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. Diabetologia 33131733–1742, 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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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.

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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, monoaoylglycerides, cholesterol 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 (25). 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 ester activity in adipose tissue, consistent with a total deficiency of HSL (22). Mice described in the current study were derived by breeding F1 SV12BALB/c hybrid mice onto a C57BL/6 background for five generations (95% C57BL6). 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 dN(5). 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 3:30 min for 40 cycles; 5′-CTGCGTTCTCCAGGAG GATTG-3′ (sense) and 5′-AGGGTGAAGCTCTCATCAC-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 was collected and 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) and determined after acid-ethanol (0.2 mmol/l HCl in 75% ethanol) extraction. 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 fluorinometric method using the SYBR Green I nucleic acid dye (Molecular Probes, Eugene, OR) and to determine protein content by the spectrophotometric biichonionic 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 medium containing 0.7% 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% CO2, 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-phosphate by glycerol-3-phosphate dehydrogenase, a reaction that is coupled with the first of two NAD+ reduction steps; 2) dihydroxyacetone-P is converted to glyceraldehyde-3-phosphate by triose-P isomerase; and 3) glyceraldehyde-3-phosphate is converted to 1,3-bisphosphoglycerate by glyceraldehyde-3-phosphate dehydrogenase coupled with the second NAD+ reduction reaction. Briefly, 40 µl of the 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 KH2PO4, 30 mmol/l sodium ascorbate, 1.7 mmol/l dithiotheritol [DTT], and 4.3 mmol/l MgCl2 [pH 9.0]) with 58 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 0.2 mmol/l glyceraldehyde-3-phosphate dehydrogenase [1,700 units/ml], 0.4 µl triose phosphate isomerase [100,000 units/ml], and 110 µl (pH 8.0) at 1°C until assayed. Islet pellets were stored at 80°C until use.

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 200g. 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 just before use) and transferred into each well. Luminescence was read for 40 s. All reagents and enzymes were purchased from Roche (Laval, Quebec, Canada).

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The body weights of the fasted and fed male mice. Body weight and blood metabolic parameters in cAMP assay. BSA as the standard. Samples 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 mmol/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 considered significant at \( P < 0.05 \).

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

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### Table 1

<table>
<thead>
<tr>
<th></th>
<th>Fasted Age 4 months</th>
<th>Fed Age 4 months</th>
<th>Fasted Age 7 months</th>
<th>Fed Age 7 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSL(^{+/+})</td>
<td>29.4 ± 0.5</td>
<td>31.8 ± 1.0</td>
<td>34.3 ± 1.0</td>
<td>38.7 ± 1.3</td>
</tr>
<tr>
<td>HSL(^{-/-})</td>
<td>29.8 ± 0.7</td>
<td>31.8 ± 0.8</td>
<td>32.2 ± 1.2</td>
<td>36.8 ± 1.9</td>
</tr>
<tr>
<td>Glycemia (mmol/l)</td>
<td>6.8 ± 0.4</td>
<td>6.4 ± 0.3</td>
<td>6.6 ± 0.6</td>
<td>7.2 ± 0.2</td>
</tr>
<tr>
<td>Insulin (pmol/l)</td>
<td>5.3 ± 3</td>
<td>199 ± 10</td>
<td>76 ± 15</td>
<td>406 ± 48</td>
</tr>
<tr>
<td>FFA (mmol/l)</td>
<td>0.47 ± 0.03</td>
<td>0.39 ± 0.03</td>
<td>0.36 ± 0.02</td>
<td>0.28 ± 0.02</td>
</tr>
<tr>
<td>TG (mmol/l)</td>
<td>0.63 ± 0.06</td>
<td>1.15 ± 0.12</td>
<td>0.72 ± 0.07</td>
<td>1.16 ± 0.14</td>
</tr>
</tbody>
</table>

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.

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**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. * \( P < 0.05 \); † \( P < 0.01 \); ‡ \( P < 0.001 \).
HSL is 95% higher in fasted and fed 4-month-old male HSL$^{-/-}$ mice compared with HSL$^{+/+}$ mice, and 65 and 50% in fasted and fed 7-month-old male HSL$^{-/-}$ mice (Table 2).

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

<table>
<thead>
<tr>
<th>Protein (μg/islet)</th>
<th>Age 4 months</th>
<th>Protein (μg/islet)</th>
<th>Age 7 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSL$^{+/+}$</td>
<td>Fasted</td>
<td>HSL$^{+/+}$</td>
<td>Fasted</td>
</tr>
<tr>
<td>2.63 ± 0.11</td>
<td>2.94 ± 0.19</td>
<td>2.75 ± 0.14</td>
<td>2.94 ± 0.19</td>
</tr>
<tr>
<td>HSL$^{-/-}$</td>
<td>Fasted</td>
<td>HSL$^{-/-}$</td>
<td>Fasted</td>
</tr>
<tr>
<td>2.36 ± 0.10</td>
<td>3.34 ± 0.11*</td>
<td>2.60 ± 0.18</td>
<td>3.69 ± 0.16*</td>
</tr>
</tbody>
</table>

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$^{-/-}$ 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).

**Fig. 2.** Lipolysis rates and TGL and NCEH activities in isolated islets from HSL$^{-/-}$ and HSL$^{+/+}$ mice. Shown are lipolysis and TGL and NCEH activity data for 4- and 7-month-old male mice (Fig. 2A–C) for fasted mice and Fig. 2D–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. 2A and D). Lipolysis, however, was 38% lower in islets from fasted 7-month-old HSL$^{-/-}$ mice compared with HSL$^{+/+}$ mice (Fig. 2A). No difference in basal islet lipolysis was observed between the genotypes in fed 7-month-old mice (Fig. 2D). 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...
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. 2C and F). TGL activity was only mildly reduced in 4-month-old fed HSL−/− mice (Fig. 2E), but markedly reduced in 7-month-old fed and fasted male HSL−/− mice (Fig. 2B 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 mmol/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 60% 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. 5A). In fed 7-month-old HSL−/− mice, there was moderate impairment in GSIS (Fig. 5A). 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. 5B). 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...
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. 6A 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. 6A 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...
of exogenous palmitate (Fig. 6A and B). These results are therefore consistent with the view that the effect of 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.

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 $\beta$-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 $\beta$-cell lipase activities, including their responsiveness to PKA activators such as GLP-1, warrant further investigation. Of relevance, isoproterenol, a $\beta$-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 $\beta$-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 $\beta$-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$^{-/}$ promoter variant (39–42). This variant promoter in vitro is associated with 40% less efficiency in HSL expression (41). The HSL$^{-/}$ mutant 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$^{-/}$ 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 $\beta$-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.

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REFERENCES


