly isolated islets (10 replicates per genotype), cultured as described above, were washed in KRBH containing 0.07% BSA and 2.8 mmol/l glucose. The islets were then transferred into the same medium in a 48-well plate on ice to a final volume of 0.2 ml. Once all islets were transferred, the plate was incubated for 3 h at 37°C in a humidified atmosphere containing 5% CO_2, after which the sample media were collected for glycerol determination by an enzymatic luminescence detection method based on the reduction of NAD^+ to NADH in a series of enzymatic reactions. The reactions are as follows: *1*) glycerol is converted to glycerol-3-PO_4 by glycerol kinase; *2*) glycerol-3-PO_4 is converted to dihydroxyacetone-PO_4 by glycerol-3-PO_4 dehydrogenase, a reaction that is coupled with the first of two NAD^+ reduction steps; *3*) dihydroxyacetone-PO_4 is converted to glyceraldehyde-3-PO_4 by triose-PO_4 isomerase; and *4*) glyceraldehyde-3-PO_4 is converted to 1,3-biphosphoglycerate by glyceraldehyde-3-PO_4 dehydrogenase coupled with the second NAD^+ reduction reaction. Briefly, 40 μl of lipolysis medium from each sample and the glycerol standards were incubated with 40 μl of conversion mix (for 1 ml, 0.67 ml of buffer containing 45 mmol/l triethanolamine, 1.6 mmol/l KH_2PO_4, 30 mmol/l sodium arsenate, 1.7 mmol/l dithiothreitol [DTT], and 4.3 mmol/l $MgCl_2$ [pH 9.0] combined with 88 μl of 90 mmol/l NAD, 94 μl of 16.5 mmol/l ATP, 24 μl glyceraldehyde-3-phosphate dehydrogenase [800 units/ml], 12 μl glycerol kinase [500 units/ml], 1.4 μl glycerol-3-phosphate dehydrogenase [1,700 units/ml], 0.4 μl triose phosphate isomerase [100,000 units/ml], and 110 μl H_2O) for 1 h at 37°C with shaking. The conversion reaction was then stopped by placing the samples on ice and adding 0.4 ml H_2O. For NADH detection, 50 μl of each sample and standard were transferred to a 96-well plate on ice. The plate was then placed in a luminometer (Wallac 1420; Perkin-Elmer, Woodbridge, Ontario, Canada) and 0.2 ml of luminescence mix (for 10 ml mix: 5 ml of 67.3 mmol/l raffinose and 0.4 mmol/l DTT in 0.2 mol/l K_3PO_4 at pH 7, 1.0 ml of 4.7 mmol/l myristic aldehyde in 1% Triton X-100, 5% BSA fraction V at pH 7.0 [1% triton/5% BSA], 0.72 ml luciferase [1.5 mU/ml] in raffinose solution as described above, 0.36 ml NAD(P)H:flavin mononucleotide oxidoreductase [1.7 units/ml] in 1% Triton/5% BSA, and 2.8 ml H_2O; plus addition of 120 μl of 0.1 mmol/l flavin mononucleotide in 20 mmol/l K_3PO_4 at pH 7.0 just before use) was injected into each well. Luminescence was read after 40 s. All reagents and enzymes were purchased from Roche (Laval, Quebec, Canada).

Assays for activities of triglyceride lipase and neutral cholesteryl ester hydrolase. Batches of 100 islets were washed twice in PBS and centrifuged for 5 min at 200*g*. Islet pellets were stored at −80°C until assayed. Islet homogenates were then prepared in 120 μl of homogenization buffer (0.25 mmol/l sucrose, 1 mmol/l DTT, 1 mmol/l EDTA at pH 7.0 with 20 μg/ml leupeptin, 20 μg/ml antipain, and 1 μg/ml pepstatin A). Triglyceride lipase (TGL) and neutral cholesteryl ester hydrolase (NCEH) activity assays were performed on the islet homogenates according to the method of Osterlund et al. (25). These assays are based on measurements of [^{3}H]oleic acid released from tri-[^{3}H]oleoylglycerol and cholesteryl-[^{14}C]oleate (Amersham Pharmacia Biotech, Baie d'Urfé, Quebec, Canada), respectively. Briefly, radiolabeled and labeled substrates were emulsified with phospholipids by sonication (25). Fat-depleted BSA was added to the substrate emulsification mix as a fatty acid acceptor. For each assay, 100 μl of emulsified substrate/BSA mix were added to 100 μl of the homogenized lipase samples. The mixture was incubated at 37°C for 30 min without shaking, after which the released fatty acids were extracted and radioactivity was counted in liquid scintillation fluid, as previously described (25). Protein concentrations of the homogenized sam-

TABLE 1
Body weight and blood glucose, plasma insulin, and lipid levels in fasted and fed male $HSL^{-/-}$ and $HSL^{+/+}$ mice ages 4 and 7 months

	Age 4 months				Age 7 months			
	Fasted		Fed		Fasted		Fed	
	$HSL^{+/+}$	$HSL^{-/-}$	$HSL^{+/+}$	$HSL^{-/-}$	$HSL^{+/+}$	$HSL^{-/-}$	$HSL^{+/+}$	$HSL^{-/-}$
Body weight (g)	29.4 ± 0.5	29.8 ± 0.7	31.8 ± 1.0	31.8 ± 0.8	34.3 ± 1.0	32.2 ± 1.2	38.7 ± 1.3	36.8 ± 1.9
Glycemia (mmol/l)	6.8 ± 0.4	6.1 ± 0.3	6.4 ± 0.3	6.3 ± 0.3	6.6 ± 0.6	5.8 ± 0.4	7.2 ± 0.2	7.8 ± 0.6
Insulin (pmol/l)	53 ± 3	38 ± 3*	199 ± 10	148 ± 4	76 ± 15	41 ± 5*	406 ± 48	401 ± 43
FFA (mmol/l)	0.47 ± 0.03	0.42 ± 0.05	0.39 ± 0.03	0.43 ± 0.02	0.36 ± 0.02	0.24 ± 0.02*	0.28 ± 0.02	0.18 ± 0.01*
TG (mmol/l)	0.63 ± 0.06	0.33 ± 0.04*	1.15 ± 0.12	1.40 ± 0.13	0.72 ± 0.07	0.25 ± 0.04*	1.16 ± 0.14	0.85 ± 0.10

Data are means ± SE (n = 6–20 per group). *$P < 0.05$ vs. $HSL^{+/+}$ for the same nutritional state and age by unpaired, two-tailed Student's t test.

ples were determined with the Bio-Rad protein assay kit (Hercules, CA) using BSA as the standard.

cAMP assay. Batches of 10 freshly isolated islets (six replicates for each condition per genotype), cultured as described above, were distributed in 12-well plates and preincubated for 30 min at 37°C in 1 ml of KRBH (pH 7.4) containing 0.07% BSA, 2.8 mmol/l glucose, and 1 mmol/l 3-isobutyl-1-methylxanthine (IBMX). The islets were then incubated for 30 min at 37°C in 1 ml of the same medium containing 1 mmol/l IBMX and 2.8 or 16.7 mmol/l glucose in the absence or presence of 10 nmol/l GLP-1. IBMX was used to prevent the degradation of formed cAMP over the 30-min incubation time. The media were removed and the islets were then lysed in 0.2 ml of lysis buffer. The lysates were stored overnight at 4°C and the cAMP content was measured the next day using the cAMP Enzymeimmunoassay Biotrack System from Amersham Biosciences (Little Chalfont, Buckinghamshire, U.K.).

Statistical analysis. Data are expressed as means ± SE. Differences among means were evaluated using the two-tailed Student's t test or, for multiple comparisons, one-way ANOVA with Bonferroni post hoc testing. Differences were considered significant at $P < 0.05$.

RESULTS

Body weight and blood metabolic parameters in fasted and fed male mice. The body weights of the $HSL^{-/-}$ mice were not different from those of the wild-type animals at age 4 or 7 months (Table 1). Fasting, but not fed, plasma insulin levels were lower in the HSL-null mice at both ages. In addition, reduced TG levels were observed in fasted animals only (both ages). Plasma FFA levels were lower in fed and fasted 7-month-old male $HSL^{-/-}$ mice, but all other fed blood metabolic parameters were not different between the $HSL^{-/-}$ and $HSL^{+/+}$ mice at either age. The effect of HSL deletion in causing lowered plasma lipid levels was much more evident in the older mice (Table 1).

Intraperitoneal glucose tolerance remains normal in HSL-deficient male mice. IPGTT was unchanged in the $HSL^{-/-}$ mice at age 4 months (Fig. 1*A*) and possibly mildly improved at age 7 months (Fig. 1*B*). The glucose tolerance was maintained in association with lower plasma insulin levels in both the 4- and 7-month-old $HSL^{-/-}$ mice (Fig. 1*C* and *D*). The areas under the curve of the plasma insulin

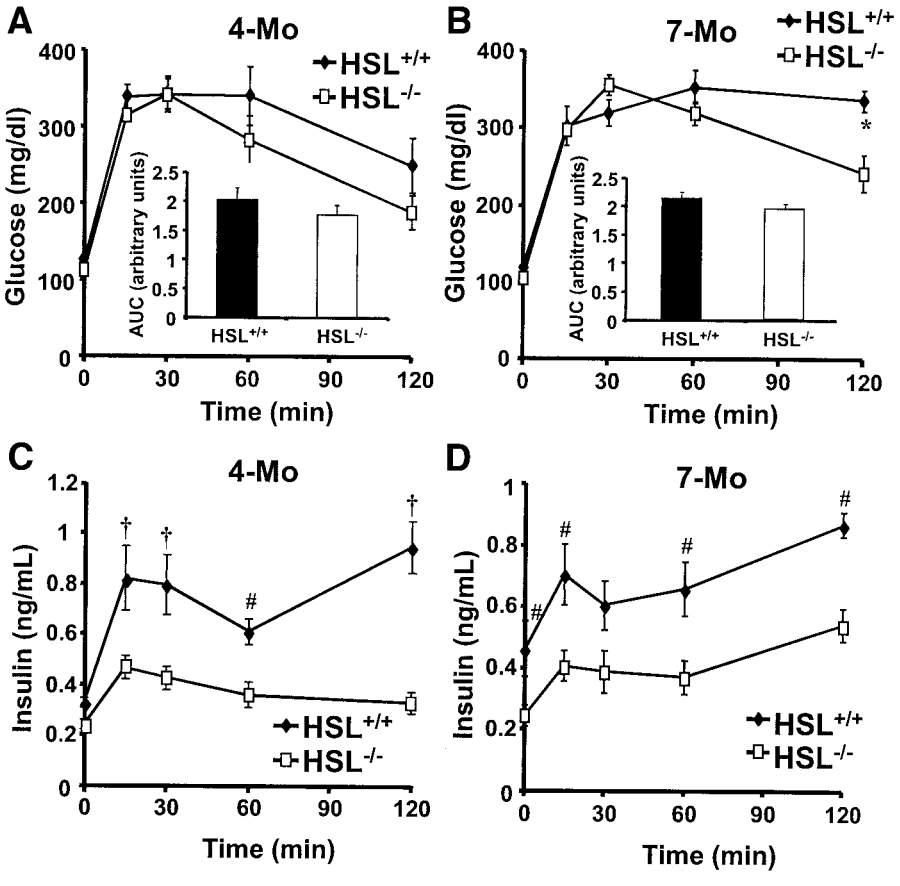

FIG. 1. Plasma glucose and insulin during an IPGTT in $HSL^{+/+}$ and $HSL^{-/-}$ mice. Plasma glucose (*A* and *B*) and insulin (*C* and *D*) levels during IPGTT tests in 16 h–fasted 4- and 7-month-old male $HSL^{+/+}$ and $HSL^{-/-}$ mice are shown. Data are means ± SE of six animals per group. *$P < 0.05$; #$P < 0.01$; †$P < 0.001$.

TABLE 2
Islet protein, DNA, insulin, and triglyceride contents in fasted and fed male $HSL^{-/-}$ and $HSL^{+/+}$ mice ages 4 and 7 months

	Age 4 months				Age 7 months			
	Fasted		Fed		Fasted		Fed	
	$HSL^{+/+}$	$HSL^{-/-}$	$HSL^{+/+}$	$HSL^{-/-}$	$HSL^{+/+}$	$HSL^{-/-}$	$HSL^{+/+}$	$HSL^{-/-}$
Protein (μg/islet)	2.63 ± 0.11	2.94 ± 0.19	2.75 ± 0.14	2.94 ± 0.19	2.36 ± 0.10	3.34 ± 0.11*	2.60 ± 0.18	3.69 ± 0.16*
DNA (ng/islet)	26.8 ± 2.1	35.2 ± 3.9	26.3 ± 1.9	30.4 ± 2.9	32.3 ± 2.5	55.1 ± 4.1*	31.2 ± 2.3	51.9 ± 3.4*
Insulin (ng/islet)	241 ± 15	258 ± 14	234 ± 38	223 ± 50	263 ± 23	375 ± 30*	210 ± 20	330 ± 38*
Insulin (ng/μg protein)	93.0 ± 3.7	90.5 ± 4.7	86.2 ± 3.4	77.3 ± 3.9	113.4 ± 3.7	114.2 ± 3.3	83.1 ± 4.4	90.4 ± 3.6
TG (ng/islet)	32.1 ± 3.2	52.6 ± 7.6*	28.6 ± 2.3	62.5 ± 6.4*	26.7 ± 3.0	62.5 ± 4.1*	26.0 ± 1.8	56.6 ± 9.1*
TG (ng/μg protein)	10.9 ± 0.8	18.8 ± 1.4*	10.8 ± 0.5	21.2 ± 2.3*	11.3 ± 1.3	18.7 ± 1.2*	10.0 ± 0.7	15.3 ± 2.4*

Data are means ± SE (n = 10–15 per group). *$P < 0.05$ vs. $HSL^{+/+}$ for the same nutritional state and age by unpaired, two-tailed Student's t test.

concentrations were, respectively, 50 and 45% lower in the 4- and 7-month-old $HSL^{-/-}$ mice in comparison with the $HSL^{+/+}$ mice. These findings suggest that fasted $HSL^{-/-}$ mice are more insulin sensitive. Of interest, the apparent improvement in insulin sensitivity in the $HSL^{-/-}$mice was well matched by reduced insulin secretion, so that glucose tolerance was minimally changed.

Total protein, DNA, insulin, and triglyceride contents of isolated islets from $HSL^{-/-}$ and $HSL^{+/+}$ male mice. Total islet protein and DNA were increased by 1.4- and 1.7-fold, respectively, in 7-month-old male $HSL^{-/-}$ mice (Table 2). The greater increase in DNA content is consistent with the islets of the $HSL^{-/-}$ mice being larger due to increased cell number. There was a nonsignificant trend for the $HSL^{-/-}$ islets to be larger in the 4-month-old mice. Islet insulin content, corrected for protein, was similar in $HSL^{-/-}$ and control mice. Because the insulin content per islet at age 7 months was 1.4-fold greater in HSL-deficient mice due to the larger size of their islets, insulin secretion studies in subsequent experiments were expressed as a function of the islet insulin content. Islet TG content corrected for protein was, respectively, 70 and 95% higher in fasted and fed 4-month-old male $HSL^{-/-}$ compared with $HSL^{+/+}$ mice, and 65 and 50% in fasted and fed 7-month-old male $HSL^{-/-}$ mice (Table 2).

Basal lipolysis and lipase activities in isolated islets of $HSL^{-/-}$ and $HSL^{+/+}$ male mice. The islet basal lipolysis, TGL, and NCEH activity data for 4- and 7-month-old male mice are shown in Fig. 2*A*–*C* for fasted mice and Fig. 2*D*–*F* for fed mice. HSL deletion had no effect on basal (measured at 2.8 mmol/l glucose) lipolysis (as assessed by glycerol release determinations) in islets isolated from fasted and fed 4-month-old mice (Fig. 2*A* and *D*). Lipolysis, however, was 38% lower in islets from fasted 7-month-old $HSL^{-/-}$ mice compared with $HSL^{+/+}$ mice (Fig. 2*A*). No difference in basal islet lipolysis was observed between the genotypes in fed 7-month-old mice (Fig. 2*D*). In view of the fact that lipolysis in islets from 7-month-old fasted control mice was higher than that of fed control mice and that lipolysis was similar in both fed and fasted islets obtained from HSL-null mice, lipolysis of the $HSL^{-/-}$ mice, therefore, might not have responded to regulatory signals present during fasting.

HSL is responsible for part of the TGL activity and most of the NCEH activity in adipocytes (26,27). The TGL and NCEH activities within the islets of the $HSL^{-/-}$ and

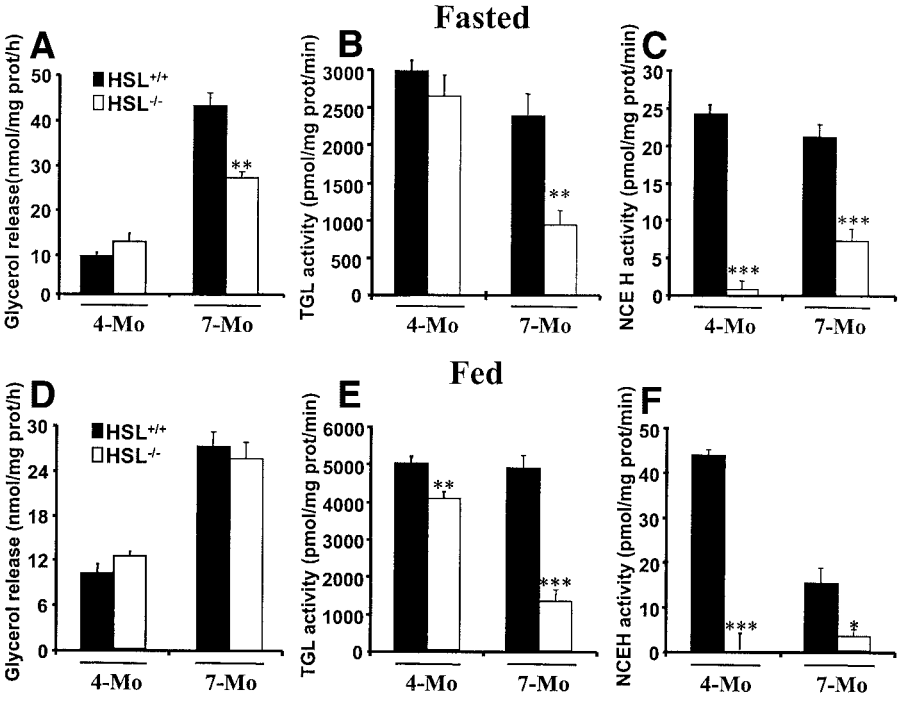

FIG. 2. Lipolysis rates and TGL and NCEH activities in isolated islets from $HSL^{-/-}$ and $HSL^{+/+}$ mice. Shown are lipolysis and TGL and NCEH activities in isolated islets (100 per well) from fasted (*A–C*) and fed (*D–F*) male $HSL^{-/-}$ and wild-type mice at ages 4 and 7 months. *A* and *D*: Lipolysis was measured as the rate of glycerol release into the incubation medium over 3 h at 2.8 mmol/l glucose. The islets were then recovered for TGL (*B* and *E*) and NCEH (*C* and *F*) activities. Data are means ± SE of 10 determinations from two separate islet isolations from nine mice per genotype. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ for the same nutritional state and age.

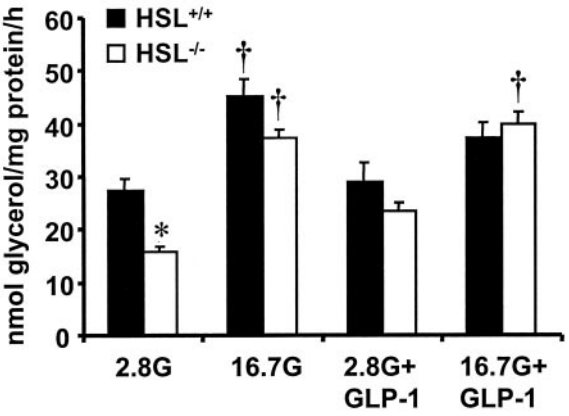

FIG. 3. Lipolysis rates in response to elevated glucose (G) and GLP-1 in isolated islets from fasted 7-month-old $HSL^{-/-}$ and $HSL^{+/+}$ mice. Lipolysis was measured over 3 h at 2.8 and 16.7 mmol/l glucose in the absence or presence of 10 nmol/l GLP-1. Data are means ± SE of 8–15 determinations from three ($HSL^{-/-}$) and two ($HSL^{+/+}$) separate experiments. *$P < 0.05$ vs. $HSL^{+/+}$ at 2.8 mmol/l glucose; †$P < 0.001$ vs. same genotype and condition at 2.8 mmol/l glucose.

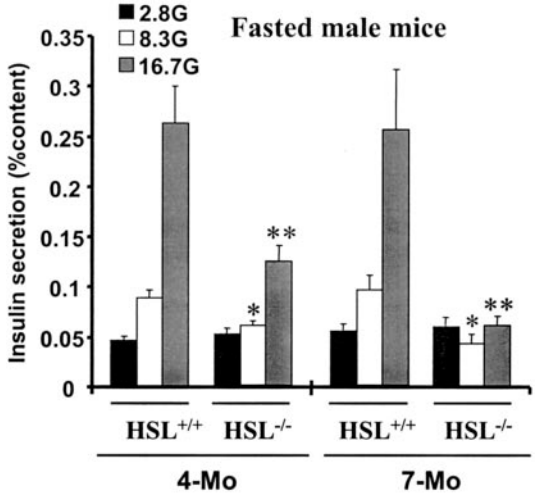

FIG. 4. Isolated islets from fasted male $HSL^{-/-}$ mice show defective GSIS. Insulin secretion was measured in freshly isolated islets from fasted 4- and 7-month-old male $HSL^{-/-}$ and $HSL^{+/+}$ mice. Groups of 10 islets were incubated for 1 h in 2.8, 8.3, or 16.7 mmol/l glucose, after which insulin release into the incubation media and islet total insulin content were measured. Data are means ± SE of 15 determinations in three separate experiments. *$P < 0.01$, **$P < 0.005$ vs. $HSL^{+/+}$ for the same age.

$HSL^{+/+}$ mice of this study were consistent with this pattern of activities, as NCEH activity was essentially abolished or markedly reduced in, respectively, 4- and 7-month-old fasted and fed male $HSL^{-/-}$ mice (Fig. 2*C* and *F*). TGL activity was only mildly reduced in 4-month-old fed $HSL^{-/-}$ mice (Fig. 2*E*), but markedly reduced in 7-month-old fed and fasted male $HSL^{-/-}$ mice (Fig. 2*B* and *E*).

The basal lipolysis and lipase activity data taken together are consistent with the view that there is more than one lipase responsible for TG hydrolysis in islet tissue. The fact that glycerol release remained unaltered in 7-month-old fed $HSL^{-/-}$ mice despite reductions in TGL and NCEH activities suggests that a significant proportion of the released glycerol may have originated from lipolysis of complex lipids (e.g., DAG, phospholipids) other than TG and cholesteryl esters. This explanation could also reconcile the finding that, although glycerol release was unaltered, TG levels were elevated in islets from fed $HSL^{-/-}$ mice. Importantly, despite the existence of lipase activity other than HSL in islets, knockout of the HSL gene was still associated with markedly reduced islet TG lipase activity in 7-month-old mice. Furthermore, when these mice were fasted (the only situation in which insulin secretion was found to be substantially altered; see below), basal lipolysis was also reduced.

Elevated glucose stimulates lipolysis in islets of $HSL^{-/-}$ and $HSL^{+/+}$ mice. The effects of high glucose (16.7 mmol/l) and 10 nmol/l GLP-1 on lipolysis in isolated islets from 7-month-old fasted $HSL^{-/-}$ and $HSL^{+/+}$ mice are shown in Fig. 3. High glucose increased lipolysis by 65 and 134% in $HSL^{+/+}$ and $HSL^{-/-}$ islets, respectively. GLP-1 failed to stimulate lipolysis at low or high glucose in $HSL^{+/+}$ mice, but resulted in a 47% increase ($P < 0.001$) in glycerol release at low glucose in the $HSL^{-/-}$ mice. GLP-1 did not potentiate glycerol release in the $HSL^{-/-}$ mice at high glucose. These results confirmed the presence of glucose-stimulated lipolysis in mouse islets, as was recently observed in rat islets (28), and demonstrated that this can occur via a lipase other than HSL. GLP-1 had no clear effect on lipolysis, but an effect that is difficult to detect with the method used cannot be entirely ruled out, as is suggested by the trend for higher glycerol release by GLP-1 stimulation at basal glucose in $HSL^{-/-}$ mice.

Glucose-stimulated insulin secretion is impaired in isolated islets from fasted male $HSL^{-/-}$ mice. GSIS was assessed in freshly isolated islets from overnight fasted male $HSL^{-/-}$ and $HSL^{+/+}$ mice at ages 4 and 7 months (Fig. 4). GSIS (secretion above basal) in the 4-month-old $HSL^{-/-}$ mice was reduced by 83 and 66% at 8.3 and 16.7 mmol/l glucose, respectively, whereas, in the 7-month-old $HSL^{-/-}$ mice, it was abolished at both elevated glucose concentrations (Fig. 4). Thus, the secretory defect worsened with increasing age. In contrast, insulin secretion in response to a depolarizing concentration of KCl (35 mmol/l) in islets isolated from fasted 7-month-old male $HSL^{-/-}$ mice compared with $HSL^{+/+}$ mice remained unaltered (data not shown).

Defect in glucose-stimulated insulin secretion in isolated islets from $HSL^{-/-}$ mice is dependent on nutritional state and sex. GSIS in freshly isolated islets from fed 4-month-old $HSL^{-/-}$ mice was not different between $HSL^{-/-}$ and $HSL^{+/+}$ mice (Fig. 5*A*). In fed 7-month-old $HSL^{-/-}$ mice, there was moderate impairment in GSIS (Fig. 5*A*). Secretion above basal at 16.7 mmol/l glucose was 32% lower in the fed 7-month-old $HSL^{-/-}$ mice, in comparison with moderately severe and total loss of GSIS in islets from fasted 4- and 7-month-old $HSL^{-/-}$ mice, respectively (Fig. 4). Thus, the secretory defect was more apparent in islets from fasted animals.

The islet phenotype of 7-month-old female $HSL^{-/-}$ mice was very different from that of the corresponding male mice. Instead of the marked loss of GSIS that was seen in fasted male $HSL^{-/-}$ mice, GSIS was increased in fasted female $HSL^{-/-}$ compared with $HSL^{+/+}$ mice (Fig. 5*B*). There was also a small increase in basal insulin secretion in fed female $HSL^{-/-}$ mice compared with $HSL^{+/+}$ mice. In contrast to the male mice (Table 1), fasting blood glucose, plasma insulin, and plasma FFA levels of 7-month-old female $HSL^{-/-}$ mice were not different from those seen in

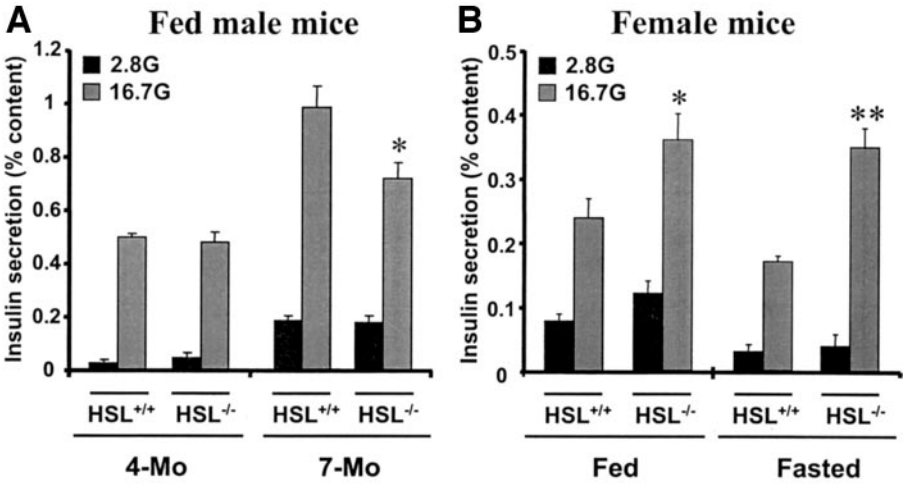

FIG. 5. The defect in GSIS in isolated islets of HSL-deficient mice is dependent on nutritional state and sex. Glucose-stimulated insulin secretion was measured, as described in the legend of Fig. 4, in isolated islets from fed 4- and 7-month-old male $HSL^{-/-}$ and $HSL^{+/+}$ mice (*A*) and fasted or fed 7-month-old female $HSL^{-/-}$ and $HSL^{+/+}$ mice (*B*). Data are means ± SE of 10 determinations in two separate experiments. *$P < 0.05$, **$P < 0.005$ vs. $HSL^{+/+}$.

$HSL^{+/+}$ mice (glycemia: 5.3 ± 0.3 vs. 5.6 ± 0.2 mmol/l; insulinemia: 57 ± 15 vs. 62 ± 12 pmol/l; FFA levels: 0.25 ± 0.01 vs. 0.26 ± 0.02 mmol/l). Plasma TG levels, however, were lower in fasting female $HSL^{-/-}$ compared with $HSL^{+/+}$ mice (0.38 ± 0.07 vs. 0.57 ± 0.08 mmol/l). This difference, although following the same trend as the male mice, was less marked and did not reach statistical significance ($P = 0.12$). Thus, female $HSL^{-/-}$ mice showed little phenotypic alterations in comparison with male mice, possibly due to better compensation through changes in the expression and/or activity of other lipases or due to the fact that female mice have more adipose tissue with a different pattern of lipid deposition than do male mice (22).

Provision of exogenous FFA restores normal glucose-stimulated insulin secretion in isolated islets from fasted $HSL^{-/-}$ mice. GSIS to 8.3 and 16.7 mmol/l glucose in isolated islets from fasted 4- and 7-month-old $HSL^{+/+}$ mice was markedly augmented by incubation in the presence of 0.4 mmol/l palmitate/0.5% BSA (Fig. 6*A* and *B*). GSIS in islets from fasted $HSL^{-/-}$ mice, incubated in the absence of palmitate, was markedly diminished at age 4 months and abolished at age 7 months (Fig. 6*A* and *B*), consistent with the results of the earlier experiment (Fig. 4). Importantly, this consistent GSIS defect in $HSL^{-/-}$ islets from fasted mice was completely (4-month-old mice) or partially (7-month-old mice) reversed by the provision

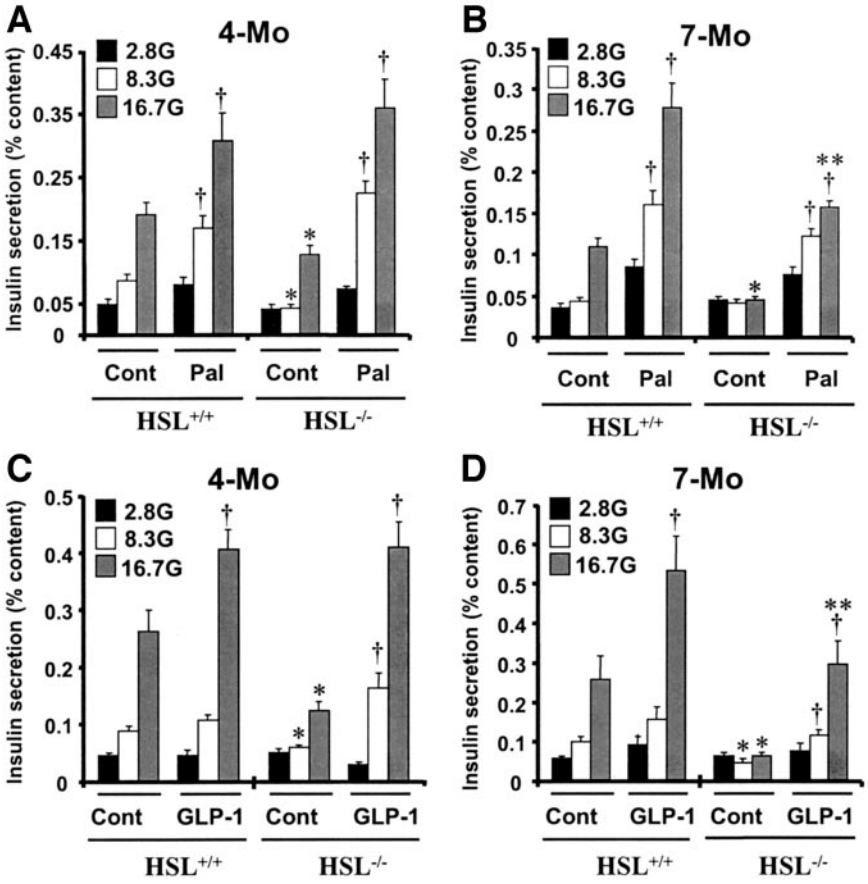

FIG. 6. Exogenous palmitate and GLP-1 independently restore normal GSIS in isolated islets from fasted male HSL-deficient mice. Groups of 10 islets from 4- and 7-month-old $HSL^{-/-}$ or $HSL^{+/+}$ mice were incubated for 1 h in 2.8, 8.3, or 16.7 mmol/l glucose with and without 0.4 mmol/l palmitate/0.5% BSA (Pal; *A* and *B*) or with and without 10 nmol/l GLP-1 (*C* and *D*). Data are means ± SE of 10 determinations in two separate experiments. *$P < 0.05$, **$P < 0.01$ vs. $HSL^{+/+}$ for the same condition; †$P < 0.05$ vs. same genotype without palmitate or GLP-1 treatment.

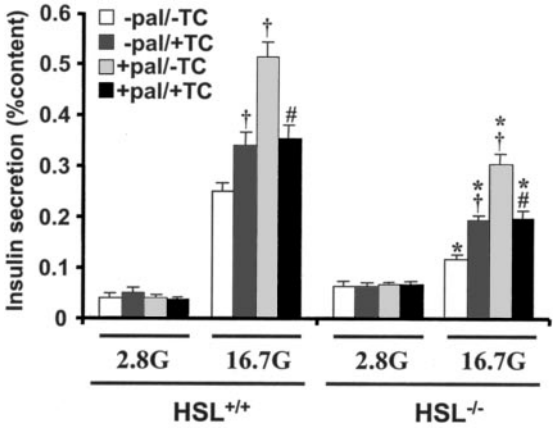

FIG. 7. Triacsin C (TC) suppresses the restoration of GSIS promoted by exogenous palmitate (pal) in isolated islets from fasted male HSL-deficient mice. Groups of 10 islets from 7-month-old $HSL^{-/-}$ or $HSL^{+/+}$ mice were incubated for 1 h in 2.8 or 16.7 mmol/l glucose (G) with or without 0.4 mmol/l palmitate/0.5% BSA and with or without 10 μmol/l triacsin C. Data are means ± SE of 12 determinations in three separate experiments. *$P < 0.005$ vs. $HSL^{+/+}$ for the same condition; †$P < 0.01$ vs. same genotype without palmitate and triacsin C; #$P < 0.05$ vs. same genotype with palmitate without triacsin C treatment.

of exogenous palmitate (Fig. 6*A* and *B*). These results are therefore consistent with the view that the defect in GSIS in the islets from the fasted $HSL^{-/-}$ mice was due to limited availability of FFAs from endogenous sources for channeling into lipid signaling via long-chain acyl-CoA (LC-CoA). Provision of exogenous FFA overrode this poor access to endogenous lipid sources.

Consistent with this interpretation, the results in Fig. 7 show that the fatty acyl-CoA synthase inhibitor, triacsin C, suppressed the restoration of GSIS promoted by exogenous palmitate in 7-month-old fasted $HSL^{-/-}$ mice. Triacsin C (10 μmol/l) in the absence of exogenous palmitate slightly enhanced GSIS in islets from HSL-null and control mice without altering basal insulin release (Fig. 7). The reason for this action of the drug is unknown.

GLP-1 reverses the defect in glucose-stimulated insulin secretion in islets from fasted male $HSL^{-/-}$ mice. One of the possible mechanisms of action by which GLP-1 augments GSIS is via activation of HSL, thus increasing the availability of FFAs for lipid-signaling pathways (16,17). This hypothetical pathway is via GLP-1's activation of adenylate cyclase, which causes an increase in cAMP production. cAMP activates PKA, which in turn activates HSL (16,17). Thus we assessed the effect of GLP-1 on GSIS in islets from fasted $HSL^{-/-}$ mice, with the expectation that its effect would be diminished.

We first showed that there were no differences in cAMP production in islets isolated from $HSL^{-/-}$ compared with $HSL^{+/+}$ mice, basally or when stimulated by 10 nmol/l GLP-1. Thus GLP-1, similarly and significantly, increased islet cAMP content in all groups (P at least <0.05). The cAMP levels after a 30-min incubation, which were all measured in the presence of 1 mmol/l IBMX to inhibit phosphodiesterases, were 16.2 ± 0.6 and 20.3 ± 0.5 vs. 21.5 ± 0.5 and 23.4 ± 0.5 pmol/mg protein, basal and stimulated, in 4-month-old $HSL^{-/-}$ and $HSL^{+/+}$ mice, and 11.4 ± 0.4 and 14.1 ± 0.6 vs. 11.4 ± 0.4 and 14.6 ± 0.8 pmol/mg protein, basal and GLP-1-stimulated conditions, in 7-month-old $HSL^{-/-}$ and $HSL^{+/+}$ mice, respectively. The effect of GLP-1 on glycerol release is shown above.

GLP-1 was associated with augmentation of GSIS in $HSL^{+/+}$ mice, particularly in the 7-month-old mice (Fig. 6*C* and *D*). Interestingly, GLP-1 had an effect similar to that of exogenous palmitate in the $HSL^{-/-}$ mice in that it totally restored GSIS in isolated islets from fasted 4-month-old male $HSL^{-/-}$ mice (Fig. 6*C*) and partially restored GSIS in islets from fasted 7-month-old male mice (Fig. 6*D*). These results indicated that HSL is not essential for the action of GLP-1 to augment GSIS. Action of GLP-1 via an alternate lipase, however, cannot be excluded.

DISCUSSION

The fatty acid supply to β-cells for lipid-signaling processes can be from exogenous and endogenous sources. The results of this study in HSL-deficient mice show roles for HSL in the maintenance of both exogenous and endogenous FFA supplies and, in at least some circumstances, the preservation of normal GSIS. Thus, with respect to the exogenous FFA supply and consistent with previous reports (29,30), HSL deletion in male mice was associated with markedly reduced plasma FFA and TG levels during fasting. For the endogenous supply, there was evidence of reduced access to islet TG stores, as the TG stores were increased in association with reduced islet TG lipase activity and lipolysis, particularly in 7-month-old mice. Given that the TG lipase activity was most affected in older mice, and plasma lipid levels were most reduced in the fasting state in male mice, β-cell islet fatty acid deprivation was almost certainly greatest in the older fasted male mice. Therefore, the finding of the greatest loss of GSIS in the fasted 7-month-old male $HSL^{-/-}$ mice, together with the reversibility of this loss with FFA replacement, are completely consistent with the essential role of fatty acid supply in normal insulin secretion (3). Furthermore, the triacsin C experiment showed that activation of FFAs to LC-CoA is necessary for this fatty acid effect, consistent with the malonyl-CoA/LC-CoA signaling pathway of insulin secretion as proposed by Prentki and Corkey (31). These findings are also consistent with our previous study, in which triacsin C diminished FFA augmentation of insulin secretion stimulated by glucose or nonfuel secretagogues (32). The distal mechanism by which LC-CoA promoted insulin secretion, however, is not known. Possibilities include a direct LC-CoA effect on exocytosis processes or indirect actions via acylation of proteins or esterification into lipid-signaling molecules such as phosphatidate and DAG (31,32). Importantly, the results underscore a role for β-cell HSL in the provision of an endogenous FFA supply when exogenous fatty acid supplies are low. The importance of lipolysis for the formation of a lipid-derived coupling signal was confirmed by the fact that inhibition of lipolysis in rat islets by orlistat reduced insulin secretion (16).

The islet NCEH and TGL activity data are consistent with lipase activity results in other tissues, which show HSL is responsible for most or all of the NCEH and part of the TGL activity (26,27,33). However, it remains unknown if DAG hydrolase activity is reduced in $HSL^{-/-}$ islets. Although HSL is the main source of DAG hydrolase activity in adipocytes (33), this activity in liver and skeletal muscle may occur via another enzyme(s) (26,33). Interestingly, the relative importance of HSL-TGL activity to

overall TGL activity increased as the HSL$^{-/-}$ mice aged. This provides an explanation for the detection of reduced basal lipolysis in the older mice only. The higher islet TG content in the islets from 4-month-old HSL$^{-/-}$ mice, however, strongly suggests abnormal lipolysis was also present in these islets, although our in vitro assay (glycerol release) was unable to detect it. Clearly the lipase and lipolysis results are indicative of at least one other lipase with TGL activity in islets. The degree to which this other enzyme(s) is upregulated in the β-cell of the HSL$^{-/-}$ mice to compensate for the total deficiency of HSL is unknown at this time.

Of considerable interest is the demonstration of glucose-stimulated lipolysis in islets from HSL$^{+/+}$ and HSL$^{-/-}$ mice. Thus, glucose-stimulated lipolysis has now been shown to occur in mouse and rat islets (28). This process may prove to be important for signal transduction in GSIS. The finding that glucose increased lipolysis in the HSL$^{-/-}$ islets is indicative of an alternate lipase being responsive to glucose. Why this did not prevent loss of GSIS, however, is not known. It may have been due to differences in subcellular localization of the lipases. Interestingly, islet HSL has recently been shown to be associated with insulin secretory granules (34).

Inhibition of lipolysis in rat islets by orlistat has been shown to inhibit the incretin action of GLP-1 (17). It was therefore a surprise that GLP-1 was able to rescue the loss of GSIS in isolated islets from fasted male HSL$^{-/-}$ mice. Hence, HSL is not essential for the incretin action of GLP-1. Although it is possible that GLP-1 was able to overcome the effect of fatty acid deprivation via signaling pathways independent of fatty acid metabolism (35), the findings could also be consistent with an action of GLP-1 to stimulate an alternative lipase. As discussed above, the failure of GLP-1 to significantly alter glycerol release does not rule out this possibility. Given that fatty acid deprivation has previously been shown to block insulin secretion to both fuel and nonfuel secretagogues like GLP-1 (36), we favor the latter possibility. Clearly, the characteristics of other islet β-cell lipase activities, including their responsiveness to PKA activators such as GLP-1, warrant further investigation. Of relevance, isoproterenol, a β-agonist believed to promote lipolysis through HSL, has been shown to stimulate lipolysis in adipocytes of HSL-knockout mice (26,27). This supports the notion of another cAMP-mediated lipase in adipose tissue and perhaps in the β-cell as well.

Several groups have now generated HSL$^{-/-}$ mice, and much heterogeneity is reported in the metabolic phenotype, particularly in relation to the measurement of insulin sensitivity (23,33,37) and β-cell function (23,33). This is also true within our own HSL$^{-/-}$ mouse strain. Insulin sensitivity assessed in the first study by the intraperitoneal insulin tolerance test (fed mice) was reduced (23), whereas, in the current study, insulin sensitivity, as estimated from the IPGTT data (fasted mice), was enhanced in the HSL$^{-/-}$ mice, as glucose levels during the tolerance test remained unaltered in the face of reduced circulating insulin in HSL-null mice. Furthermore, GSIS in isolated islets from fasted HSL$^{-/-}$ mice was impaired in male mice but enhanced in female mice. Although at first sight the data and literature seem confusing, some patterns relating to nutritional status and sex are now evolving. With respect to nutritional status, serum FFA and TG levels are usually unchanged (30,33) in the fed state, but markedly reduced in the fasted state (30,37) in HSL$^{-/-}$ compared with HSL$^{+/+}$ mice, as was also observed in the current study. Similarly, hepatic TG content has been reported as unchanged (30) or increased (29,33) in the fed state and decreased in the fasted state (22,30,37,38) in HSL$^{-/-}$ mice. With the well-known inverse correlations between plasma FFA and hepatic TG content and insulin sensitivity (2), it is conceivable that these abnormally wide fluctuations in whole animal lipid partitioning with nutritional status cause swings between relatively reduced (fed state) and enhanced (fasting state) insulin sensitivity in the HSL$^{-/-}$ mice compared with wild-type mice. Thus, our favored hypothesis is that the differences in insulin sensitivity between our two studies are due to differences in the nutritional status (fed versus fasted) at the time of assessment. The degree to which insulin sensitivity changes with the nutritional state, however, may also relate to the amount of fat in the diet and whether the mice are male (37) or female (33).

The finding of reduced and enhanced GSIS in islets from male and female HSL$^{-/-}$ mice, respectively, is difficult to explain, but the results are consistent with those previously reported in 4-month-old female mice (33). It may be that the islets from the female HSL-null mice are not as deprived of their fatty acid supply during fasting as it appears from plasma lipid levels. There does seem to exist some sexual dimorphism in the effects of disturbances in HSL expression, as was evident in studies of the human HSL −60C/G promoter variant (39–42). This variant promoter in vitro is associated with 40% less efficiency in HSL expression (41). The HSL −60C/G promoter variant is associated with obesity in women (42) and lower fasting plasma FFA levels (39) and higher insulin sensitivity in males (40,42). HSL deletion in mice is associated with marked changes in adipocyte metabolism, with evidence of a reduction in the size of white adipose tissue depots and marked alterations in adipose tissue expression of both insulin sensitizing and insulin resistance−inducing adipokines (29). Considering that the total fat mass is approximately twofold greater in female compared with male HSL$^{-/-}$ mice (22) and the marked differences that are known to occur in fat cell distribution between males and females (43), the overall balance of disturbed adipokine metabolism with HSL deletion could also be quite different between males and females, thus providing further reason for the observed metabolic dimorphism between male and female HSL$^{-/-}$ mice, as well as the HSL −60C/G promoter variant in humans (39–42).

It is of interest to discuss the findings of a recent report in which HSL was overexpressed specifically in β-cells in mice (44). These mice, having been fed a high-fat diet, had impaired glucose tolerance with poor GSIS. It was concluded that excess HSL activity in the islet prevented detoxification of the increased fat supply, as the potentially protective diversion of fatty acid to TG stores was prevented (44). This study, in which HSL was overexpressed (44), and the current study, in which it was absent, highlight the importance of normal lipolysis pathways with regulation of lipid stores for normal insulin secretion. The

possibility should also be considered that the provision of fatty acids through lipolysis, at least via HSL in male mice, is important for normal GSIS upon refeeding.

ACKNOWLEDGMENTS

This work was supported by grants from the National Institute of Health (M.P. and B.E.C.; R01-DK-63356), the Juvenile Diabetes Research Foundation (M.P.; 1-2001-920), and the Canadian Institute of Health Research (M.P.; MOP-12653). M-L.P. is the recipient of a fellowship from Fondation pour la Recherche Médicale.

We thank Mélanie Fortier for advice for the glycerol release measurements, Johane Morin for expert technical assistance, and Pellegrino Masiello for critical review of the manuscript.

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