Rapid Publication

A Role for Hormone-Sensitive Lipase in Glucose-Stimulated Insulin Secretion

A Study in Hormone-Sensitive Lipase-Deficient Mice

Raphaël Roduit,[1] Pellegrino Masiello,[2] Shu Pei Wang,[3] Hong Li,[3] Grant A. Mitchell,[3] and Marc Prentki[1]

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Endogenous lipid stores are thought to be involved in the mechanism whereby the β-cell adapts its secretory capacity in obesity and diabetes. In addition, hormone-sensitive lipase (HSL) is expressed in β-cells and may provide fatty acids necessary for the generation of coupling factors linking glucose metabolism to insulin release. We have recently created HSL-deficient mice that were used to directly assess the role of HSL in insulin secretion and action. HSL$^{-/-}$ mice were normoglycemic and normoinsulinemic under basal conditions, but showed an ~30% reduction of circulating free fatty acids (FFAs) with respect to control and heterozygous animals after an overnight fast. An intraperitoneal glucose tolerance test revealed that HSL-null mice were glucose-intolerant and displayed a lack of a rise in plasma insulin after a glucose challenge. Examination of plasma glucose during an insulin tolerance test suggested that HSL-null mice were insulin-resistant, because plasma glucose was barely lowered after the injection of insulin. Freshly isolated islets from HSL-deficient mice displayed elevated secretion at low (3 mmol/l) glucose, failed to release insulin in response to high (20 mmol/l) glucose, but had a normal secretion when challenged with elevated KCl. The phenotype of heterozygous mice with respect to the measured parameters in vitro was similar to that of wild type. Finally, the islet triglyceride content of HSL$^{-/-}$ mice was 2–2.5 fold that in HSL$^{-/+}$ and HSL$^{+/+}$ animals. The results demonstrate an important role of HSL and endogenous β-cell lipolysis in the coupling mechanism of glucose-stimulated insulin secretion. The data also provide direct support for the concept that some lipid molecule(s), such as FFAs, fatty acyl-CoA or their derivatives, are implicated in β-cell glucose signaling. *Diabetes* 50:1970–1975, 2001

From the [1]Molecular Nutrition Unit, Department of Nutrition, University of Montreal, the Centre de Recherche du CHUM and Institut du Cancer, Montreal, Quebec, Canada; the [2]Department of Experimental Pathology, University of Pisa, Pisa, Italy; and the [3]Department of Pediatrics, Research Center, Saint Justine Hospital, Montreal, Quebec, Canada.

Address correspondence and reprint requests to Dr. Grant A. Mitchell, Division of Medical Genetics, Hôpital Sainte Justine, 3175 Chemin de la Côte Sainte Catherine, Montreal, Quebec H3T 1C5, Canada. E-mail: mitchell@justine.umontreal.ca.

Received for publication 22 December 2000 and accepted in revised form 5 July 2001. Posted on the World Wide Web at http://diabetes.diabetesjournals.org on 6 August 2001.

R.R., P.M., and S.P.W. each contributed equally to this work.

AUC, area under the curve; BAT, brown adipose tissue; BSA, bovine serum albumin; FFA, free fatty acid; HBSS, Hanks' balanced salt solution, HSL, hormone-sensitive lipase; IPGTT, intraperitoneal glucose tolerance test; KRBH, Krebs-Ringer bicarbonate HEPES buffer; TG, triacylglycerol; WAT, white adipose tissue.

Hormone-sensitive lipase (HSL) is a multifunctional cytoplasmic enzyme that catalyzes the hydrolysis of triacylglycerols (TGs), diacylglycerols, and cholesterol esters (1,2). This enzyme is considered crucial in regulating lipolysis in fat cells because of its sensitivity to neurotransmitters and hormones, particularly to catecholamines and insulin, which respectively stimulate or inhibit its activity (1–3).

Activation of HSL occurs during adaptive thermogenesis in brown adipose tissue (BAT) (4) and is associated with the release of free fatty acids (FFAs) from white adipose tissue (WAT) into the circulation (2). The latter effect influences carbohydrate as well as lipid utilization and storage in several tissues, including liver, muscles, and possibly pancreatic β-cells (2). Besides adipocytes, HSL activity has been documented in myocardium, skeletal muscles, brain, adrenal gland macrophages, and testis (2,5). Recently, evidence that HSL is present and active in rat pancreatic β-cells (6) has reinforced the idea that endogenous lipolysis participates in the regulation of insulin secretion through the generation of FFA or other lipid signaling molecules (7,8).

We created HSL-deficient mice by targeted disruption of the HSL gene (9). The adipose tissue phenotype of these mice included reduced abdominal fat mass and marked heterogeneity in adipocyte size in both WAT and BAT. The lipolytic rate in isolated HSL$^{-/-}$ adipocytes was impaired after β-adrenergic stimulation, but it was normal under basal conditions (9). The animals' body weight and cold tolerance were unchanged, suggesting that HSL-independent lipolytic pathway(s) likely exist in fat cells (9). Similar phenotypic features, including sterility in male animals, were recently reported in an independently derived HSL-deficient mouse strain (10,11).

Here we explored some metabolic characteristics of these animals related to insulin secretion and action, reasoning that HSL-null mice might provide insights into the involvement, if any, of HSL in obesity and type 2 diabetes. Our in vivo and in vitro studies indicate that HSL deficiency affects both insulin secretion and action, and that HSL-null mice, though not diabetic, are glucose-intolerant. Interestingly, pancreatic β-cells of HSL$^{-/-}$ mice

do not secrete insulin in response to glucose but are responsive to a depolarizing concentration of KCl. This reveals that mobilization of endogenous lipid stores via HSL is important for glucose-stimulated insulin secretion.

RESEARCH DESIGN AND METHODS

HSL-deficient mice. Our HSL-deficient mice (9) have no detectable immunoreactive HSL and no measurable HSL activity. We used 5-month-old male mice derived from breeding the F1 mice (9) onto a C57BL/6 background for five generations.

Intraperitoneal glucose and insulin tolerance tests. Glucose (1.5 g/kg body wt; 8% solution) was administered intraperitoneally to conscious animals after an overnight fast. Blood samples (~60 μl) were collected from the tail vein into heparinized capillaries at 0, 30, 60, 120, and 210 min after glucose injection. Glycemia was then measured with a blood glucose meter (Medisense, Bedford, MA). Plasma obtained after centrifugation of blood samples was stored at −20°C until assayed for insulin.

Human recombinant insulin (Eli-Lilly, Indianapolis, IN) (0.75 units/kg body wt) was injected intraperitoneally to fed conscious mice after appropriate dilution with saline. A drop of blood withdrawn from the tail vein at 0, 15, 30, and 60 min after insulin administration served for determination of glycemia.

Isolation of mouse pancreatic islets. Mice were killed by cervical dislocation and used for islet isolation, by collagenase digestion of the total pancreas, as described previously (12). Briefly, after cannulation of the bile duct performed under a dissecting microscope, 2 ml of a collagenase solution (2 mg/ml) dissolved in Hanks' balanced salt solution (HBSS) containing 10 mmol/l HEPES (pH 7.4) were injected to dilate the pancreas, which was then removed and incubated in the same solution at 37°C for 25 min. Three pancreases per genotype group were pooled before incubation. After disruption of the digested tissue by vigorous shaking, filtration through a gauze, and sequential washes in HBSS/HEPES, islets were hand-picked under a stereomicroscope. At the end of the isolation step, two separate batches of 100 islets per each genotype were used for TG extraction. The remaining islets were put in culture for 2 h at 37°C in a humidified atmosphere containing 5% CO_2 and at a concentration of 15–20 islets/ml in regular RPMI-1640 medium containing 3 mmol/l glucose supplemented with 10% fetal calf serum, 10 mmol/l HEPES (pH 7.4), and 1 mmol/l sodium pyruvate. Islets were then used for static incubation experiments.

Insulin secretion experiments. The freshly isolated islets, cultured as described above for 2 h only to allow recovery from the isolation procedure, were washed in phosphate-buffered saline and preincubated for 30 min in Krebs-Ringer bicarbonate buffer with 10 mmol/l HEPES (KRBH; pH 7.4) containing 0.07% bovine serum albumin (BSA; Fraction V RIA grade; Sigma, St. Louis, MO) at 3 mmol/l glucose. Groups of 10 islets (6 replicas per genotype) were distributed in 12-well plates and submitted to three consecutive 45-min incubations in 1 ml of KRBH with 0.07% BSA. The first, second, and third incubations were carried out at 3, 20, and 3 mmol/l glucose plus 35 mmol/l KCl, respectively. At the end of each incubation period, media were withdrawn for insulin determination. Finally, 1 ml of cold acid ethanol mixture (ethanol/H_2O/concentrated HCl, 150:47:3 vol/vol/vol) was added to extract islet insulin content.

Islet triglyceride determinations. Batches of 100 freshly isolated islets were subjected to lipid extraction in a chloroform/methanol (2:1 vol/vol) mixture, essentially according to Folch et al. (13). For the assay, the dry material derived from evaporation of the chloroform phase was resuspended in water in the presence of a detergent (thesit 20% wt/vol in chloroform). TGs were measured enzymatically with a commercial kit (GPO Trinder; Sigma). Triolein, dissolved in chloroform/methanol and processed similarly to samples, was used as a standard.

FFA measurements. FFAs were determined from 10-μl plasma samples using a commercial kit (Wako Chemicals).

Insulin measurements. Plasma insulin was determined using a commercial kit based on the enzyme-linked immunosorbent assay technique (ALPCO Mouse Insulin ELISA kit; ALPCO, Windham, NH). The detection limit of the optimized assay was 0.06 ng/ml.

Statistical analysis. Statistical significance was evaluated by two-tailed Student's t test. Differences with $P < 0.05$ were deemed significant.

RESULTS

Metabolic features of the animals. No significant difference related to the HSL genotype of the mice was found for body weight ($HSL^{+/+}$ 33.2 ± 1.1 g, $HSL^{+/-}$ 33.6 ± 0.6 g, $HSL^{-/-}$ 33.5 ± 1.2 g; $n = 8$), basal glycemia (Fig. 2, time 0) or insulinemia (Fig. 3, time 0) after an overnight fast. In the fed state, glycemia (Fig. 4, time 0) and insulinemia (not shown) were also not different among the HSL genotype groups. Basal FFAs were measured before the intraperitoneal glucose tolerance test (IPGTT) in the same plasma samples used for basal glycemia and insulinemia determinations. In fasting HSL-deficient mice, circulating FFA levels were reduced by 25–30% ($P < 0.01$) with respect to both wild type and heterozygous animals (Fig. 1), as expected from their reduced adipose tissue lipolysis (9,10).

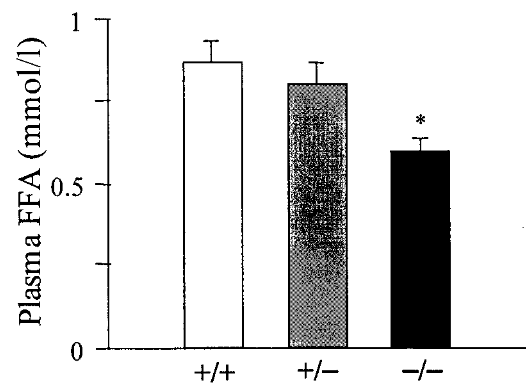

FIG. 1. Plasma FFAs are reduced in HSL-null mice. FFAs were measured in blood samples from overnight-fasted HSL-null ($HSL^{-/-}$), heterozygous ($HSL^{+/-}$), and wild-type ($HSL^{+/+}$) mice at time 0 of the IPGTT. Data are means + SE of eight animals. *$P < 0.01$ vs. $HSL^{+/-}$ and $HSL^{+/+}$ mice.

HSL-deficient mice are glucose-intolerant and secrete less insulin than normal mice in response to a rise in blood glucose. Figure 2 shows plasma glucose during an IPGTT carried out in overnight-fasted $HSL^{+/+}$, $HSL^{+/-}$, and $HSL^{-/-}$ mice. Plasma glucose (Fig.2*A*) was higher in HSL-null mice versus the two other groups at all time points investigated after the glucose load, although statistical significance was reached only at times 120 and 210 min. Calculation of the integrated plasma glucose levels, i.e., area under the curve (AUC) shown in Fig. 2*B*, confirmed that a significant reduction of glucose tolerance was present in $HSL^{-/-}$ mice with respect to both the $HSL^{+/+}$ ($P < 0.01$) and $HSL^{+/-}$ ($P < 0.02$) genotypes. After the intraperitoneal glucose administration, plasma insulin (Fig. 3*A*) increased to a similar extent in $HSL^{+/+}$ and $HSL^{+/-}$ mice, but not in $HSL^{-/-}$ animals. Panel *B* indicates that the calculated AUC for plasma insulin was significantly lower in HSL-deficient mice in comparison with the other two groups ($P < 0.01$). Thus, HSL-deficient mice are mildly glucose-intolerant and appear to have a decreased capability to release insulin in response to a rise in blood glucose. There were no statistically significant differences between $HSL^{+/-}$ and $HSL^{+/+}$ mice.

Insulin tolerance test. Figure 4 shows plasma glucose levels after an intraperitoneal administration of insulin (0.75 units/kg body wt) in fed animals. Insulin injection caused a decrease with respect to basal glucose values by ~40% in the $HSL^{+/+}$ and $HSL^{+/-}$ groups at 30 and 60 min, whereas the decrease in plasma glucose in the $HSL^{-/-}$ group was significantly less ($P < 0.02$ vs. the wild-type genotype). This suggests that HSL-null mice are insulin-resistant, although further testing will be necessary to directly prove it.

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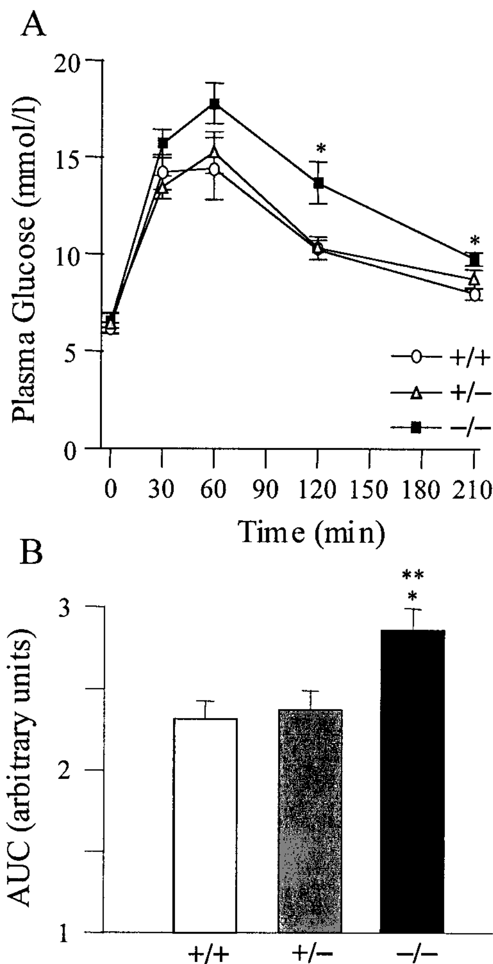

FIG. 2. HSL-null mice are glucose-intolerant. *A*: Plasma glucose levels during an IPGTT were measured in $HSL^{-/-}$, $HSL^{+/-}$, and $HSL^{+/+}$ mice. Glucose (1.5 g/kg body wt) was injected intraperitoneally after an overnight fast. Data are the means + SE obtained from eight animals per condition. *$P < 0.02$ vs. control ($HSL^{+/+}$). *B*: AUC of the data shown in *A*. *$P < 0.01$ vs. control; **$P < 0.02$ vs. heterozygous mice.

Isolated pancreatic islets from HSL-deficient mice do not secrete insulin in response to glucose and have an elevated TG content. Pancreatic islets isolated from either $HSL^{-/-}$ or $HSL^{+/+}$ mice were of similar size and appearance. Aliquots of pools from three mice were submitted to three consecutive 45-min static incubations in KRBH medium, first with 3 mmol/l glucose, and then with 20 mmol/l glucose, and finally with 3 mmol/l glucose plus 35 mmol/l KCl (Fig. 5). Of note, islets from mice lack the priming effect of glucose or time-dependent potentiation seen in rat and human islets (14). Consequently, in our protocol the KCl insulin secretory response is not predicted to be influenced by the previous exposure to high glucose. Insulin release was stimulated ~12-fold over basal value in wild-type islets by high glucose and 7-fold by a depolarizing K^+ concentration. In contrast, islets from $HSL^{-/-}$ mice showed a three- to fourfold increased basal secretion and were unresponsive to high glucose, while maintaining normal responsiveness to the nonfuel secretagogue KCl. The insulin content of the islets isolated from $HSL^{-/-}$ mice (141 ± 10 ng/islet), extracted at the end of the secretion experiment, was not significantly different from that of controls (164 ± 14 ng/islet). The insulin release of islets from heterozygotes challenged with the same stimuli was comparable to that of controls (data not shown).

Triglyceride levels were determined in islets freshly isolated from the three genotypes and belonging to the same pools used for the secretion experiments. The islet TG content was approximately doubled in $HSL^{-/-}$ mice with respect to both $HSL^{+/+}$ or $HSL^{+/-}$ animals (Fig. 6).

DISCUSSION

The availability of HSL-deficient mice provides an excellent opportunity to assess the importance of this enzyme in the regulation of lipid and glucose homeostasis, through both the pleiotropic effects of circulating FFAs released from fat stores and the activation of endogenous lipolysis in nonadipose tissues. We focused on the possible in vivo and in vitro alterations of insulin secretion in HSL-deficient mice, but we also obtained valuable information about the overall sensitivity of these animals to the action of insulin.

For the discussion of our results, it is useful to summarize the previously reported phenotypic features of HSL-null mice, as observed by Osuga et al. (10) and ourselves

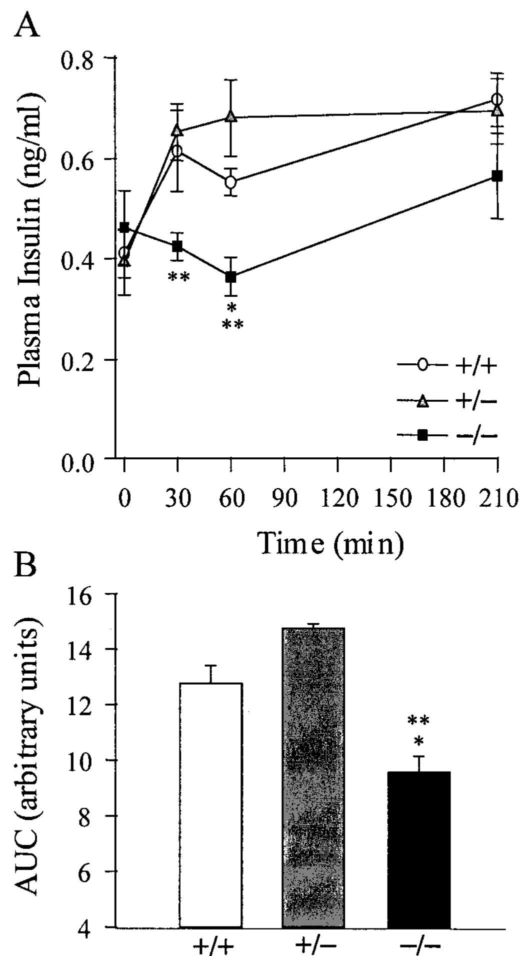

FIG. 3. Lack of a rise in plasma insulin during an IPGTT in HSL-null mice. The experimental conditions are those described in the legend for Fig. 2. Data are the means + SE of 5–7 determinations per point (eight mice per group from two separate experiments; the number of determinations was less than that of animals because of paucity of plasma). *A*: At time 30 min, **$P < 0.02$ vs. $HSL^{+/-}$ mice; at time 60 min, *$P < 0.01$ vs. both $HSL^{+/+}$ and $HSL^{+/-}$ mice. *B*: AUC of the data shown in *A*. *$P < 0.01$ vs. both control and heterozygous mice.

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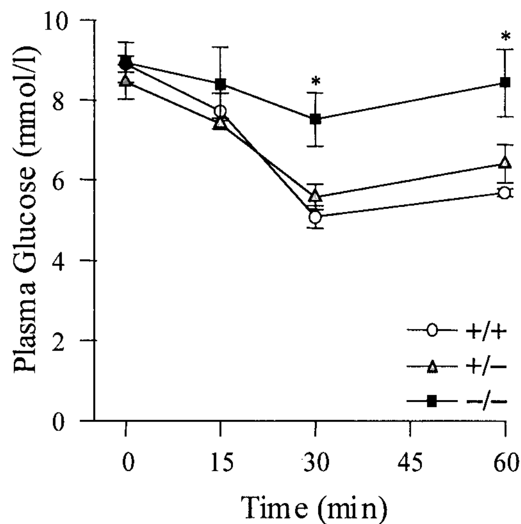

FIG. 4. Examination of plasma glucose during an insulin tolerance test suggests that HSL-null mice are insulin-resistant. Plasma glucose levels were measured during an insulin tolerance test in fed HSL$^{-/-}$, HSL$^{+/-}$, and HSL$^{+/+}$ mice. Insulin (0.75 units/kg body wt) was injected intraperitoneally in fed animals. Data are mean values + SE obtained from five mice. *P < 0.02 vs. control (HSL$^{+/+}$) mice.

(9). Common characteristics of these two independently created HSL-deficient strains are: *1*) adipose tissue alterations with histological evidence of increased size and striking heterogeneity of adipocyte diameters; *2*) preservation of basal WAT lipolysis but loss of that stimulated by β-adrenergic agents; *3*) maintenance of a substantial WAT triacylglycerol lipase activity but complete lack of neutral cholesterol esterase activity; *4*) no statistically significant difference in body weight and WAT mass, despite the histologically evident fat accumulation in adipocytes; *5*) increased mass of BAT; and *6*) male sterility caused by defective spermatogenesis.

The present work shows that HSL$^{-/-}$ mice, in both the fasted and fed states, displayed the same glycemia and

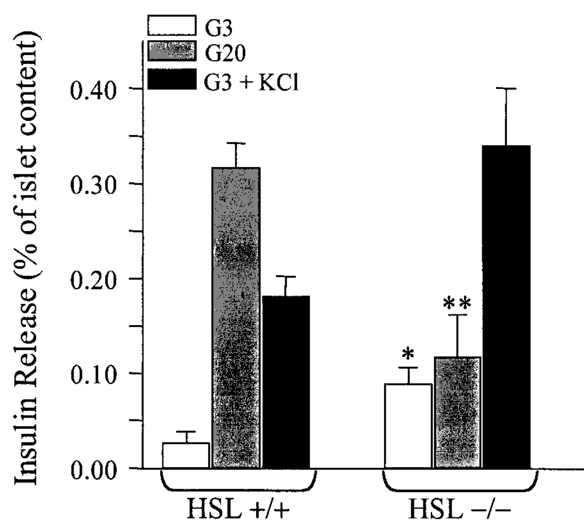

FIG. 5. Freshly isolated pancreatic islets of HSL-null mice do not release insulin in response to glucose. Islets from fed animals were isolated from three pooled pancreases of HSL$^{-/-}$ mice and C57BL/6 HSL$^{+/+}$ controls. Groups of 10 islets were incubated in KRBH buffer for three consecutive 45-min periods in the presence of either 3, 20, and 3 mmol/l glucose plus 35 mmol/l KCl, respectively. Means + SE of six determinations. *P < 0.02 and **P < 0.004 vs. the 3 and 20 mmol/l control situations in the HSL$^{+/+}$ group, respectively.

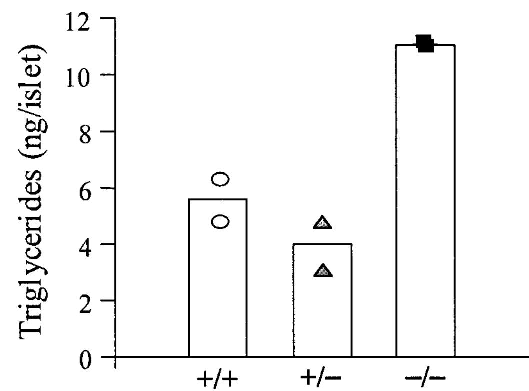

FIG. 6. The islet triacylglycerol content of HSL-null mice is increased. Means of two separate experiments for HSL$^{-/-}$ (■), HSL$^{+/-}$ (▲), and HSL$^{+/+}$ (○), each containing 100 freshly isolated islets obtained from three pooled pancreases per genotype condition. Thus, the mean values for each genotype corresponds to the average TG content of six mice.

insulinemia as control animals but had reduced plasma FFA concentrations. Challenged with an intraperitoneal glucose load, HSL$^{-/-}$ mice revealed a moderate reduction in glucose tolerance. This is likely because of impairments of both insulin secretion, which is substantially blunted after glucose administration, and insulin effectiveness on target tissues, as assessed by the insulin tolerance test.

The lack of a significant rise in circulating insulin in fasted HSL$^{-/-}$ mice after a glucose challenge could be caused, in part, by the lower levels of blood FFAs. Thus, in fasted humans (15) and rats (16), efficient glucose-stimulated insulin secretion is absolutely dependent on an elevated level of circulatory FFAs. However, it must be emphasized that the β-cells of HSL$^{-/-}$ mice have an intrinsic defective responsiveness to glucose, because islets isolated from these animals were insensitive to high glucose in vitro, while maintaining a normal response to a depolarizing concentration of K^+. Because islets of HSL-deficient mice contain higher amounts of TG, we suggest that impaired β-cell lipolysis contributes to secretory dysfunction. The fact that β-cells of HSL-null mice responded normally to a nonnutrient stimulus (elevated K^+) discounts the possibility that a critical lipid molecule necessary for exocytosis in general (17) may have been depleted in these animals. Thus, major lipid depletion of islet with leptin impaired the secretory action of glucose (18). In addition, the secretory response of the perfused pancreases of fasted rats, but not fed animals, to both fuel and nonfuel stimuli requires the presence of exogenous FFAs (16,17). Hence, the glucose signaling process involving HSL is different from a permissive role of exogenous fatty acids for secretion in general (for both fuel and nonfuel stimuli), as documented by McGarry et al. (17) in islets from fasted rats. This conclusion stems from the observation that insulin release in response to elevated KCl remained intact in islets from HSL-deficient mice.

The key question is, How are β-cell HSL and endogenous lipolysis implicated in glucose-stimulated insulin secretion? Lipid-derived signals are thought to participate in the transduction process implicated in the stimulation of insulin release promoted by fuel stimuli (7,19). Besides the C-kinase activator diacylglycerol, long-chain acyl-CoA esters or their derivatives, such as some phospholipid signaling molecules, have been proposed to act as cou-

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pling factors in fuel signaling (20–25). Changes in lipid partitioning in the β-cell promoted by high glucose in particular, increased lipid esterification processes (19), or protein acylation (22,26) might be components of the ATP-sensitive K^+ channel–independent pathway of glucose signaling (27). Within the framework of this hypothesis, β-cell TG stores could provide, via lipolysis, an essential endogenous source of diacylglycerol, various phospholipid signaling molecules, and long-chain acyl-CoA esters (the activated intracellular form of FFAs), which are the substrates of FFA-metabolizing enzymes (2,28).

The hypothesis proposing a role for lipid signaling in the coupling mechanisms of glucose-stimulated insulin secretion has been challenged mainly by studies in which tumoral β(INS) cells were used (29,30). However, in these studies, in which fat oxidation was altered by various adenoviral constructs, not only the glucose-induced rise in insulin release but also the fat esterification processes remained intact (29,30). Because alterations in fat oxidation do not change the action of glucose on lipid partitioning in terms of fat esterification in INS cells, it can be concluded that these previous studies do not contradict the lipid signaling hypothesis proposing that fatty acyl-CoA themselves, or some of their derivatives, are implicated in the signal transduction process of glucose (8,19). Cell lines with glucose-sensing are invaluable to test various aspects of β-cell function because tissue limitation and heterogeneity is often a problem in islet studies. Nonetheless, recent evidence indicates that tumoral β-cell lines are apparently not optimal to test the lipid signaling hypothesis, and that this model of fuel-sensing (8,19) should be further tested in normal islet tissue.

To date, the role of endogenous TG in the regulation of insulin secretion has remained elusive, although the following evidence indicates that they may be important: *1*) islet TG content correlates with the enhancement of insulin secretion in obesity (31); *2*) glucose-stimulated insulin secretion is abrogated in islets depleted of TG by overexpression of the leptin gene (18); *3*) pharmacological antilipolytic agents partially inhibit glucose-induced insulin secretion in vivo (16); and *4*) we recently observed that the antilipolytic agent 3,5-dimethylpyrazole inhibits insulin release in response to both nutrient secretagogues and cAMP agonists in isolated rat islets, the inhibition being relieved by a synthetic diacylglycerol (32). Hence, our results in HSL-deficient mice provide direct support for the hypothesis that endogenous TG stores, via HSL-mediated lipolysis, normally play an important role in glucose-induced insulin release.

The data also suggest that insulin action is impaired in HSL-null mice, as revealed by the insulin tolerance test. There is much evidence to indicate that fat deposition in muscle causes insulin resistance (8,33–35). In addition, skeletal muscle TG and long-chain acyl-CoA contents in humans are inversely correlated to whole-body insulin action (35,36). The possibility of insulin resistance in HSL-deficient mice is particularly interesting in view of the association, in some families, of the HSL locus with the metabolic syndrome (37) and the impairment of lipolysis reported in familial combined hyperlipidemia (38). Further work is needed to directly show altered insulin action in HSL-deficient mice and to understand how it is related to defective muscle lipolysis and/or HSL expression.

Why are HSL-deficient mice nondiabetic and only mildly glucose-intolerant, given that they apparently exhibit both defective secretion in response to elevated glucose and altered insulin action? At this stage, it can only be speculated that a compensation phenomenon occurs in these animals. In accordance with this view, secretion at low glucose is elevated in HSL-null mice. The biochemical nature of this phenomenon remains to be explained. The secretory response to other fuel stimuli and possibly some neurohormonal agonists might be increased. A change in neural regulation of insulin release is another possibility. Additional work is required to characterize the insulin secretion of HSL-deficient islets at low and high glucose in response to various agents, particularly to fatty acids and glucagon-like peptide 1. With respect to the role of HSL in the signaling process of cAMP agonists, of note is a recent report showing that the lipase inhibitor orlistat reduces glucagon-like peptide 1–induced secretion in β(HIT) cells (39).

In conclusion, these results have uncovered an important role of β-cell HSL and lipolysis in the coupling mechanism of glucose. They also raise important questions concerning the requirement for β-cell HSL activity and endogenous islet TG stores for glucose-stimulated insulin secretion in the face of a relatively modest decrease in circulating FFAs. The data also provide direct support for the concept that some lipid molecule(s) (e.g., FFAs themselves, fatty-acyl-CoA, or their derivatives) is implicated in pancreatic β-cell glucose signaling (19,22).

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ACKNOWLEDGMENTS

This work was supported by grants from the Canadian Institute of Health Research (to G.A.M. and M.P.), the Canadian Diabetes Association (to M.P.), the Juvenile Diabetes Research Foundation (to M.P.), and the Canadian Genetic Diseases Network (to G.A.M.). M.P. is a Canadian Institute of Health Research Scientist. R.R. is the recipient of a Swiss National Science Foundation fellowship.

We thank Dr. Vincent Poitout for advice for the triglyceride measurements, Linge Pan and Johane Morin for expert technical assistance, and Alix Zutter for helpful discussions.

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