

β -Cell Lipases and Insulin Secretion

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Lipids have been implicated in β -cell stimulus-secretion coupling. Thus, lipases in β -cells would be required to generate coupling factors from intracellular lipids. Indeed, we found that glucose stimulates lipolysis in rodent islets and clonal β -cells. Lipolysis and diglyceride lipase activity in islets are abolished by orlistat, a pan-lipase inhibitor. Moreover, orlistat dose-dependently inhibits glucose- and forskolin-stimulated insulin secretion, while leaving glucose oxidation and the rise in ATP-to-ADP ratio intact. In an effort to identify β -cell lipase(s), we found that hormone-sensitive lipase (HSL), the rate-limiting enzyme for acylglyceride hydrolysis in adipocytes, is active in rodent β -cells. To further address the role of HSL, a global and β -cell-specific inactivation, respectively, of the lipase has been created in mice. Whereas our line of HSL null mice is moderately glucose intolerant due to reduced peripheral insulin sensitivity, it exhibits normal islet metabolism and insulin secretion. Preliminary analysis of the β -cell-specific HSL knockout has revealed no evidence for disturbed islet function. Thus, studies of ours and others indicate that there is a complex lipid regulatory component in β -cell stimulus-secretion coupling. The role of HSL and other lipases needs to be further clarified to provide a balanced view of the role of lipids and lipolysis in β -cells. *Diabetes* 55 (Suppl. 2):S24–S31, 2006

Lipids play a complex role in glucose homeostasis under both normal and pathological conditions. While it is undisputed that lipids are critical in the development of the late complications of all forms of diabetes (i.e., the small and large vessel diseases of diabetes), a view has also emerged that lipids may play a primary role in how metabolic abnormalities in type 2 diabetes evolve. This notion includes the concept of lipotoxicity (1), in which lipids are thought to exert negative effects on the insulin-secreting β -cells as well as interfere with the peripheral actions of the hormone. Thus, increased levels of circulating lipids, i.e., free fatty acids (FFAs) and triglycerides, could perturb cellular function directly or indirectly via inappropriate accumulation in

nonadipose cells, e.g., β -cells and liver and skeletal muscle.

In addition to the “bad” role played by lipids, it has also become increasingly clear that lipids are in fact required for proper function of pancreatic β -cells. Prentki and Corkey (2,3) have proposed a model linking glucose to lipid metabolism in the β -cell—glucose stimulation leading to a rise in malonyl-CoA levels. This metabolite, which in fat-synthesizing cells is the starting point for fatty acid synthesis, blocks the transport of long-chain acyl-CoA into the mitochondrion, via inhibition of carnitine palmitoyl transferase 1 (4). As a result, levels of this lipid moiety in the cytoplasm should rise and could potentially constitute a coupling factor of glucose stimulus to secretion. At that point, an unexplored area was how long-chain acyl-CoAs were generated in the β -cell for participation in stimulus-secretion coupling. If lipids were involved, one or more lipases would be required to release them from intracellular stores, similar to processes in the adipocyte. Indeed, islets do contain triglycerides, an accumulation of which may increase during the pathogenesis of diabetes (5), but the molecular machinery surrounding these depots has not been clarified. Furthermore, hydrolysis of these intracellular stores may drive basal hyperinsulinemia, a hallmark of insulin resistance. This is, at least initially, a beneficial process, since it serves to compensate for impaired peripheral sensitivity to the hormone, thereby preventing hyperglycemia.

Further support for the notion of a “good” role for lipids in glucose homeostasis comes from studies in leptin-treated rats (6); the rats, made hyperleptinemic by recombinant adenoviruses containing leptin cDNA, display pancreatic islets depleted of lipids, which are unresponsive to glucose (7). Remarkably, the glucose responsiveness is restored by adding exogenous FFAs. McGarry and colleagues (8) also showed that glucose responsiveness in fasted rats both in vivo and in vitro depends on the presence of FFAs. This result was later replicated in humans as well (9). An important conclusion from these experiments was that a fatty acid-derived factor may play a pivotal role in insulin secretion; this factor was lost in the referred experiments either by leptin treatment or by fasting. Again, how such a factor is generated within cells remains unknown, but the action of a lipase could be assumed.

While the critical role of ATP-sensitive K^+ (K_{ATP}) channels for glucose-stimulated insulin secretion (GSIS) has long been recognized, it has now been widely acknowledged that nutrients also act via K_{ATP} channel-independent or amplifying pathways (10). These pathways involve coupling factors that may or may not be the same as those used by the K_{ATP} -dependent or triggering pathway of insulin secretion. K_{ATP} -independent pathways have been suggested to be primarily responsible for the second phase of GSIS. There is an intense search for the identity of the coupling factors responsible for K_{ATP} -independent insulin

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ATGL, adipocyte triglyceride lipase; FFA, free fatty acid; GSIS, glucose-stimulated insulin secretion; HSL, hormone-sensitive lipase; K_{ATP} channel, ATP-sensitive K^+ channel.

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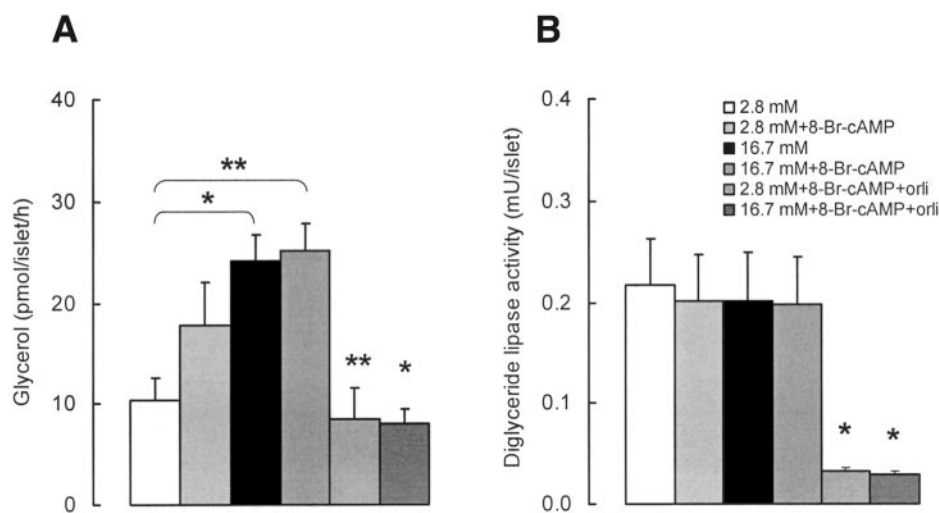


FIG. 1. Lipolysis and diglyceride lipase activity in isolated rat islets. **A:** Freshly isolated rat islets were cultured for 3 h at the indicated glucose concentrations, in the presence or absence of 1 mmol/l 8-bromoadenosine-3',5'-cyclic monophosphate (8-Br-cAMP) and/or 200 μmol/l orlistat (orli). Lipolysis was monitored by glycerol release into the incubation buffer. **B:** Islets from the experiment in **A** were homogenized and lipase activity toward a diglyceride substrate was determined. * $P < 0.05$ and ** $P < 0.01$ for any condition with orlistat vs. without the inhibitor. Data are from Mulder et al. (14).

secretion. Lipids have emerged as a prime candidate (3), but others, including the ATP-to-ADP ratio (11), glutamate (12), and NADPH (13), have also been put forth.

Our contribution to this field of research is examination of how a potential lipid-derived factor is produced within the pancreatic β -cell. Here, we will describe studies that show that the activity of one or more lipases is required for a full insulin secretory response from β -cells. Also, we have attempted to clarify the identity of this lipase(s). These studies include the finding of expression and activity of hormone-sensitive lipase (HSL) in pancreatic β -cells and the subsequent global and β -cell-specific genetic inactivation of HSL in mice.

LIPASES ARE REQUIRED FOR A FULL INSULIN SECRETORY RESPONSE FROM β -CELLS

To explore whether lipases play a role in β -cell stimulus-secretion coupling (14), we used orlistat, previously known as tetrahydrolipstatin, which is an inhibitor of a wide spectrum of lipases. The drug is a lipophilic molecule that irreversibly binds to the catalytic site of a great number of lipases (15). Orlistat is widely used in the treatment of obesity (16), because it inhibits intestinal lipases, hereby preventing uptake of lipids and restricting caloric input. Since orlistat, at therapeutic doses, is not taken up into the circulation, undesired systemic effects are avoided. However, at high concentrations in vitro, orlistat traverses biological membranes (17) and can therefore be used as a tool to explore the function of lipases in cells (18). Indeed, orlistat has previously been shown to inhibit cAMP-induced insulin secretion in the clonal β -cell line HIT-T15 (19) and GLP-1-stimulated secretion in rat islets (20).

Our first aim was to investigate whether lipolysis takes place in primary rat β -cells (14). Such a process would require a lipase or at least acylglyceride hydrolytic activity. The existence of lipolytic activity was assumed but not shown in previous studies (19,20). We found that elevated glucose promotes glycerol release, an index of lipolysis, from rat islets (Fig. 1A); the glycerol release is potentiated by addition of 8-bromoadenosine-3',5'-cyclic monophos-

phate, a cAMP analog with enhanced stability. In contrast, glycerol release is abolished by addition of 200 μmol/l orlistat. Concurrently, diglyceride lipase activity (21) is dramatically inhibited by orlistat in extracts of these islets (Fig. 1B).

Although these experiments demonstrated that lipolysis does occur in pancreatic β -cells and that lipase activity can be detected and blocked in the same cells, the next step was to examine whether these processes were involved in insulin secretion. First, we assayed insulin secretion in 1-h static incubations of rat islets in response to glucose with or without forskolin in the presence of increasing concentrations of orlistat (Fig. 2). We found that the lipase inhibitor concentration-dependently inhibits insulin secretion under these conditions. Interestingly, when 1 mmol/l palmitate is added to the islets incubated with 16.7 mmol/l glucose and 200 μmol/l orlistat, the insulin secretory response is recovered. This suggests that palmitate can substitute for a lipid-derived intracellular signal, the formation of which is blocked by orlistat. Our results agreed with work using another antilipolytic agent: 3,5-dimethylpyrazole. This drug inhibits insulin secretion induced by glucose, cAMP-raising agents, and α -ketoisocaproic acid (22).

We next examined the effect of orlistat on the dynamics of insulin secretion in rat islets (14). Orlistat significantly inhibited the second but not first phase of insulin secretion (-25% ; $P < 0.05$) without impairing glucose metabolism. This suggested that a lipid-derived factor, the production of which is blocked by orlistat, predominantly acts during second phase, possibly via the K_{ATP} -independent amplification of insulin secretion. In agreement with this notion, we observed no effect of orlistat on the glucose-induced rise in the ATP-to-ADP ratio, a prerequisite for GSIS. However, an effect on intracellular Ca^{2+} cannot be excluded, particularly if different Ca^{2+} channels control the two phases of insulin secretion (23).

We have recently extended these studies and found that glucose concentration-dependently stimulates glycerol release from INS-1 cells and that this correlates well with

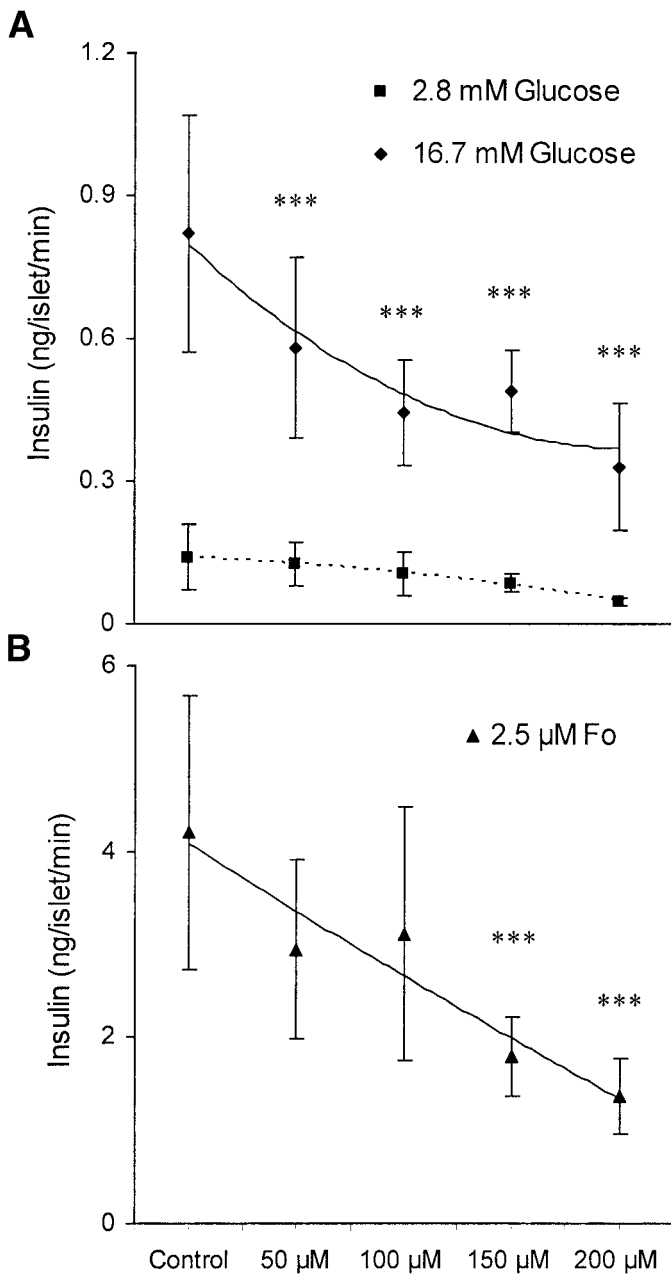


FIG. 2. Static incubation of isolated rat islets. **A:** Rat islets cultured overnight were preincubated for 1 h at 2.8 mmol/l glucose, after which the conditions were changed to HEPES-buffered saline solution containing 2.8 mmol/l glucose or 16.7 mmol/l glucose with increasing concentrations of orlistat, or **(B)** 16.7 mmol/l glucose and 2.5 μmol/l forskolin with increasing concentrations of orlistat. Three to five independent experiments were performed for each condition. ****P* < 0.001 vs. control. Data are from Mulder et al. (14).

insulin release (24); similar findings were made in islets from C57BL/6J mice (24).

HORMONE-SENSITIVE LIPASE IS ONE ENZYME ACCOUNTING FOR LIPASE ACTIVITY IN β-CELLS

The studies summarized thus far demonstrated that β-cells display lipase activity. This was not a surprising finding, since it had long been recognized that β-cells store triglycerides and use lipids as fuel when ambient glucose is low (25,26). Furthermore, that lipolysis does occur in β-cells was suggested by studies from Corkey’s group (19).

They measured acidification of and efflux of fatty acids from HIT-T15 cells in response to glucose and the incretin hormone GLP-1. This was interpreted as intracellular release of fatty acids from lipid stores, i.e., lipolysis, presumably by HSL, given that cAMP mediated the effect observed. However, the identity of these one or more lipases remained unconfirmed. To resolve this, we decided to examine whether β-cells express HSL. In adipocytes, a well-defined signaling cascade controlling HSL has been established (27): activation of adrenergic and/or glucagon receptors raises intracellular cAMP levels with subsequent protein kinase A activation and phosphorylation of HSL. This phosphorylation results in translocation of HSL to the lipid droplet, where it hydrolyzes acylglycerides. The activation is made possible by a concurrent phosphorylation of perilipin (28), a protein that coats the lipid droplet, and removal of which allows the translocation of HSL to the lipid surface. These processes are reversed by insulin, which via activation of PDE3B hydrolyzes cAMP in the adipocytes (29). Thus, triglyceride hydrolysis in adipocytes is stimulated by a rise in intracellular cAMP.

We were able to demonstrate that both HSL mRNA and protein are expressed in various preparations of rodent β-cells (30). Interestingly, the molecular form of HSL in β-cells is slightly larger than the predominant form in adipocytes (89 vs. 84 kDa). More recently, we found that the β-cell HSL isoform is encoded by a transcript that comprises an additional exon (A), which is spliced to exon 1 and introduces an upstream start codon. These additional 5’-base pairs encode a 43-amino acid peptide (31). Western blotting of protein from human islets also revealed expression of HSL (H. Lindvall, L. Stenson Holst, H.M., C.H., unpublished data), demonstrating that HSL expression in islets is not a rodent-specific phenomenon. Morphological analysis of rodent islets revealed that HSL is strongly expressed in β-cells (30), but it is also present in the other islet cell types. Using an antibody to HSL to block diglyceride lipase activity indicated that ~25% of such activity in INS-1 cells is accounted for by HSL (30,32). Our group has also shown that chronic exposure to high glucose increases expression and activity of HSL in INS-1 cells and rat islets and, in parallel, stimulates lipolysis, as determined by glycerol release from INS-1 cells (32). On the other hand, prolonged high-fat feeding of C57BL/6J mice results in decreased HSL expression in islets (33). Interestingly, this is paralleled by triglyceride accumulation and perturbed insulin secretion both in vivo and in vitro; after normalization of the diet, HSL expression increases, and this is accompanied by a reduction in triglyceride levels and restored GSIS.

HSL KNOCKOUT MICE: A SUMMARY OF THE PHENOTYPE

To further explore the role of HSL in metabolism, including pancreatic β-cell function, the next logical step was to target the HSL locus with a view to inactivate the enzyme. Remarkably, to date, four independent lines of HSL null mice have been reported (34–37), presumably reflecting the interest in the role of this enzyme in metabolism. While there initially was some disappointment regarding the absence of a dramatic phenotype in HSL null mice—the mice did not become obese (34)—interesting observations have subsequently been made in these mice. Overall, the different lines exhibit many similarities but some distinct

TABLE 1
Glucose metabolism in HSL null mouse lines

	Ishibashi line	Mitchell line	Zechner line	Holm line
Fasting plasma glucose	Decreased*	Unchanged	Increased	Increased
Fasting plasma insulin	Unchanged	Decreased	Decreased	Increased
Glucose tolerance	ND	Decreased ²	ND	Unchanged [†]
Insulin secretion in vivo	ND	Decreased ²	ND	Increased [‡]
Insulin-induced hypoglycemia	ND	Attenuated	ND	Attenuated
Whole body glucose uptake [§]	ND	Unchanged	Unchanged	Unchanged
Hepatic glucose production	ND	Increased [¶]	Increased [¶]	Decreased [#]
Muscle glucose uptake in vitro	ND	ND	ND	Decreased
Islet function in vitro				
GSIS	ND	Increased ^{**} /decreased ^{††}	ND	Unchanged ^{‡‡}
Glucose oxidation	ND	ND	ND	Unchanged
Palmitate oxidation	ND	ND	ND	Unchanged
Cholesterol hydrolase activity	ND	Decreased	ND	Decreased
Triglyceride lipase activity	ND	Decreased	ND	ND
Diglyceride lipase activity	ND	ND	ND	Unchanged
Acylglyceride content	ND	Increased	ND	Unchanged
Glucose-stimulated lipolysis	ND	Unchanged ^{§§}	ND	Unchanged

*Statistically significant only in male mice; †oral glucose tolerance test; ‡intravenous glucose tolerance test; §hyperinsulinemic-euglycemic clamp; ||change denotes degree of inhibition during hyperinsulinemic-euglycemic clamp; ¶fasted mice; #fed mice; **fed and fasted 7-month-old female mice; ††male mice, pronounced in islets from fasted mice; ‡‡male and female mice, fed or fasted, freshly isolated islets, or islets cultured overnight; §§fed and fasted 4- and 7-month-old male mice. ||no response to glucose in either wild-type or HSL null mice. ND, not determined or reported in this line.

differences exist, particularly with respect to glucose metabolism.

All lines exhibit male infertility. This is presumably caused by the absence of HSL from testes (34,38,39), where it is normally expressed, as first described by us (40). While HSL deficiency causes abnormal spermatogenesis (38), the function of HSL in testis is unresolved. An initial and puzzling finding was that release of both FFAs and glycerol from adipocytes, albeit at substantially reduced rates, was observed both in vivo and in vitro (34–36,41), despite the lack of HSL from adipocytes, where it was believed to be the sole and rate-limiting acylglyceride hydrolase. This strongly suggested the existence of one or more additional lipases in adipocytes. Using an elegant multiple approach, Zechner's group (42) was able to identify a novel adipocyte triglyceride lipase (ATGL) in adipose tissue in HSL null mice. In fact, the lipase was independently discovered by three different groups and has been named desnutrin or ζ -isoform of Ca^{2+} -independent phospholipase A₂ (43,44). It also occurs in normal mice and humans and is primarily responsible for hydrolysis of triglycerides to diglycerides.

Interestingly, accumulation of diglycerides in adipocytes is a consistent finding in the different HSL null lines (36,37). This finding has led to the notion that the primary role of HSL is to hydrolyze diglycerides. It was previously known that another lipase, monoglyceride lipase, is responsible for an unregulated hydrolysis of monoglycerides to FFAs and glycerol in adipocytes (45). Furthermore, neutral cholesterol ester hydrolase activity is abolished in adipocytes from HSL null adipocytes (34,37), suggesting that an important role for HSL is to control breakdown of this class of lipids. An important unresolved issue is whether ATGL is controlled by hormonal signals.

GLUCOSE HOMEOSTASIS IN HSL NULL MICE

There are distinct differences between the existing HSL null mouse lines with regard to glucose homeostasis

(Table 1). The differences have been difficult to understand and reconcile. HSL null mice created by Mitchell's group (35) were initially reported to be glucose intolerant (46); basal glycemia and insulinemia are normal. The mice exhibited retarded glucose elimination due to a virtually lost insulin response. Follow-up studies have shown that the glucose intolerance of these HSL null mice improves with further breeding (47); the poor insulin secretion in vivo in the fasted state, however, remains. HSL null mice created by the Zechner group (48) exhibit increased fasting plasma glucose levels, while insulin levels are decreased. In the Ishibashi line (34), fasting plasma glucose levels are either unchanged in female mice or decreased in male mice, while insulin levels are unaltered (49). HSL null mice created in our laboratory are slightly, but significantly, hyperglycemic with hyperinsulinemia in the fasted state (37). In contrast to the Mitchell line, our mice are not overtly glucose intolerant (Fig. 3). Instead, they show an exaggerated insulin response in both intravenous and oral glucose tolerance tests (37), which suggests that our HSL null line is insulin resistant. This interpretation is supported by the observation of a diminished disposal of glucose during an insulin tolerance test. The Mitchell mice also exhibit impaired insulin action (46). Moreover, a hyperinsulinemic-euglycemic clamp in our HSL null line showed a reduced insulin response in liver: glucose production is not appropriately turned off by insulin (37). In addition, there are indications of reduced insulin sensitivity in other peripheral tissues. Thus, insulin-stimulated glucose uptake in soleus muscle and lipogenesis in isolated adipocytes are reduced in HSL null mice (37). Finally, there is a doubling in β -cell mass with increased insulin content, which presumably arises as an adaptation to insulin resistance in the mice.

To add to the confusion, while a hyperinsulinemic-euglycemic clamp in Zechner's line of HSL null mice (36) confirmed that there is no impact on whole-body glucose uptake (48), their work indicated that insulin sensitivity is

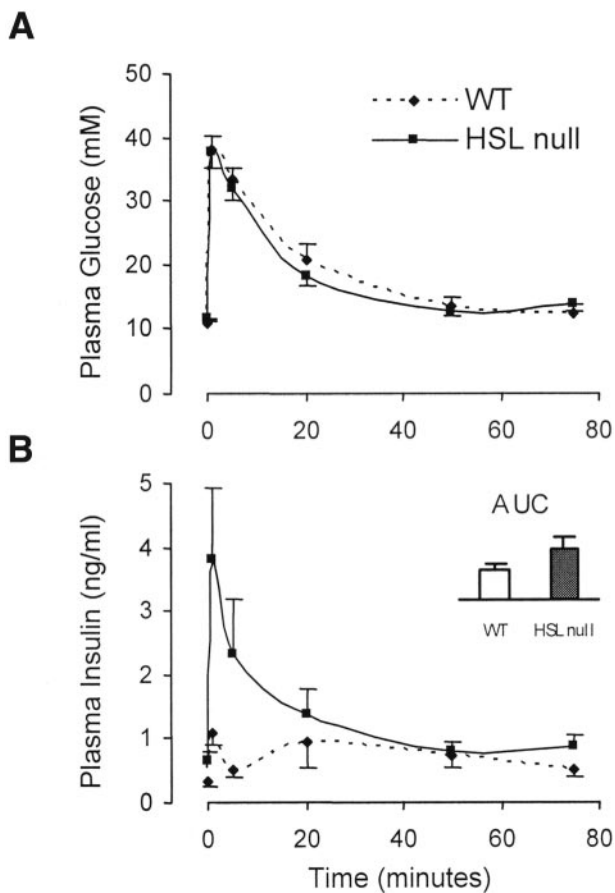


FIG. 3. Intravenous glucose tolerance test in HSL null mice. Glucose (1 g/kg) was injected into the tail vein of female anesthetized HSL wild-type (WT) and null mice, and retro-orbital blood samples were drawn at indicated time points for determination of plasma glucose (A) and insulin (B). Six mice were examined twice. Insulin levels were significantly higher in HSL null mice at 0 and 1 min after glucose administration ($P < 0.05$; two-tailed unpaired t test). Insert in B shows area under the curve (AUC) for plasma insulin during the intravenous glucose tolerance test. Data are from Mulder et al. (37).

increased in liver; this was suggested to be the result of decreased hepatic triglyceride levels. Possible explanations for this discrepancy is that our line of HSL null mice exhibits increased (37), and not decreased (36), hepatic acylglyceride levels. Our clamp was performed in the fed state and at a considerably higher rate of insulin infusion. In the fed state, hepatic acylglyceride levels are higher, and this could be even more pronounced when HSL is absent. Furthermore, glucose from the intestine may contribute significantly to endogenous glucose production. Indeed, increased hepatic insulin sensitivity was later reproduced in the Mitchell HSL null line under fasted conditions (50), similar to those that were examined in Zechner's HSL null mice (48). Apparently, insulin sensitivity has been examined under different conditions in the HSL null lines.

INSULIN SECRETION IN HSL NULL MICE

As reviewed above, at the whole animal level, there are discrepancies with regard to insulin secretion (Table 1). While our line of HSL null mice responds vigorously to glucose and arginine in vivo (37), the mice created by Mitchell exhibit a perturbation of insulin secretion in vivo (46,47). In contrast, insulin secretion from perfused islets is similar in both HSL null and wild-type mice generated in

our laboratory (37,51). Moreover, in static incubations of islets from our HSL null line (Fig. 4), insulin secretion in response to glucose under K_{ATP} -dependent and -independent conditions, in response to GLP-1, carbacholine, palmitate, or α -ketoisocaproic acid, is unchanged (37,51). Insulin secretion was examined in islets freshly isolated from 4-week-, 4-month-, and 7.5-month-old fed female mice and from 7.5-month-old fed or fasted male mice; insulin secretion in 7.5-month-old mice was examined both in freshly isolated and overnight cultured islets. All efforts produced the same result: HSL wild-type and null islets responded similarly in terms of insulin secretion (51). Furthermore, the intracellular glucose-induced Ca^{2+} response is indistinguishable between the two genotypes. It should be mentioned, though, that early generations of our HSL null mice exhibited a perturbed GSIS; this was rescued by addition of palmitate. However, with subsequent breeding, this phenotype was lost. This cannot be attributed to back crossing, because we were still using mice from the F2 generation produced by breeding of heterozygous male and female HSL^{+/-} mice (51).

Contrary to our observations, Prentki and coworkers (46,47) have observed perturbed insulin secretion in vitro in the mice created by Mitchell (35). However, the phenotype is variable. GSIS is increased in islets isolated from fed or fasted female mice and decreased in islets from male mice (47). Furthermore, this decrease depends on the nutritional state of the animals: it is much less pronounced in fed than fasted mice. Overall, fasting markedly reduces fractional secretion of insulin, but the specific response to glucose is lost in male HSL null islets. Adding exogenous palmitate to these islets restores GSIS (47).

We also examined metabolism in islets from our line of HSL null mice (51) and found that, with the exception of neutral cholesterol ester hydrolase activity, there are no alterations in the absence of HSL (Table 1). Analysis included fatty acid and glucose oxidation, acylglyceride content, and diglyceride lipase activity. Interestingly, we did not observe any stimulation by glucose of lipolysis in either wild-type or HSL null islets, as assessed by glycerol release. This result was at odds with the situation in rat islets, where glucose and 8-Br-cAMP enhance lipolysis (14). However, we have later also observed glucose-stimulated lipolysis in islets from C57BL/6J mice (24). The Mitchell mice only exhibit a reduction in unstimulated glycerol release from islets in 7-month-old fasted HSL null mice (47). Remarkably, glycerol release from islets is significantly stimulated in both genotypes—a stimulation that is not further potentiated by addition of GLP-1. Triglyceride lipase activity is most clearly impaired in 7-month-old mice, which raises some questions since HSL is predominantly a diglyceride lipase (36), and, if anything, one would expect redundancy to increase with age. Finally, acylglyceride levels are increased in islets from Mitchell's (47), but not our (51), line of HSL null mice (Table 1).

In sum, the analyses of insulin secretion in islets lacking HSL have generated more questions than clear answers. In our line of HSL null mice, diglyceride lipase activity is unchanged despite the fact that HSL is likely to be more efficient as a diglyceride lipase (36,37). In contrast, Mitchell's HSL null mice exhibit reduced triglyceride lipase activity in islets, again despite the fact that HSL is a more efficient diglyceride lipase (36,37). Why did our line of HSL null mice lose their initial insulin secretory phenotype? Why is a challenge with age and food restriction necessary

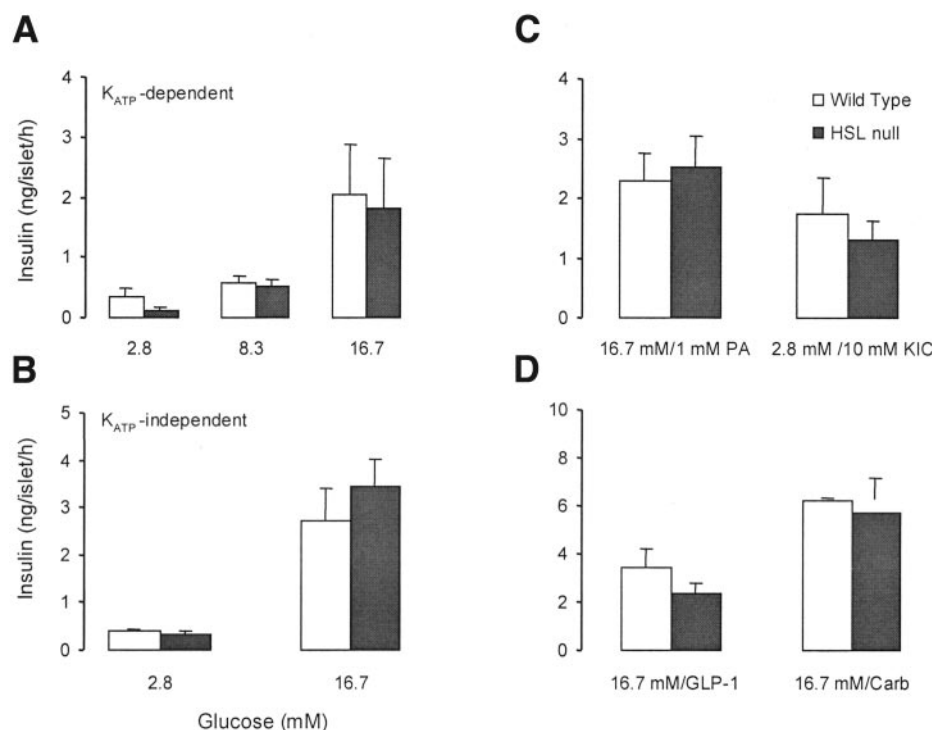


FIG. 4. Static incubation of islets from wild-type and HSL null mice. Freshly isolated islets from 4-month-old female mice were incubated for 1 h with the respective secretagogue, after which a sample from the buffer was taken for insulin determination. Eight replicates of batches of three islets were used for each condition; three independent experiments were performed. Under K_{ATP} -independent conditions, 35 mmol/l KCl and 250 μ mol/l diazoxide were added; 100 nmol/l GLP-1 and 100 μ mol/l carbacholine (Carb) were used. KIC, α -ketoisocaproic acid; PA, palmitic acid. Data are from Fex et al. (51).

for the phenotype to become apparent in the Mitchell line? Do these challenges make lipids less available for mobilization of coupling factors? Why do the two mouse lines differ with respect to ability of glucose to stimulate lipolysis? What is the mechanism of perturbed GSIS in Mitchell's HSL null line if glucose stimulates lipolysis equally in islets from the two genotypes?

The reasoning here focuses on β -cells as a separate entity. However, β -cells are integrated in whole-body physiological processes. Thus, it should be borne in mind that lipids can be derived from different sources, i.e., plasma (FFAs and lipoproteins) as well as intracellular stores. We cannot assume that these sources are identical in the different HSL null lines. However, if we disregard this level of complexity, some potential explanations for the discrepancies can be discussed. One possibility is that one or more unknown genes on the allele containing the mutated HSL locus and that has been transferred to the HSL null, but not wild-type, offspring accounts for the phenotype. This should be ameliorated by back-crossing the mice onto an inbred pure genetic background. In fact, we have back-crossed our mice onto C57BL/6J for 10 generations, but this has not reinstated the initial perturbed insulin secretory phenotype (S.L., C.H., unpublished data). The Mitchell mice were back-crossed for five generations, which should have provided some unifying of the genetic background (46,47). The genetic background of the two lines is different. This is relevant because it has become increasingly clear that gene targeting in different genetic backgrounds may produce divergent results (52). Finally, genetic redundancy may play an important role, particularly since the targeting of the HSL locus is present from the gastrula stage. The discovery of ATGL has fuelled this notion (42). Indeed, using RT-PCR, we have identified

ATGL mRNA in mouse and rat islets, including HSL null islets (Fig. 5), and clonal β -cells. Studies are ongoing in our laboratory to resolve the role of this novel lipase in β -cells.

β -CELL-SPECIFIC KNOCKOUT OF HSL

To circumvent some of the problems inherent in a classic global gene knockout, particularly genetic redundancy, we have created a conditional HSL knockout in β -cells. For this purpose, we have cross-bred the floxed HSL mouse line created by Zechner's group (36) with transgenic mice expressing cre under control of the rat insulin-2 promoter (53). Thus, when the rat insulin-2 promoter is transactivated during embryonic life (approximately embryonic day 9–11), cre is expressed in β -cells and excises an essential fragment from the HSL gene, which hereby produces a truncated and nonfunctional transcript.

At this point, preliminary analysis of the mice has been made. We can confirm that recombination has occurred in β -cell HSL knockout islets, since we detect a truncated, but no full length, HSL transcript using RT-PCR. The mice breed normally and produce offspring that is grossly

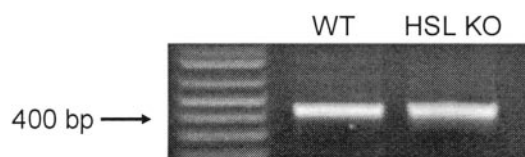


FIG. 5. RT-PCR in islets from wild-type (WT) and HSL null mice. PCR products were resolved on a 1% agarose gel. RT-PCR, using primers to amplify a 418-base pair (bp) fragment of the ATGL transcript, generated a PCR product of the expected size. This indicates that ATGL is expressed also in mouse islets.

normal. Fed plasma glucose and insulin levels are similar in β-cell HSL knockout and wild-type mice (i.e., floxed mice). The pancreatic islets in the mice appear normal when immunostained for insulin. Thus far, there is no indication of a striking secretory and metabolic phenotype in the β-cell HSL knockout mice. However, a lesson learned from Prentki's HSL studies is that specific conditions and challenges need to be applied to reveal a phenotype in islets lacking HSL (46,47).

WHAT IS CERTAIN ABOUT LIPASES AND LIPOLYTIC ACTIVITY IN β-CELLS?

Based on the studies reviewed above, we would like to summarize what can be stated with certainty about lipases and lipolytic activity in β-cells and their possible importance for insulin secretion: 1) β-Cells express lipases: one of them is HSL (30), another is ATGL (Fig. 5). 2) Lipases are involved in insulin secretion: pharmacological inhibition of lipase activity in β-cells impairs insulin secretion (14,22). 3) The exact role of lipolysis in insulin secretion is unclear, because most observations are circumstantial and some are contradictory (14,19). Thus, the Mitchell HSL null mice exhibit an abrogation of GSIS despite intact glucose-stimulated lipolysis (47), whereas islets in our HSL null line normally secrete insulin without increasing lipolysis in response to glucose. 4) The role of cAMP/protein kinase A for activation of HSL (27) and other lipases in β-cells, and thereby lipolysis, is undecided: whereas we found that lipolysis increases in rat islets (14) and INS-1 cells (32) in response to cAMP, GLP-1 was as efficacious as palmitate to rescue insulin secretion in the absence of HSL (47).

Much effort has thus been devoted to assess whether, besides their established chronic toxic effects, lipids exert an acute regulatory role in β-cells. In our view, little evidence so far supports such a notion, including the hypothesis that lipases are an active provider of long-chain acyl-CoA (2). Perhaps, lipids should be considered as a permissive factor in β-cell stimulus-secretion coupling—a concept supported by earlier studies from the laboratories of Unger (7) and McGarry (8). Such a view can also be reconciled with some of the observations made in the joint efforts to elucidate the role of HSL in β-cells, which have been reviewed here. Now we need to turn our attention to other potential lipases in β-cells, so that a balanced view of the role of lipids in β-cell stimulus-secretion coupling can be attained.

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