Glucose and Insulin Stimulate Heparin-releasable Lipoprotein Lipase Activity in Mouse Islets and INS-1 Cells

A POTENTIAL LINK BETWEEN INSULIN RESISTANCE AND b-CELL DYSFUNCTION*

Received for publication, November 28, 2000, and in revised form, December 20, 2000
Published, JBC Papers in Press, January 11, 2001, DOI 10.1074/jbc.M010707200

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Lipoprotein lipase (LpL) provides tissues with triglyceride-derived fatty acids. Fatty acids affect b-cell function, and LpL overexpression decreases insulin secretion in cell lines, but whether LpL is regulated in b-cells is unknown. To test the hypothesis that glucose and insulin regulate LpL activity in b-cells, we studied pancreatic islets and INS-1 cells. Acute exposure of b-cells to physiological concentrations of glucose stimulated both total cellular LpL activity and heparin-releasable LpL activity. Glucose had no effect on total LpL protein mass but instead promoted the appearance of LpL protein in a heparin-releasable fraction, suggesting that glucose stimulates the translocation of LpL from intracellular to extracellular sites in b-cells. The induction of heparin-releasable LpL activity was unaffected by treatment with diazoxide, an inhibitor of insulin exocytosis that does not alter glucose metabolism but was blocked by conditions that inhibit glucose metabolism. In vitro hyperinsulinemia had no effect on LpL activity in the presence of low concentrations of glucose but increased LpL activity in the presence of 20 mM glucose. Using dual-laser confocal microscopy, we detected intracellular LpL in vesicles distinct from those containing insulin. LpL was also detected at the cell surface and was displaced from this site by heparin in dispersed islets and INS-1 cells. These results show that glucose metabolism controls the trafficking of LpL activity in b-cells independent of insulin secretion. They suggest that hyperglycemia and hyperinsulinemia associated with insulin resistance may contribute to progressive b-cell dysfunction by increasing LpL-mediated delivery of lipid to islets.

Lipoprotein lipase (LpL)* catalyzes the rate-limiting step for clearance of circulating triglycerides. The 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 is the major physiological determinant of fatty acid delivery to peripheral tissues in vivo. The enzyme is highly glycosylated and functions as a head-to-tail dimer (2, 3) under well-defined conditions (4–6). Functional LpL binds to proteoglycans at the cell surface and capillary endothelium and is displaced from these sites by heparin (7–9). LpL is synthesized by a variety of cells (adipocytes, myocytes, and monocyte/macrophages) where its expression is differentially regulated by nutritional, hormonal, and developmental signals (10–14). LpL mRNA was recently identified in human and mouse islets and the rat insulinoma cell line INS-1 (15).

LpL regulation is complex and varies between cells. In adipocytes, LpL is increased by activation of protein kinase C (α, β, δ, and ε isoforms) and inhibition of protein kinase C decreases LpL synthesis (16). Insulin increases adipocyte LpL activity by both post-transcriptional and post-translational mechanisms (17). This effect is attenuated by rapamycin, an inhibitor of the mTOR (mammalian target of rapamycin) signaling pathway (18). In cardiac muscle cells, the stimulatory effect of insulin on LpL requires dexamethasone and appears to exert its effects by altering the cytoskeleton (19, 20). However, insulin does not regulate LpL in murine peritoneal macrophages and macrophage cell lines (7).

Glucose is known to regulate LpL in adipocytes (21, 22). Incubation of rat adipocytes in the absence of glucose produces a 49-kDa form of LpL that is catalytically inactive and not secreted (21). In the same cells, incubation in the presence of glucose produces a 55-kDa catalytically active form of LpL that is secreted. Glucose is essential for glycosylation, which is required for LpL enzymatic activity (23), and increases the rate of LpL translational and post-translational events. Glucose regulation of LpL expression in macrophages differs from adipocytes. Saritpaur et al. (24) demonstrated that glucose increases LpL expression in J774 macrophages in a protein kinase C- and AP1-dependent manner. LpL regulation in pancreatic islets has not been studied.

LpL mRNA was recently identified in human and mouse islets as well as insulinoma cells (15). LpL expression was shown to be inversely related to insulin secretion, indicating that LpL may be a physiologically relevant provider of fatty acids to b-cells. In this study, we address the regulation of LpL in b-cells. Our results show that glucose metabolism controls the intracellular trafficking of LpL-containing vesicles through mechanisms that function independent of the exocytosis of insulin. Hyperglycemia-induced translocation of LpL to the cell
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Acute exposure to an elevated glucose concentration stimulates LpL activity in pancreatic islets. Two triplicate groups containing 200 pancreatic islets/group were preincubated in basal (3 mM) glucose for 30 min. Subsequently, one triplicate group was maintained under basal glucose and one triplicate group was exposed to elevated (20 mM) glucose for the indicated time periods. Following the incubation periods, the islets were dissolved in detergent extract buffer and the extract was assayed for LpL activity as previously described. Identical results were seen in three independent experiments. Data are expressed as mean ± S.E. * p < 0.05 versus 1 h.

surface, a process amplified by hyperinsulinemia, represents a potential mechanism underlying the progressive β-cell lipotoxicity associated with insulin resistance.

EXPERIMENTAL PROCEDURES

Materials—Male C57BL/6 mice were obtained from Harlan Sprague-Dawley (Indianapolis, IN). CMRL-1066 and RPMI-1640 tissue culture media, penicillin, streptomycin, Hanks’ balanced salt solution, and L-glutamine were obtained from Life Technologies. Fetal bovine serum was from HyClone (Logan, UT). Collagenase type P was obtained from Roche Molecular Biochemicals. Ficoll, D-mannoheptulose, diazoxide, purified LpL, and low molecular weight (M, 3000–5000) heparin were from Sigma. Rat sera, a source of apolipoprotein C-II, was obtained from Amersham Pharmacia Biotech. For Western blot analysis, the primary antibody was chicken-anti-bovine milk LpL (25) and guinea pig-anti-human insulin (Linco). The secondary antibodies were FITC-conjugated donkey anti-rabbit IgG and Cy3-conjugated donkey anti-chicken IgG (Jackson ImmunoResearch). For immunohistochemical studies, the primary antibodies were rabbit-anti-human LpL (26, 27) and guinea pig-anti-human insulin (Linco). The secondary antibodies were FITC-conjugated donkey anti-rabbit IgG and Cy3-conjugated donkey anti-guinea pig IgG (Jackson ImmunoResearch). CellTiter 96 AQueous One Solution containing MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium inert salt reagent) was purchased from Promega (Madison, WI).

Pancreatic Islet Isolation and Culture—Islets were isolated from 6- to 8-week-old male C57BL/6 mice by collagenase digestion as previously reported (28). Islets were cultured overnight in an atmosphere of 95% air, 5% CO2 in CMRL-1066 tissue culture medium (CMRL) containing 5.5 mM glucose, 2 mM L-glutamine, 10% (v/v) heat-inactivated fetal bovine serum, 100 units/ml penicillin, and 100 g/ml streptomycin. Mouse islets (200 per experimental condition) were cultured in 35-mm sterile dishes.

Cell Culture—All experiments were performed in triplicate. INS-1 and RINm5F cells were maintained under conditions previously described (15, 29) in 12-well tissue culture plates (3 × 105 cells/well). INS-1 cells, established by Asfari et al. (30), were a kind gift from Chris Newgard (Dallas, TX). Isolated pancreatic mouse islets (200) were seeded into a 35-mm dish containing 1 ml of culture media. The islets were washed with CMRL (without fetal calf serum, containing 0.1% bovine serum albumin and 3 mM glucose) and incubated for 30 min prior to acute exposure (4 h) to 3 or 20 mM glucose. For studies using diazoxide or mannoheptulose, these agents were present during the preincubation and glucose stimulation period. For determining the effect of room temperature, preincubation and glucose stimulation protocols were performed in a gassed, humidified chamber at 25 °C. Glucose stimulation was performed by replacing preincubation media with stimulation media (containing 0.1% bovine serum albumin and 3 or 20 mM glucose).

After glucose stimulation, islets were collected and media replaced with 125 μl of CMRL containing 100 μg/ml heparin and incubated for 30 min. The heparin-containing media were then collected, a detergent solution was added to the plates, and the islets were incubated until visually dissolved. The heparin-containing media and the detergent extracts were assayed for LpL activity (described below). The stimulation culture supernatant was assayed for insulin by radioimmunoassay. INS-1 cells (3 × 105) were seeded in 12-well culture plates 48 h prior to glucose stimulation. Preincubation and acute glucose stimulation of INS-1 cells were performed as described with mouse islets, except cells were cultured in RPMI media, and the absence of glucose was used for the basal conditions. The effect of prolonged insulin exposure (24 h) was performed by adding exogenous porcine insulin (0.25, 0.5, or 1 μU) to the incubation media. After acute exposure to an elevated glucose concentration or prolonged exposure to exogenous insulin, heparin was added to the incubation media to a final concentration of 100 μg/ml and incubated for 30 min. The culture media were then collected, a detergent solution was added, and cells were incubated until visually dissolved. The heparin-containing culture supernatant and the detergent extract were assayed for LpL activity as described below. The culture supernatant was assayed for insulin.

LpL Enzyme Activity Assay—Lipase activity was measured by an in vitro assay in which radiolabeled fatty acids esterified to glycerol are cleaved and recovered after a chloroform/methanol/heptane-based extraction (17). The units of activity are reported as moles of free fatty acid released per specific number of islets or cells per unit time. LpL activity is distinguishable from other lipase activities by its sensitivity to high molar salt concentration (4). "Heparin-releasable" LpL activity is the amount of activity in the supernatant of heparin-treated islets or cells. "Detergent-extractable" is the amount of activity after detergent solubilization of remaining cells or islet pellets following heparin treatment. Detergent solubilization involves incubating β-cells or islet pellets with a detergent solution containing 20 g/liter deoxycholate for 30 min at 37 °C. "Total LpL activity" is the amount of activity after detergent solubilization of remaining cells or islet pellets following heparin treatment. Detergent solubilization involves incubating β-cells or islet pellets with a detergent solution containing 20 g/liter deoxycholate for 30 min at 37 °C. Total LpL activity is the amount of activity after detergent solubilization of cells or islet pellets that have not been exposed to heparin-treatment.

Cellular Metabolism Assay—Metabolism was determined using an assay based on the ability of NAHD or NADPH generated by substrate flux to reduce a tetrazolium substrate and produce a stable colored product. INS-1 or RINm5F cells were cultured in 96-well culture plates (2 × 103 cells/well). At the start of the experiment, the cells were washed with culture media and stimulated with glucose as described above. Subsequently, 20 μl/ml tetrazolium reagent was added to the media and the optical density at 540 nm was determined after 4 h on a Molecular Devices Thermomax plate reader (Sunnyvale, CA).

Immunohistochemistry—Dispersed islets were obtained by dispase digestion of freshly isolated mouse islets and cultured for 24 h in 5.5 mM glucose. Duplicated aliquots of dispersed islet cells were treated with or without heparin (100 μg/ml) for 30 min prior to centrifugation onto
glass slides and fixation in Bouin’s solution. INS-1 cells (30,000) were seeded into 8-well collagen I-coated culture slides (Becton Dickinson, Bedford, MA) and cultured for 48 h in the absence of glucose. Paired wells containing INS-1 cells were treated with or without heparin (100 μg/ml) for 30 min prior to fixation in Bouin’s solution. Fixed cells were assayed for insulin and LpL localization using antibodies as described above followed by FITC-conjugated donkey anti-primary antibodies that allowed fluorescence detection. Visualization of localization of LpL and insulin in cells was performed with a Sarastro 2000 dual-laser Zeiss Axioskop confocal microscope.

RESULTS

Acute Glucose Exposure Stimulates LpL Activity but Not Total LpL Mass—To determine whether glucose acutely stim-
ulates LpL activity in islets, we first measured total LpL activity in mouse islets. In preliminary experiments, LpL enzyme activity (defined by salt inhibition and apoCII dependence) was detected in islets and shown to be linearly dependent on the number of islets assayed (data not shown). Glucose caused a time-dependent stimulation of mouse islet total LpL activity (Fig. 1). LpL enzyme activity was 91% higher in the presence of 20 mM glucose in comparison to basal glucose (3 mM) following a 4-h incubation period (*p, 0.01). The same effect was seen at 8 h. Glucose-stimulated insulin secretion from these islets at 1, 4, and 8 h increased over basal secretion by 12-, 20-, and 2.5-fold, respectively (data not shown).

Western blotting (Fig. 2) of mouse islets (lanes 2 and 3), and the β-cell lines INS-1 (lanes 4 and 5), and RINm5F (lanes 6 and 7) showed that these cell types produce an LpL protein with the same molecular weight as LpL from differentiated 3T3-L1 adipocytes (lane 1). However, total LpL mass was not increased in any of these cell types after incubation in 20 mM glucose for 4 h (compare lanes 2 versus 3, 4 versus 5, and 6 versus 7). These results suggest that post-translational mechanisms contribute
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to the acute induction of LpL enzyme activity in β-cells.

Acute Glucose Exposure Stimulates LpL Translocation to the Plasma Membrane—In adipocytes, physiologically relevant LpL is thought to reside in a cellular compartment at or close to the cell surface. LpL at this site is released to the media by heparin. To determine whether glucose affects the proportion of LpL that is accessible to heparin, we examined LpL enzyme activity and LpL protein mass from glucose-stimulated islets and INS-1 cells after treatment with 100 μg/ml heparin. Heparin-releasable LpL activity from mouse islets (Fig. 3A) and INS-1 cells (Fig. 3B) was significantly higher after 4-h exposure to 20 mM glucose than with basal glucose treatment. Heparin-releasable LpL protein mass also increased in INS-1 cells treated with 20 mM glucose for 4 h (Fig. 4, lanes 3 versus 5).

These data suggest that glucose acutely promotes the translocation of active LpL to the surface of β-cells.

Effects of Insulin Secretion and Cellular Metabolism on Glucose-stimulated LpL Activity—Because insulin has been shown to regulate LpL activity and expression in adipocytes (17), we studied the relationship between glucose-stimulated insulin secretion and LpL activity in islets and β-cells. Diazoxide, an agent known to hyperpolarize β-cells and inhibit insulin secretion, blocked glucose-stimulated insulin secretion (Fig. 5A) but did not affect glucose-stimulated LpL activity (Fig. 5B) or glucose metabolism (data not shown). Inhibition of glucose metabolism by incubation at 25 °C or in the presence of mannohexitol (10 mM) prevented the stimulation of LpL activity by glucose (Fig. 5B) as well as glucose-stimulated insulin secretion (Fig. 5A). Incubation at 25 °C or in the presence of mannohexitol decreased glucose metabolism by 50% and 100%, respectively (data not shown).

In 3T3-L1 adipocytes, prolonged exposure to exogenous insulin (10^{-12} to 10^{-6} M) increases heparin-releasable LpL activity (17). Treatment of INS-1 cells with 0.25–1.0 μM exogenous insulin caused a dose-dependent increase in heparin-releasable LpL activity above that produced by 20 mM glucose alone (Fig. 6). This effect required elevated glucose concentrations, because exogenous insulin at 1.0 μM did not stimulate LpL activity at low glucose concentrations (1 mM).

LpL and Insulin Reside in Separate Vesicles in Pancreatic β-Cells—Diazoxide treatment blocks glucose-stimulated insulin secretion but not glucose-stimulated induction of LpL activity, implying that LpL and insulin reside in separate and distinct vesicles. To address this issue directly, we used fluorescent immunohistochemistry to localize these proteins in β-cells. Dual-laser confocal fluorescent microscopic images of islets (Fig. 7) showed that LpL (red) and insulin (green) reside in separate vesicles within insulin-expressing β-cells. Membrane-associated LpL present on β-cells (Fig. 7A, white arrows) was not observed in heparin-treated cells (Fig. 7B). Similar results were seen in INS-1 cells (Fig. 8). Serial confocal microscopy sections showed that both control (panels A–C) and heparin-treated (panels D–F) INS-1 cells contained insulin (green) and LpL (red) residing in separate vesicles. However, INS-1 cells were polarized with respect to LpL expression with the
heparin-treated (Bouin’s solution. Insulin (FITC, green) and LpL (Cy3, red) expression were detected using fluorescence immunohistochemistry observed using a dual-laser confocal microscope. Serial section images (0.5 μm in thickness and separated by 1.5 μm) demonstrate separate and distinct areas of localization for insulin and LpL. Heparin treatment (D) results in the removal of apical membrane-associated LpL from adherent, cultured INS-1 cells (white arrows, A). Images shown are representative of the appearance of cells on multiple different slides.

FIG. 8. LpL is located in INS-1 cells. Control, untreated (A–C) and heparin-treated (D–F) adherent, cultured INS-1 cells were fixed with Bouin’s solution. Insulin (FITC, green) and LpL (Cy3, red) expression were detected using fluorescence immunohistochemistry observed using a dual-laser confocal microscope. Serial section images (0.5 μm in thickness and separated by 1.5 μm) demonstrate separate and distinct areas of localization for insulin and LpL. Heparin treatment (D) results in the removal of apical membrane-associated LpL from adherent, cultured INS-1 cells (white arrows, A). Images shown are representative of the appearance of cells on multiple different slides.

DISCUSSION

β-Cell lipid metabolism is critical for the normal regulation of insulin secretion and may be involved in the obesity-related β-cell failure that leads to type 2 diabetes. Fatty acids are required for glucose-stimulated insulin secretion in some systems (31). In islets and rodents, short term exposure to elevated fatty acid concentrations enhances glucose-stimulated insulin secretion (32–34). Long term exposure decreases glucose-stimulated insulin secretion (35, 36). Basal insulin secretion is stimulated by chronic exposure of islets to fatty acids (37–39). β-Cell failure in Zucker diabetic fatty rats is preceded by accumulation of islet triglyceride (40), an effect that can be reversed by therapies associated with a decrease in islet triglycerides (41). Cytosolic acyl-CoA may serve as an effector molecule in the control of glucose-stimulated insulin secretion, perhaps through the activation of protein kinase C isoforms (42). Fatty acids stimulate islet ceramide synthesis, a process that may lead to apoptosis in islets (43).

Fatty acids affect islet function, but the origin of islet-associated fat is poorly understood. Cells can obtain fatty acids through three mechanisms: de novo lipogenesis, importing circulating free fatty acids, or hydrolyzing lipoprotein-associated triglycerides through the action of LpL. The first two are unlikely to be utilized by islets. The first, de novo lipogenesis, is extremely energy inefficient (44). The second, importing free fatty acids from the plasma, is not a major source of fat for most tissues. The bulk of circulating free fatty acids are derived from the hydrolysis of triglycerides in adipose tissue, transported to the liver, converted back to triglycerides, and secreted in the form of lipoproteins (45). LpL, by hydrolyzing lipoprotein-associated triglycerides in the capillaries of peripheral tissues, is probably the major provider of fatty acids to skeletal muscle, heart, and adipose tissue (46). We recently provided an initial description of LpL expression in islet cells and showed that manipulating LpL expression in these cells can affect insulin secretion (15). These data suggest that β-cells, like parenchymal cells in other tissues, derive physiologically relevant stores of fatty acids through the action of LpL.

In the current study, we demonstrate that β-cells from pancreatic islets and β-cell lines express functional LpL activity with properties similar to that described in adipocytes. LpL activity was inhibited by salt, dependent on a source of apolipoprotein C-II for activity, and heparin-releasable. LpL in detergent extracts and a heparin-releasable fraction from mouse islets and two β-cell lines, INS-1 and RINm5F, had the same apparent molecular weight as LpL from differentiated 3T3-L1 adipocytes. Like adipocytes, β-cells showed an increase in LpL enzyme activity after exposure to glucose. Total and heparin-releasable LpL activities of islets were increased 2-fold in the presence of elevated glucose (20 mM) compared with basal glucose levels. Glucose treatment had no effect on total LpL protein content but caused an increase in the heparin-releasable fraction of LpL protein. These results imply that glucose acutely induces the translocation of active LpL from intracellular stores to the cell surface of islets.

Like glucose-stimulated insulin secretion, glucose-stimulated LpL activation required glucose metabolism. Incubating β-cells either in the presence of mannoheptulose or at room temperature, interventions that decrease glucose metabolism, prevented the induction of LpL activity by glucose. Glucose metabolism appears to be tightly linked to the regulation of LpL activity, because the 50% reduction in glucose metabolism associated with incubation at room temperature completely inhibited LpL activity. Unlike glucose-stimulated insulin secretion, glucose-stimulated LpL activation was unaffected by treatment with diazoxide, an agent that opens islet KATP channels and prevents insulin secretion. These results provide physiological evidence that functional LpL is not located in insulin secretory granules.

We also provide visual evidence that LpL and insulin exist in discrete secretory organelles. Our immunohistochemical studies show that LpL protein is associated with β-cells in intact islets as well as in dispersed primary β-cells and INS-1 cells. However, intracellular LpL is localized to secretory granules that are distinct from those containing insulin. Unlike insulin, LpL is also found at the cell surface where it can be released by treatment with heparin.
Exposure of β-cells to elevated levels of exogenous insulin for 24 h stimulated heparin-releasable LpL activity by ~25% above that of glucose. This effect of insulin required the presence of elevated concentrations of glucose, because insulin had no effect at basal glucose concentrations. In 3T3-L1 adipocytes, insulin increases heparin-releasable LpL activity without affecting LpL transcription, mRNA levels, or protein synthetic rate, consistent with post-translational activation of LpL (17). The current data suggest that hyperinsulinemia may have similar effects on β-cell LpL activity in the setting of hyperglycemia.

Based on the current results, our working model for how islet LpL may contribute to β-cell dysfunction is shown in Fig. 9. Under basal conditions (left panel), monomeric islet LpL dimerizes and translocates to the capillary endothelium. At this site, LpL acts on circulating lipoproteins to yield free fatty acids. These are transported to the β-cell by poorly understood mechanisms that may involve fatty acid transporters (47). Intracellular fatty acids are then re-esterified to yield triglyceride, the neutral lipid that accumulates in the β-cells of diabetic animals. Fatty acids can also be released from intracellular triglyceride stores through the activity of hormone-sensitive lipase, known to be expressed and active in β-cells (48). In the setting of hyperglycemia and hyperinsulinemia associated with peripheral insulin resistance (right panel), translocation of active LpL to the capillary endothelium is increased, and more triglyceride-derived fatty acids are delivered to the β-cell. Because fatty acids and glucose compete as respiratory substrates in many tissues (49), chronically increasing β-cell fatty acids would decrease glucose metabolism leading to decreased insulin secretion. Increasing fatty acids may also directly impair β-cell function and promote apoptosis (40, 50–51) consistent with this notion of chronic lipotoxicity, islets from mice with LpL deficiency secrete more insulin than islets from animals with normal LpL activity. These LpL-deficient mice have higher fasting insulin levels than wild-type mice in the setting of normal LpL activity. These LpL-deficient mice have higher fasting insulin levels than wild-type mice in the setting of normal LpL activity. These LpL-deficient mice have.

In summary, β-cell LpL is regulated by glucose and insulin. The promotion of LpL activity requires glucose metabolism and involves altered trafficking of secretory granules that are distinct from those containing insulin. This demonstration of a regulated, physiologically relevant pathway for delivering lipid to β-cells provides a framework for clarifying the mechanisms underlying the insulin secretory failure that characterizes type 2 diabetes.

Acknowledgments—We thank Richard Hresko for supplying the 3T3-L1 adipocytes and Joan Fink for technical assistance.

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