Relative Hypoglycemia and Hyperinsulinemia in Mice with Heterozygous Lipoprotein Lipase (LPL) Deficiency

ISLET LPL REGULATES INSULIN SECRETION*

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Lipoprotein lipase (LPL) provides tissues with fatty acids, which have complex effects on glucose utilization and insulin secretion. To determine if LPL has direct effects on glucose metabolism, we studied mice with heterozygous LPL deficiency (LPL+/−). LPL+/− mice had mean fasting glucose values that were up to 39 mg/dl lower than LPL+/+ littermates. Despite having lower glucose levels, LPL+/− mice had fasting insulin levels that were twice those of +/+ mice. Hyperinsulinemic clamp experiments showed no effect of genotype on basal or insulin-stimulated glucose utilization. LPL message was detected in mouse islets, INS-1 cells (a rat insulinoma cell line), and human islets. LPL enzyme activity was detected in the media from both mouse and human islets incubated in vitro. In mice, +/+ islets expressed half the enzyme activity of +/+ islets. Islets isolated from +/+ mice secreted less insulin in vitro than +/+ and −/− islets, suggesting that LPL suppresses insulin secretion. To test this notion directly, LPL enzyme activity was manipulated in INS-1 cells. INS-1 cells treated with an adeno-associated virus expressing human LPL had more LPL enzyme activity and secreted less insulin than adeno-associated virus-β-galactosidase-treated cells. INS-1 cells transfected with an antisense LPL oligonucleotide had less LPL enzyme activity and secreted more insulin than cells transfected with a control oligonucleotide. These data suggest that islet LPL is a novel regulator of insulin secretion. They further suggest that genetically determined levels of LPL play a role in establishing glucose levels in mice.

Lipoprotein lipase (LPL) catalyzes the rate-limiting step for clearance of triglycerides from the blood. Hydrolysis of lipoprotein-associated triglycerides in the capillary beds of peripheral tissues such as muscle and adipose tissue produces free fatty acids that are available for local uptake (1). LPL enzyme activity is probably the major factor controlling movement of exogenous fatty acids into peripheral tissues. The overexpression of LPL in mouse muscle (2) increases tissue lipid as well as mitochondria and peroxisomes, the sites of fatty acid metabolism. Mice with homozgyous LPL deficiency (LPL−/−) (3, 4) die soon after birth with minimal tissue lipid. Mice deficient in adipose tissue LPL develop adipose tissue lipid stores but only by inducing de novo fatty acid biosynthesis from glucose (5). These results suggest that tissue lipid content plays important roles in normal physiology and that LPL is essential for the acquisition of exogenous fatty acids by tissues.

Fatty acids and glucose compete as respiratory substrates in many tissues (6). In muscle, fatty acids inhibit glucose utilization and oxidation. In liver, fatty acids inhibit glucose oxidation and promote gluconeogenesis. In the pancreatic beta cell (7), fatty acids have complex effects that differ depending on the duration of exposure. Since LPL is the dominant provider of fatty acids to tissues and fatty acids alter insulin secretion and glucose utilization, defects in the LPL gene could affect glucose metabolism.

Recent reports in mice and humans suggest that LPL genotype affects glucose metabolism. The reason for the death of LPL−/− mice soon after birth (3, 4) is unknown. Merkel and colleagues (8) reported that LPL−/− mice are profoundly hypoglycemic (mean glucose of 15 mg/dl), although the underlying mechanisms are unknown. LPL−/− humans are rare, but human heterozygous LPL deficiency (LPL+/−) occurs in about 3% of unselected subjects of various ethnic backgrounds (9, 10). These individuals have elevated triglycerides and decreased high density lipoprotein cholesterol (11). It is not yet clear whether such individuals are at increased risk for atherosclerosis, ischemic heart disease, or diabetes. Recently, Nordestgaard and colleagues (12) screened Danish subjects for mutations in the LPL gene. As expected, LPL+/− humans had higher triglycerides and lower high density lipoprotein cholesterol. Unexpectedly, these unrelated LPL+/− humans had reduced plasma glucose concentrations compared with LPL+/+ humans. Two previous studies of related LPL+/− humans found no effect on glucose levels (13, 14).

We tested the hypothesis that LPL has a direct effect on glucose metabolism in mice. Our data show that LPL+/− mice are relatively hypoglycemic. Since LPL provides fatty acids to muscle (the major site of insulin-stimulated glucose disposal) and fatty acids compete with glucose as substrates, we expected lower plasma glucose concentrations in LPL+/− mice.

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§ The abbreviations used are: LPL, lipoprotein lipase; BSA, bovine serum albumin; RT-PCR, reverse transcriptase-polymerase chain reaction; NEFA, non-esterified fatty acids; AAV, adeno-associated virus.
Infusion was continued during a 1-h control period, and 20 was begun for measurement of the rate of appearance of glucose. The infusion approximately 170 mg/dl, the average blood glucose in a freely feeding, dextrose infusion rate varied in order to maintain the blood glucose at and continued for at least 90 min for each experimental period. A was taken from the tail for determination of glucose-specific activity at and 20 min prior to and at the end of the control period. This approximation was based on measurement of specific activity during identical conditions in the same type of mice in our laboratory (19). The infusion was calculated by dividing the infusion rate of 3-3Hglucose by the specific activity at the same time. Glucose production was calculated by subtracting the cold glucose infusion rate from Rg.

EXPERIMENTAL PROCEDURES

Animals—The mice used in the studies were animals that carry one disrupted allele of the LPL gene as described by Coleman et al. (3) and their unaffected littermates. These animals (originally C57BL/6J-129Sv hybrids) have been continuously mated with C57BL/6J mice; N6 and N7 generation descendants from this cross into the C57BL/6J background were used to compare glucose metabolism in isolated mouse islets, experiments also included islets from mice lacking LPL in all tissues except muscle. LO-MC mice, deficient in native mouse LPL but expressing human LPL driven by the mouse MCK promoter (5, 15), were a gift from J. Breslow (New York, NY). Following sacrifice a clearly visible lipid film was present at the bottom of the tube despite the fact that each tube contained only 10 mice islets. This film was carefully resuspended, which required several minutes for each tube, in reaction mixtures provided in kit form for the determination of triglyceride content (see below). Each individual assay for islet triglyceride content was performed in triplicate.

For RNA preparation, islets were counted by hand using a microscope to ensure that samples were not contaminated by acinar tissue. Total RNA was prepared using guanidinium isothiocyanate and sedimentation in cesium chloride as described (22). RT-PCR was performed using AMV RT for first strand synthesis, Tag polymerase for the PCR step, and primers as described in Table II. Mouse Islet Manipulation—Human pancreatic islets were obtained from the Islet Core of the Washington University Diabetes Research and Training Center, which has approval from the Human Studies Committee for these procedures. Cultured islets were counted with a microscope and aliquoted to tubes for determination of LPL enzyme activity (100 islets per tube) or used for preparation of total RNA exactly as described above.

LPL Enzyme Activity—Islets were assayed for LPL enzyme activity in two ways. To determine if islets secrete LPL activity, mice islets (30 islets per aliquot) and human islets (100 islets per aliquot) were washed with Krebs-Ringer bicarbonate buffer (115 mM NaCl, 5 mM KCl, 2.5 mM CaCl2, 1 mM MgCl2, 24 mM NaHCO3, and 25 mM Hepes, pH 7.4) containing 3 mg glucose and 0.1% BSA. Islets were placed in 10 × 75-mm siliconized borosilicate tubes in 200 µl of Krebs with 3 mM glucose, 0.1% BSA and incubated for 30 min. Buffer was then replaced with Krebs containing 3 mg glucose, 0.1% BSA and incubated for 30 min. The buffer was then removed and assayed for insulin content.

For for analysis of triglyceride content, islets were placed in glass tubes, and lipids were extracted with 2:1 (v/v) chloroform:methanol. The original extraction procedure was as described (18). Following lipid extraction, a clearly visible lipid film was present at the bottom of the tube despite the fact that each tube contained only 10 mouse islets. This film was carefully resuspended, which required several minutes for each tube, in reaction mixtures provided in kit form for the determination of triglyceride content (see below). Each individual assay for islet triglyceride content was performed in triplicate.

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LPL Enzyme Activity—Islets were assayed for LPL enzyme activity in two ways. To determine if islets secrete LPL activity, mice islets (30 islets per aliquot) and human islets (100 islets per aliquot) were washed with Krebs containing 3 mg glucose and 0.1% BSA and then incubated in the same buffer for 30 min as described above for insulin secretion studies. Islets were centrifuged at 300 × g and then the buffer was aspirated. Islet LPL activity, determined as the salt-inhibitable capacity of samples to hydrolyze radiolabeled fatty acids from a phospholipid-stabilized triolein emulsion as described (23). To determine the effect of LPL genotype on islet LPL activity, islets from LPL−/− and LPL+/+ mice (100 islets per aliquot) were directly homogenized in sample assay buffer and activity assayed.

Adeno-associated Virus Overexpression of LPL in INS-1 Cells—A recombinant adeno-associated virus (AAV) containing the human LPL cDNA driven by the cytomegalovirus immediate early promoter was generated by Avigen Corp. (Alameda, CA) using techniques described for other recombinant AAV vectors (24). In preliminary experiments, transfection of AAV-LPL into both C2C12 and COS cells resulted in the dose-dependent expression of LPL enzyme activity. The generation of AAV-LPL-secreting activity, determined as the salt-inhibitable capacity of samples to hydrolyze radiolabeled fatty acids from a phospholipid-stabilized triolein emulsion as described (23). To determine the effect of LPL genotype on islet LPL activity, islets from LPL−/− and LPL+/+ mice (100 islets per aliquot) were directly homogenized in sample assay buffer and activity assayed.

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TABLE I

| Glucose (mg/dl) | LPL+/+ | LPL+/- | p |
|----------------|--------|--------|---|
| Experiment 1   | 181 ± 9| 142 ± 8| 0.0145 |
| Experiment 2   | 183 ± 5| 155 ± 5| 0.0021 |
| Triglycerides (mg/dl) | | | |
| Experiment 1   | 54 ± 4| 82 ± 9| 0.0108 |
| Experiment 2   | 46 ± 4| 84 ± 9| 0.0035 |
| Cholesterol (mg/dl) | | | |
| Experiment 1   | 60 ± 5| 60 ± 8| 0.9855 |
| Experiment 2   | 80 ± 7| 73 ± 5| 0.4359 |
| NEFA (mEq/liter) | | | |
| Experiment 1   | 0.80 ± 0.04| 0.77 ± 0.06| 0.6615 |
| Experiment 2   | 0.65 ± 0.05| 0.75 ± 0.05| 0.0832 |
| Body weight (g) | | | |
| Experiment 1   | 29 ± 3| 25 ± 3| 0.167 |
| Experiment 2   | 31 ± 1| 31 ± 2| 0.8681 |

Comparisons are with LPL+/+ mice in the same experiment by unpaired, two-tailed t test with significant differences as indicated.

Animals were fasted for 4 h before collection of serum. Data are expressed as mean ± S.E. for 6–14 mice of both sexes between the ages of 2 and 4 months.

RESULTS

Serum chemistries for LPL+/+ and +/− mice between the ages of 2 and 4 months are shown in Table I. LPL+/+ mice in two separate experiments had fasting serum glucose values that were 15–22% (28–39 mg/dl, p = 0.0145 and 0.0021) lower than LPL+/+ mice. As expected, fasting triglycerides were 52–83% (p = 0.0108 and 0.0035) higher in LPL+/− mice, but there were no significant differences in NEFA, body weight, or cholesterol.

More detailed characterization of glucose metabolism was carried out in mice over the age of 12 months since the larger caliber of the tail vein at this age simplifies glucose tolerance testing. Blood glucose values in chow-fed mice over the age of 12 months were 181 ± 3 mg/dl for LPL+/+ (n = 39) versus 170 ± 3 mg/dl for LPL+/− (n = 34) (p = 0.0137). In two additional groups over the age of 12 months matched for weight and sex, the blood glucose after a 4-h fast was as follows: group 1, 187 ± 5 for +/+ versus 163 ± 3 mg/dl for +/− (p = 0.0003); group 2, 191 ± 9 for +/+ versus 169 ± 4 for +/− (p = 0.036). These mice underwent insulin tolerance testing after a 4-h fast. Data for group 1 are shown in Fig. 1. Similar results were seen for group 2. Both genotypes had similar glucose excursions although values in the +/− mice tended to be lower throughout the test and returned to significantly lower levels in LPL+/− mice at 150 min after the glucose injection.

Insulin levels were higher in LPL+/− compared with +/+ mice (Fig. 2). In chow-fed mice between the ages of 2 and 4 months (left side of figure), fasting serum insulin levels were 0.81 ± 0.21 for +/+ versus 1.90 ± 0.44 for +/− (Mann-Whitney two-tailed p = 0.0251). High feeding is known to elevate insulin levels in mice. When mice were fed a high fat diet for 6 weeks, insulin levels were elevated in both genotypes but remained higher in LPL+/− mice (right side of figure). In a large group of mice with the same mean weight by genotype, insulin levels were 2.09 ± 0.37 (n = 30) for +/+ mice versus 4.38 ± 1.03 (n = 25) for +/− mice (Mann-Whitney two-tailed p = 0.0286). Thus, heterozygous LPL deficiency is associated with relative hypoglycemia in the setting of hyperinsulinemia.

Hyperinsulinemia can be due to insulin resistance. To address this issue, hyperinsulinemic clamp experiments were carried out in chow-fed LPL+/+ (n = 6) and LPL+/− (n = 7) mice after a 4-h fast. During the clamp period, glucose and insulin levels were the same in both genotypes (data not shown). The tracer-determined rates of glucose utilization were identical for the two genotypes during the basal period (Fig. 3). The insulin-stimulated rates of glucose utilization were the same for LPL+/+ and +/− mice (Fig. 3) indicating that the LPL+/− mice were not insulin-resistant.

LPL expression was detected in mouse islets by RT-PCR (Fig. 4). A single band of the correct predicted size (~573 base pairs) was seen using RNA from LPL+/+ mouse islets (lane 1) but did not appear when the RT step was omitted from the reaction (lane 2). The same band was seen using RNA from mouse heart (lane 3). Islets also contain non-insulin-producing cells raising the possibility that the LPL mRNA signal is derived solely from cells that do not synthesize insulin. However, the same RT-PCR band was also seen in INS-1 cells (lane 4), which are derived from rat pancreatic beta cells. In addition, an LPL mRNA species of the correct size for rat (22) was detected by Northern blotting using RNA from INS-1 cells (data not shown). These data suggest that insulin-producing islet cells...
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FIG. 2. Fasting insulin levels. Animals were fasted for 4 h. Serum was collected from LPL+/+ (solid squares) and LPL+/− (open circles) mice while eating a chow (left side of figure) or high fat diet (right side of figure).

FIG. 3. The effect of LPL deficiency on basal glucose utilization and insulin responsiveness in mice. Tracer determined rates of glucose utilization represent three measurements made during the last 15 min of the basal period and the last 20 min of the clamp period for LPL+/+ (solid bars, n = 6) and LPL+/− (open bars, n = 7) mice. Data are shown as mean ± S.E.

FIG. 4. LPL is expressed in pancreatic islets and INS-1 cells. Islets from LPL+/+ mice were isolated by density gradient sedimentation followed by selection with a microscope to exclude contaminating tissues. Total RNA was prepared and subjected to RT-PCR using LPL-specific primers. Products were electrophoresed in agarose gels and stained with ethidium bromide. The source of the RNA was as follows: lane 1, INS-1 cells; lane 2, LPL+/+ islets but the RT step was omitted to ensure that positive bands were not due to PCR artifact; lane 3, mouse heart; lane 4, INS-1 cells. The bands from lanes 1 and 3 were cut from this gel and sequenced. The products of the reactions yielded sequence identical to mouse LPL.

express LPL.

The amplified band from lane 1 of Fig. 4 was sequenced and found to be mouse LPL (Table II). To provide additional evidence that islets express LPL, RNA was prepared from human pancreatic islets and subjected to RT-PCR using two sets of primers. Both reactions yielded PCR products of the predicted size, and the sequence of both bands matched that of human LPL (Table II).

If LPL provides lipoprotein-derived fatty acids to beta cells, it must be secreted. To address this issue, mouse (30 islets per aliquot) and human (100 islets per aliquot) islets were washed then incubated in vitro for 30 min as described under “Experimental Procedures.” The media from these incubations were collected and assayed for LPL enzyme activity. Under these conditions, LPL activity in the medium exposed to mouse islets (n = 10 aliquots) was 10.2 ± 5 pmol/30 islets/min. LPL activity in the medium exposed to human islets (n = 4 aliquots) was 4.8 ± 2 pmol/100 islets/min.

To determine if there is an effect of LPL genotype on LPL enzyme activity in islets, islets from LPL+/− and LPL+/+ mice were homogenized and assayed for activity (Table III). Islets from LPL+/− mice contained 48% of the enzyme activity of LPL+/+ islets. The level of enzyme activity in LPL+/+ islets was 5−13% of the enzyme activity found in cardiac tissue from LPL+/+ mice (Ref. 3 and data not shown). Triglyceride content was modestly but significantly lower in LPL+/− islets (Table III); similar results were seen in three independent experiments. Overall triglyceride content of islets was substantial. A typical islet contains about 2,000 cells (27), yielding an estimated triglyceride content of 80 ng/cell. The triglyceride content of a typical adipocyte is 300−900 ng/cell (28, 29).

Under conditions of basal glucose concentration (3 mM), islets isolated from LPL-deficient mice secreted more insulin in vitro than islets from LPL+/+ mice (Fig. 5). These conditions are comparable to the fasting, basal state in intact mice, in which we detect relative hypoglycemia (Fig. 1) and hyperinsulinemia (Fig. 2). Secretion studies were performed immediately after islet isolation suggesting that islet metabolism reflected the availability of in vivo circulating substrates such as triglycerides. LPL+/− islets (open bar) cultured for 30 min in 3 mM glucose secreted 5-fold more insulin than LPL+/+ islets (closed bar). Islets were also isolated from mice expressing LPL only in muscle (L0-MCK, Refs. 5 and 15). LPL+/− islets from these mice (hatched bar) also secreted more insulin than LPL+/+ islets.

To address directly the role of LPL enzyme activity in mediating insulin secretion, LPL activity was manipulated in INS-1 cells (Figs. 6 and 7). For these experiments, cells were maintained in serum-containing medium until just prior to measurements of insulin secretion. LPL enzyme activity was detected in INS-1 cells and was unaffected by treatment with a viral vector expressing β-galactosidase (AAV-β-galactosidase). Basal LPL activity in INS-1 cells was between 4 and 10% of the activity detected in mouse islets. Treatment of these cells with a viral vector expressing human LPL (AAV-LPL) resulted in higher levels of LPL enzyme activity and lower levels of insulin secretion (Fig. 6). Similar results were seen in three independent experiments. These data indicate that increasing LPL enzyme activity in insulin-producing cells decreases basal insulin secretion. There was no effect of LPL overexpression on glucose-stimulated insulin secretion (not shown).

Decreasing LPL enzyme activity in INS-1 cells increased insulin secretion (Fig. 7). Cells treated with an LPL antisense oligonucleotide (Fig. 7, Antisense) had 32% less enzyme activity than cells treated with a control oligonucleotide of the same base composition (Fig. 7, Scrambled). Antisense-treated cells had a 28% increase in insulin secretion as compared with Scrambled-treated cells. Similar results were seen in four independent experiments.
Sequence data from RT-PCR products using LPL-specific primers and RNA isolated from mouse and human pancreatic islets

Mouse primers and sequence from mouse islet-derived RNA

| Sequence of RT-PCR product from mouse islet RNA | Upstream (nt 24) | Downstream (nt 549) |
|-------------------------------------------------|------------------|---------------------|
| Mouse LPL TGGACGCTTT | 5'TGACGCTTT | 5'TGGACGCTTT |
| 90 | CAGACGCAGGG | AAGAGATTCN |
| 9 | CGAGGAGGG | AAGAGATTCN |
| 29 | GTTGGCAGCAG | AAGAGATTCN |

Human primers and sequence from human islet-derived RNA

| Sequence of RT-PCR product from human islet RNA using primer set 1 | Upstream 1 (nt 418) | Downstream 1 (nt 627) |
|------------------------------------------------------------------|-------------------|---------------------|
| Human LPL CCTGTACAG | 5'TGGACGCTTT | 5'TGGACGCTTT |
| 49 | ACTTCAATGT | ACTTCAATGT |
| 9 | TGGTTGCTG | GACTG |
| 2 | CATTTGCTG | GACTG |

| Sequence of RT-PCR product from human islet RNA using primer set 2 | Upstream 2 (nt 1168) | Downstream 2 (nt 1338) |
|------------------------------------------------------------------|-------------------|---------------------|
| Human LPL TAAAGATTTC | 5'TGGACGCTTT | 5'TGGACGCTTT |
| 1208 | ACTGAGAGT | AAACCCATAC |
| 9 | AAACCCATAC | CAATCAGGC |

LPL enzyme activity and triglyceride content in pancreatic islets isolated from LPL wild-type (LPL+/+) and heterozygous LPL-deficient (LPL+/-) mice

Islets were isolated by density gradient separation and selected with a microscope to exclude contaminating acinar tissue. LPL enzyme activity was determined as the salt-inhibitable capacity of samples to hydrolyze radiolabeled fatty acids from a phospholipid-stabilized triolein emulsion. Triglyceride content was determined enzymatically after extraction of lipids with chloroform:methanol. Data are expressed as mean ± S.E.

|                   | LPL+/+ | n   | LPL+/- | n   |
|-------------------|--------|-----|--------|-----|
| LPL enzyme activity (pmol/mg protein/min) | 564 ± 74 | 4 | 268 ± 23* | 4 |
| Triglyceride content (μg/islet) | 160 ± 22 | 20 | 153 ± 1.6b | 19 |

* p = 0.0088 versus LPL+/+ by unpaired t test.
# p = 0.0150 versus LPL+/+ by unpaired t test.

DISCUSSION

Fatty acids affect insulin secretion. LPL provides lipoprotein-derived fatty acids to tissues. In this report, we provide evidence that LPL is expressed in islets. We also show that LPL+/+ mice have lower circulating glucose concentrations and higher insulin levels as compared with their LPL+/+ littermates. These mice have no evidence of insulin resistance.

Three lines of evidence suggest that hyperinsulinemia in LPL+/+ mice is due to an increased rate of insulin secretion. First, in the hyperinsulinemic Euglycemic Clamp experiments, insulin concentrations were equal in the LPL+/+ and +/- mice at the end of the clamp, indicating that at least under the conditions of the clamp, LPL deficiency does not affect the rate of insulin clearance. Second, islets from LPL-deficient mice secrete more insulin than islets from wild-type mice. Third, changing LPL activity in INS-1 cells is inversely related to insulin secretion, i.e., increasing LPL activity decreases insulin secretion and decreasing LPL activity increases insulin secretion.

LPL is often considered in the context of glucose metabolism because it is insulin-responsive (23, 30). Patients with poorly controlled diabetes frequently have dyslipidemia due to defects in LPL enzyme activity (31). However, screening of patients with type 2 diabetes and extreme hypertriglyceridemia has provided no evidence that genetic LPL deficiency contributes to the high frequency of lipoprotein disorders in diabetes (32). If the current results can be extrapolated to humans, they suggest an explanation for the failure to detect LPL mutations in diabetes. Genetic LPL deficiency may produce lower glucose concentrations thereby decreasing the likelihood of meeting the biochemical criteria for diabetes.

How does deficient islet LPL enzyme activity increase insulin secretion? The most obvious mechanism would involve a decrease in the provision of triglyceride-derived fatty acids to the islet. A large and frankly confusing body of literature spanning 30 years addresses the role of fatty acids in insulin secretion.

Acute treatment of pancreatic islets or intact rats with free fatty acids increases glucose-stimulated insulin secretion (33–
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Chronic treatment impairs glucose-stimulated insulin secretion (38, 39). But these effects are not limited to glucose-stimulated insulin secretion; fatty acids also alter basal insulin release. At least three independent groups have shown that chronic exposure of islets to fatty acids enhances insulin secretion (40–42), an effect that might ultimately deplete insulin stores and increase the risk for diabetes. Unger and colleagues (43) have proposed that deranged lipid metabolism may cause beta cell failure based on evidence that islet triglycerides rise in Zucker rats before the onset of overt diabetes.

These observations are difficult to reconcile with the enhanced insulin secretion seen in the setting of heterozygous LPL deficiency. Serum-free fatty acid levels are not elevated in LPL+/− mice. Since fatty acids chronically increase basal insulin secretion in cultured cells, one would expect basal insulin secretion to decrease when fewer fatty acids are delivered to the islet due to heterozygous LPL deficiency. The opposite was observed. LPL+/− islets, provided with fewer triglyceride-derived fatty acids, secrete more insulin. In this sense, our data are consistent with the concept of “lipotoxicity” in islet cells (44). Decreased islet lipid content caused by heterozygous LPL deficiency is associated with more insulin secretion. Islet triglyceride content was consistently lower in LPL+/− mice. But this difference (about 5%) appears trivial when compared with the 10-fold increase in islet triglyceride observed in Zucker rats during progression to diabetes (44).

If the decrease in provision of triglyceride-derived fatty acids in LPL+/− mice is related to their increased insulin secretion, these fatty acids might carry out their physiologic effects due to the specific site where they are found in the islet. Intracellular triglycerides are usually thought of as uniform deposits of neutral lipid. However, it is unknown if all fat is viewed equally by the cell. It is possible that fatty acids of different origins (transported as NEFA from the plasma, derived from lipoproteins through the action of LPL, released from intracellular droplets by hormone-sensitive lipase (45), or synthesized from glucose) have different metabolic effects.

If triglyceride-derived fatty acids from LPL are important determinants of insulin secretion, the downstream mechanism remains obscure. Fatty acids have been shown to decrease islet expression of islet/duodenum homeobox-1, a transcription factor important for the expression of several islet genes including insulin, glucokinase, and Glut2 (46). Perhaps LPL deficiency protects islets from this potentially toxic effect. Fatty acids may directly alter insulin release through effects on ATP-sensitive potassium channels (47).

The pancreatic potassium channel consists of the sulfonylurea receptor and an inward rectifying K subunit; mutations in the former cause persistent hyperinsulinemic hypoglycemia of infancy (48). Like LPL+/− mice, these patients have glucose-independent insulin secretion, suggesting that LPL deficiency may decrease islet potassium channel activity. In humans, spontaneous hyperinsulinemic hypoglycemia can also be caused by an activating mutation of glucokinase (49), the major regulator of glucose-mediated insulin secretion (50). Glucokinase is less likely to be involved in the phenotype associated with LPL deficiency. Glucose levels were not significantly lower throughout glucose tolerance tests in LPL+/− mice (Fig. 1), and changing LPL activity in INS-1 cells had no effect on glucose-stimulated insulin secretion.

It is possible that the effects of LPL on insulin secretion are not related specifically to fatty acids. Both insulin and LPL are secreted proteins that are stored at intracellular sites prior to secretion. Phospholipases A2 are probably important for insulin secretion (27), and one form of secretory phospholipase A2 is found in insulin secretory granules (51). LPL has phospholipase A1 activity (52), cleaving the primary ester bond of phospholipids. In fact, the mass of phospholipid and triglyceride hydrolyzed by LPL are similar during the metabolism of triglyceride-rich lipoproteins (53). ApoC-II, an activator of LPL...
activity, would probably not be available intracellularly, but LPL retains considerable activity toward phospholipids in its absence (54, 55). If LPL and insulin share the same secretory pathway, LPL could compete with phospholipase A2 for the same substrate. The presence of LPL would antagonize phospholipase A2 and decrease insulin secretion. LPL is also capable of catalyzing acyl transfer (56). This activity can produce diacylglycerol, another potential mediator of insulin secretion.

In summary, LPL is expressed in islets and inversely related to insulin secretion. This observation is physiologically relevant because both LPL/+ mice (this report) and LPL−/− mice (8) have lower glucose levels than LPL+/+ mice. Islet LPL expression thus represents a novel and direct link between glucose and lipid metabolism.

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