Localization of Lipoprotein Lipase in the Diabetic Heart
Regulation by Acute Changes in Insulin

Nandakumar Sambandam, Mohammed A. Abrahani, Edith St. Pierre, Osama Al-Atar, Margaret C. Cam, Brian Rodrigues

Abstract—Vascular endothelium–bound lipoprotein lipase (LPL) is rate limiting for free fatty acid (FFA) transport into tissues. In streptozotocin (STZ)-diabetic rats, we have previously demonstrated an increased heparin-releasable LPL activity from perfused hearts. Because heparin can traverse the endothelial barrier, conventional Langendorff retrograde perfusion of the heart with heparin could release LPL from both the capillary luminal and abluminal surfaces. To determine the precise location of the augmented LPL, a modified Langendorff retrograde perfusion was used to isolate the enzyme at the coronary lumen from that in the interstitial effluent. In response to heparin, a 4-fold increase in LPL activity and protein mass was observed in the coronary perfusate after 2 weeks of STZ diabetes. Release of LPL activity into the interstitial fluid of control hearts was slow but progressive, whereas in diabetic hearts, peak enzyme activity was observed within 1 to 2 minutes after heparin, followed by a gradual decline. Immunohistochemical studies of myocardial sections confirmed that the augmented LPL in diabetic hearts was mainly localized at the capillary endothelium. To study the acute effects of insulin on endothelial LPL activity, we examined rat hearts at various times after the onset of hyperglycemia. An increased heparin-releasable LPL activity in diabetic rats was demonstrated shortly (6 to 24 hours) after STZ injection or after withdrawal from exogenous insulin. Heparin-releasable coronary LPL activity was also increased after an overnight fast. These studies indicate that the intravascular heparin-releasable fraction of cardiac LPL activity is acutely regulated by short-term changes in insulin rather than glucose. Thus, during short periods (hours) of hypoinsulinemia, increased LPL activity at the capillary endothelium can increase the delivery of FFAs to the heart. The resultant metabolic changes could induce the subsequent cardiomyopathy that is observed in the chronic diabetic rat. (Arterioscler Thromb Vasc Biol. 1999;19:1526-1534.)

Key Words: lipoprotein lipase ■ diabetes ■ Langendorff perfused heart ■ insulin

Lipoprotein lipase (LPL) is a multifunctional enzyme that is widely distributed in various tissues.1,2 In the adult heart, endothelial cells demonstrate no positive hybridization for LPL mRNA but are immunoreactive for protein at the cell surface.3 Thus, it is thought that coronary endothelial LPL is synthesized and processed in myocytes, and the enzyme is then translocated across the interstitial space and vascular mesh onto heparan sulfate proteoglycan (HSPG) binding sites on the luminal surface of the capillary endothelium.8 At this location, “functional” LPL hydrolyzes lipoprotein triglyceride (TG) to free fatty acid (FFA) and 2-monocacylglycerol, which are transported into the heart for numerous metabolic and structural functions. In addition to its role as a lipolytic enzyme, LPL has also been implicated as a mediator for lipoproteins to interact with cell surfaces and receptors, thereby facilitating lipoprotein uptake.5-7

LPL activity is regulated in a tissue-specific manner by dietary and hormonal factors that modulate the enzyme via transcriptional, posttranscriptional, and posttranslational mechanisms.8 For example, with fasting, LPL activity decreases in adipose but increases in cardiac tissue; as a result, FFAs from circulating TGs are diverted away from storage to meet the metabolic demands of the heart.1 Insulin causes LPL activity to increase 3-fold in adipose tissue but reduces LPL activity significantly in skeletal muscle.9 In this way, insulin directs TG FFAs away from muscle oxidation and toward storage. Hence, LPL fulfills a “gatekeeping” function by carefully regulating the supply of FFAs according to the metabolic demands of different tissues.10 In the heart, it is the rapid, heparin-releasable LPL pool (localized on capillary endothelial cells) that is more sensitive to altered physiological and pathological states than is the total cellular activity (ie, the non–heparin-releasable component that represents a storage form of the enzyme).11,12

Recently, we reported that heparin perfusion of control rat hearts releases 2 pools of LPL into the medium: an initial fast phase followed by a prolonged release.13 The fast phase is considered to represent extracellularly bound LPL, whereas...
the delayed phase may originate from a separate compartment. Because heparin has been demonstrated to traverse the endothelial barrier, we suggested that conventional Langendorff retrograde perfusion of the heart with heparin can release not only LPL bound to the luminal side of the capillary endothelium but also that present at the abluminal surface, the interstitial space, and the myocyte surface. Interestingly, in streptozotocin (STZ)-diabetic rats, the initial fast phase of heparin-releasable LPL activity was significantly elevated after 2 or 12 weeks of hypoinsulinemia, whereas the second, or delayed, phase of LPL release was absent. In the present study we asked 2 questions: (1) Does the augmented LPL in the diabetic heart actually represent the functional pool of enzyme at the coronary lumen, exclusive of the abluminal, interstitial, and myocyte pools? This question is of particular importance because the presence of the enzyme at this location would permit FFA supply to the diabetic heart in the absence of glucose utilization. (2) Can changes in LPL activity be acutely regulated by a short duration (hours) of hyperglycemia or hypoinsulinemia? During diabetes, poor compliance with insulin treatment causes patients to be regularly exposed to brief periods of hyperglycemia.

**Methods**

**Experimental Animals**

All animals were cared for in accordance with the principles promulgated by the Canadian Council on Animal Care and the University of British Columbia. Adult male Wistar rats (280 to 290 g) were obtained from the University of British Columbia animal care unit (Vancouver, BC). The rats were maintained under a 12-hour light (7 AM to 7 PM)/dark cycle and supplied with a standard laboratory chow diet (PMI Feeds) and water ad libitum.

**Induction of Diabetes**

Selective β-cell death and the ensuing diabetic state can be produced after a single intravenous dose of STZ. A dose-dependent increase in severity of diabetes is produced by 25 to 100 mg/kg STZ. After an injection of STZ of 55 mg/kg IV, stable hyperglycemia develops within 24 to 48 hours and remains 2 to 3 times higher than normal, in concert with an ≈50% reduction in plasma insulin levels. Although these animals are insulin deficient, they do not require insulin supplementation for survival and do not develop ketoadiposisis. On the other hand, a 100 mg/kg dose of STZ causes intense β-cell necrosis, remarkable elevation of serum glucose within 24 hours, reduced plasma insulin to 2% to 5% of control, and 98% loss of pancreatic insulin stores. Without administration of exogenous insulin, death in most of these animals occurs within 7 to 10 days. Rats were randomly divided into nondiabetic control (CON) and diabetic (D55 and D100) groups. Halothane-anesthetized rats were injected with STZ (55 [D55] or 100 [D100] mg/kg IV, Sigma Chemical Co) or an equivalent volume (1 mL/kg) of saline. Glycosuria was determined 24 hours after STZ injection, and hyperglycemia was tested at 48 hours via glucometer. All STZ-treated rats displayed both glycosuria (>4+) and hyperglycemia (>13 mmol/L).

**Insulin Reduction**

To evaluate the effect of a chronic reduction in plasma insulin on cardiac LPL, D55 rats were kept for 2 weeks after the STZ injection, at which time they were euthanized and the hearts removed. To investigate the short-term effects of a decrease in insulin on cardiac LPL, 2 protocols were followed. In the first protocol, rats were injected with 100 mg/kg STZ. In preliminary experiments, we determined that after this dose of STZ, there is a triphasic pattern of changes in blood glucose and insulin levels in the 24-hour period after injection. An initial brief hyperglycemia is followed by a period of hypoglycemia that is brought about by massive β-cell degranulation and an enormous release of insulin. Blood glucose then rises to a hyperglycemic value of 13 mmol/L within 12 to 16 hours. Rats were followed up individually, and at the point of hyperglycemia, or 3 or 6 hours after hyperglycemia, animals were euthanized for the determination of cardiac LPL activity. One potential drawback with this approach is the varied metabolic changes that occur before progression to stable hyperglycemia. Hence, with our second protocol, rats were made severely diabetic with 100 mg/kg STZ. One day after diabetes induction, the animals were treated subcutaneously with an intermediate-acting insulin (Iletin NPH, beef and pork; Lilly) once daily. The insulin injection was given at 10 AM with the dose adjusted daily to achieve normoglycemia. Treatment was continued for 7 days. This time was necessary to ascertain the optimal insulin dose (~18 to 20 U/kg) required to maintain euglycemia for 24 hours. After the animals’ diabetes had been well controlled, insulin injection was stopped and plasma glucose closely monitored. After the last insulin injection, plasma glucose increases after 24 hours. A plasma glucose concentration of 13 mmol/L was considered a hyperglycemic value, and when this level was reached, diabetic animals were kept for either 6 or 24 hours before they were euthanized. Using this method, we were able to achieve fixed durations of hypoinsulinemia and hyperglycemia.

**Modified Langendorff Perfusion**

To localize and quantify the augmented LPL in diabetic rat hearts, a modified Langendorff retrograde perfusion technique was used to separate the coronary from the interstitial effluent. Rats were anesthetized with 50 mg/kg sodium pentobarbital IP, and the thoracic cavity was opened. The left anterior vena cava was ligated below the azygous vein followed by ligation of the right anterior vena cava. The hearts were then carefully excised, with the aorta, inferior vena cava, and lungs still attached. Rats were not injected with heparin before being killed because heparin displaces LPL bound to HSPGs on the capillary endothelium. Consequently, it was necessary to cannulate the heart quickly to avoid clotting in the coronary arteries. Immediately on excision, the beating heart was immersed in cold (4°C) Krebs-Ringer-HEPES buffer (pH 7.4). The concentrations of solutes in the buffer were (in mmol/L) 1 CaCl2, 118 NaCl, 4.96 KCl, 1.19 KH2PO4, 1.19 MgSO4·7H2O, 24 HEPES, and 10 glucose. After the aorta was cannulated and tied below the innominate artery, the hearts were perfused retrogradely by the noncirculating Langendorff technique. The perfusion fluid was continuously gassed with 95% O2/5% CO2 in a double-walled, water-heated chamber maintained at 37°C with a temperature-controlled circulating water bath. A peri-staltic pump controlled the rate of coronary flow (8 mL/min). The right and left branches of the pulmonary artery were cut before they entered the lungs, and the 2 branches were then trimmed off at their junction. Afterward, the inferior vena cava and branches of the right and left pulmonary veins were ligated, the lungs were removed, and the pulmonary artery was cannulated and tied. At this time, most of the perfusate (≈89% to 99%) starts flowing through the pulmonary cannula, whereas a small amount of fluid (≈1% to 2%) drips down to the apex of the heart. The pulmonary effluent represents the coronary perfusate, whereas the fluid collected at the apex represents interstitial transudate. To measure the release of LPL activity or protein into the medium, the perfusion solution was changed to Krebs buffer containing 1% BSA (fraction V, Boehringer Mannheim Biochemica) and heparin (5 U/mL). This concentration of heparin can maximally release cardiac LPL from its binding sites, an action mediated by the interaction of negative charges on heparin with positively charged amino residues on the enzyme. The coronary and interstitial effluents were collected separately in timed fractions and frozen until assayed for LPL activity.

**Preparation of Cardiac Myocytes**

Perfusion of the heart with heparin releases predominantly extracellular, endothelium-bound LPL; however, activity is still measurable within the heart. This heparin-nonreleasable LPL activity is located predominantly within the myocytes. To measure this fraction, calcium-tolerant myocytes were prepared from hearts (ventricles) according to a previously described procedure. In brief, hearts were removed from anesthetized rats and digested through perfusion of collagenase (228 U/mL) retrogradely through the heart. Myocytes
were made calcium-tolerant by successive exposure to increasing concentrations of calcium. Our method of isolation yields a highly enriched population of calcium-tolerant myocardial cells that are rod-shaped in the presence of 1 mmol/L Ca²⁺ with clear cross striations. Yield of myocytes (cell number) was determined microscopically by using an improved Neubauer hemocytometer. Myocyte viability (generally between 75% and 85%) was assessed through trypan blue exclusion. Cardiac myocytes from CON and diabetic rats were suspended in Joklik minimum essential medium to a cell density of 0.4×10⁶ cells/mL and incubated at 37°C under an atmosphere of 95% O₂/5% CO₂. LPL activity in cell homogenates was determined at time zero by removing a sample of cell suspension, followed by centrifugation, sonication of the cell pellet, and deproteinization using 2% triton X-100 (1:150 dilution, Chemicon Corp), followed by incubation for 1 hour at room temperature. The release of surface-bound LPL, heparin (5 U/mL) was added to the myocyte suspension. Aliquots of cell suspension (1 mL) were removed at specified intervals, and the medium was separated from cells by centrifugation (3000 g for 10 seconds). The supernatant was decanted and stored at −70°C until it was assayed for LPL activity.

Assay of LPL Activity and Mass

LPL catalytic activity in coronary perfusates, interstitial fluid, incubation medium of cardiac myocytes, and myocyte cell pellets was determined by measuring the in vitro hydrolysis of a sonicated [³H]tri olein substrate emulsion. One hundred microliters of either myocyte medium or coronary perfusate or 25 μL of interstitial fluid or myocyte cell pellets was used to measure LPL activity. Results are routinely expressed as nanomoles of oleate released per hour per milliliter (coronary perfusate or interstitial fluid) or per 10⁶ cells (myocyte medium or cells).

Changes in the amount of LPL activity do not always represent changes in quantity of immunoassayed LPL protein. To measure LPL mass, coronary perfusates (~24 mL) from CON and D55 hearts were collected between 1 and 3 minutes after heparin perfusion. LPL protein was measured by a previously described sandwich ELISA. LPL mass in coronary fluid was used to calculate LPL specific activity as milliunits per nanogram of LPL protein, where 1 mU is defined as the amount of enzyme catalyzing the release of 1 nmol oleate per minute.

Immunolocalization of LPL

Immediately on excision, CON and D55 rat hearts were perfused retrogradely by the noncirculating Langendorff technique with Krebs-Ringer-HEPES buffer for 3 minutes to clear the heart of blood. Perfusion buffer was then changed to fixative (neutral phosphate-buffered 10% formalin solution) for 2 minutes. After perfusion, hearts were stored in 10% formalin for 24 hours, followed by paraffin processing through graded ethanol and xylene. The sections were then embedded in Paraplast, sectioned at 3 μm, and mounted on positively charged glass slides. For immunostaining, sections were deparaffinized, rehydrated, and treated with 5% (vol/vol) heat-inactivated rabbit serum in Tris-buffered saline (TBS, 0.15 mol/L NaCl, pH 7.4) to block nonspecific background. Sections were incubated with the affinity-purified polyclonal antibody against LPL (1:100 dilution in TBS containing 1% wt/vol BSA) overnight at room temperature in a humid chamber. The primary antibody was then washed in TBS and further incubated for 1 hour at room temperature with the secondary biotinylated rabbit anti-chicken IgG (1:150 dilution, Chemicon Corp), followed by incubation for 1 hour with streptavidin-biotin-peroxidase complex (ABC kit, Vector Inc.). After being rinsed, sections were stained with 3,3’-diaminobenzidine hydrochloride/H₂O₂, followed by staining with 0.1% (wt/vol) nuclear fast red in 5% (wt/vol) aqueous aluminum sulfate. After a final rinse in running tap water, sections were dehydrated in ethanol, cleared in xylene, mounted in a resinous mounting medium, and photographed. Sections from CON and diabetic hearts were treated in an identical manner during all incubation and washing steps. As previously demonstrated, an absence of staining was observed when the primary antibody was omitted or replaced by preimmune chicken serum.

### Table 1. Characteristics of Diabetes (55 mg/kg STZ) at 2 Weeks After STZ Injection

<table>
<thead>
<tr>
<th></th>
<th>CON</th>
<th>STZ Injected</th>
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<tbody>
<tr>
<td>Body weight, g</td>
<td>373±6</td>
<td>345±6.3*</td>
</tr>
<tr>
<td>Heart weight, g</td>
<td>1.15±0.02</td>
<td>0.99±0.02</td>
</tr>
<tr>
<td>Plasma insulin, ng/mL</td>
<td>2.17±0.25</td>
<td>0.84±0.07*</td>
</tr>
<tr>
<td>Plasma glucose, mmol/L</td>
<td>7.0±0.11</td>
<td>15.8±0.4*</td>
</tr>
<tr>
<td>Plasma TG, mmol/L</td>
<td>1.79±0.11</td>
<td>3.68±0.41*</td>
</tr>
<tr>
<td>Plasma cholesterol, mmol/L</td>
<td>1.54±0.05</td>
<td>2.11±0.10*</td>
</tr>
</tbody>
</table>

Data are mean±SEM for the 6 to 8 CON and diabetic rats. Values are those obtained before euthanasia. Plasma parameters were from fed rats. Blood was collected from the tail vein into heparinized tubes that were centrifuged for separation of plasma. Insulin was measured by using rat insulin standards. *Significantly different from CON, P<0.05.

Effect of Food Restriction on LPL Activity

To ascertain whether hypoinsulinemia in the absence of hyperglycemia could alter LPL activity, CON rats were fasted for 16 hours (6 PM to 10 AM), and LPL activity was measured in coronary and interstitial compartments. During fasting, food was withdrawn from the animals, but they had free access to water.

Plasma Measurements

Blood samples from the tail vein were collected in heparinized glass capillary tubes. Blood samples were immediately centrifuged, and plasma was collected and stored at −20°C until it was assayed. Plasma glucose, TG, and cholesterol levels were measured with kits (Boehringer Mannheim). Plasma insulin was measured using a double-antibody radioimmunoassay kit from Linco Research Inc.

Materials

[³H]Tri olein was purchased from Amersham Canada. Heparin sodium injection (Hapalean, 1000 USP U/mL) was obtained from Organon Teknika. Joklik minimum essential medium was obtained from Gibco Canada. Collagenase (CLS 2, 325 U/mg) was purchased from Worthington Biochemical Corp. All other chemicals were obtained from Sigma. Affinity-purified chicken polyclonal antibodies against LPL purified from bovine milk were a generous gift from Dr D.L. Severson (University of Calgary).

Statistical Analysis

All data are reported as mean±SEM unless otherwise stated. One-way ANOVA followed by the Newman-Keuls test or the unpaired Student’s t test was used to determine differences between group mean values. Changes in heparin-releasable LPL activity over time were analyzed with a multivariate ANOVA followed by the Newman-Keuls test using the Number Cruncher Statistical System. The level of statistical significance was set at P<0.05.

### Results

**Chronic Diabetes**

**General Characteristics**

Induction of diabetes with 55 mg/kg STZ resulted in glycosuria. Body weight gain over 2 weeks was reduced in D55 animals relative to CON, but there was no significant difference in heart weight between CON and diabetic rats (Table 1). The STZ injection caused a reduction in plasma insulin levels that was accompanied by hyperglycemia. Both plasma TG and cholesterol levels were elevated in D55 rats (Table 1).

**Modified Langendorff Perfusion**

Retrograde perfusion of hearts from CON and 2-week D55 rats with heparin resulted in release of LPL activity into the coronary perfusate that was collected via the cannulated...
pulmonary artery (Figure 1A). This heparin-induced LPL discharge was rapid, and peak activity in both groups was observed within 1.5 minutes. On continuous perfusion of these hearts with heparin, LPL activity returned to near basal levels. In D55 hearts, peak LPL activity in the coronary perfusate was almost 3- to 4-fold as much as CON. To confirm that the elevated lipase activity was specific to LPL, the assay was performed in the presence of 1 mol/L NaCl and the absence of apo C2, and under these conditions, peak activity from CON and D55 was inhibited (data not shown). The increase in LPL activity in the coronary perfusate of D55 rats was not due to an increase in specific activity of the protein but was a consequence of a 4-fold increase in LPL protein mass as measured by ELISA (Table 2). After heparin administration, the release of LPL activity into the interstitial fluid was clearly different from that observed in the coronary perfusate (Figure 1B). Initially, the enzyme released from CON hearts was low but gradually increased over time. In D55 hearts, a peak release of enzyme was observed within 1 to 2 minutes, followed by gradual decline, such that after 10 minutes, it was lower than CON.

**Immunohistochemical studies of myocardial sections were performed to complement our observation that the augmented LPL in diabetic hearts was mainly localized at the endothelial cells.** Although the results are difficult to quantify, this technique provides information regarding cellular localization of the LPL protein within the cardiac tissue. Whereas LPL immunoreactivity was found throughout the CON and diabetic myocardium (brown stain), capillary blood vessels in the diabetic heart (Figure 2B) demonstrated a more intense LPL immunoreactivity compared with CON (Figure 2A). In contrast, little to no detectable LPL immunoreactivity was found in the larger blood vessels of CON and diabetic hearts (data not shown). Staining was not seen when the primary antibody was omitted or replaced by preimmune chicken serum (data not shown).

**Acute Diabetes**

**Insulin Depletion Study**

Induction of diabetes with 100 mg/kg STZ produced the characteristic triphasic pattern of changes in blood glucose and insulin levels in the 24-hour period immediately after injection (Figure 3, top). An initial brief hyperglycemia was followed by a period of hypoglycemia that is brought about by a release of insulin from damaged β-cells. Blood glucose rose gradually with a corresponding decline in plasma insulin, and hyperglycemia (>13 mmol/L) was usually attained within 12 to 16 hours. Interestingly, even at this early stage of diabetes, peak (Figure 3) and total (calculated as area under the curve over 10 minutes; for CON, 555±233 nmol · mL⁻¹ · 10 min⁻¹; for 13+0 rats, 1322±46 nmol · mL⁻¹ · 10 min⁻¹) heparin-releasable LPL activity in the coronary perfusate was increased when compared with CON rats. Prolonging the hyperglycemia for a further 3 or 6 hours (at which point glucose levels were >20 mmol/L) maintained this elevated peak (Figure 3) and total (for 13+3 rats, 1431±82 nmol · mL⁻¹ · 10 min⁻¹; for 13+6 rats, 1941±121 nmol · mL⁻¹ · 10 min⁻¹) enzyme activity at the coronary lumen. At both 3 and 6 hours, D100 animals did not show either the immediate peak LPL release into the interstitial transudate (as seen in D55 animals) or the gradual increase in enzyme release (as observed in CON or fasted [FAST] rats). Indeed, release of enzyme into the interstitial fluid was low throughout the entire perfusion (data not shown).

**TABLE 2. LPL Activity, Mass, and Specific Activity in Coronary Perfusate From Hearts Isolated From 2-Week CON and D55 Rats**

<table>
<thead>
<tr>
<th>Rats</th>
<th>Activity, mU</th>
<th>Mass, ng</th>
<th>Specific Activity, mU/ng</th>
</tr>
</thead>
<tbody>
<tr>
<td>CON</td>
<td>4.97±0.54</td>
<td>5.7±1.0</td>
<td>0.87±0.15</td>
</tr>
<tr>
<td>Diabetic</td>
<td>21.24±1.99*</td>
<td>22.3±5.3*</td>
<td>0.95±0.05</td>
</tr>
</tbody>
</table>

*Significantly different from CON, P<0.05.

**Measurement of LPL catalytic activity by in vitro hydrolysis of [³H]triolein and mass by ELISA were done in pooled coronary perfusate samples (24 mL) collected between 1 and 3 minutes after heparin perfusion.** For ELISA, coronary perfusate samples were lyophilized and reconstituted in 0.2 mL water. Aliquots (100 μL) of samples were then diluted in PBS and added to the microtiter wells coated with 100 μL of anti-LPL antibody. One mU is defined as the amount of enzyme catalyzing the release of 1 nmol oleate per minute. Results are mean±SEM of 6 to 8 control and diabetic rats.
Insulin Withdrawal Study

Treatment of D100 rats for 1 week with long-acting insulin resulted in an increase in body weight and a normalization of fluid intake (Figure 4), plasma glucose, and insulin levels (Figure 5). Subsequent withdrawal of insulin produced an increase in plasma glucose from 24 hours after the last injection. On reaching a glucose concentration of 13 mmol/L, STZ animals were kept for a further 6 or 24 hours before they were euthanized. At termination, both the 6- and 24-hour STZ groups were hyperglycemic and hypoinsulinemic (Figure 5). As observed with the insulin depletion study, even a brief duration of 6 hours of hyperglycemia produced an increase in peak heparin-releasable LPL activity in the coronary perfusate, which remained elevated after 24 hours of hyperglycemia (Figure 6). Total LPL activity was similarly high at these time points (for CON, 554±233; for the D100+I group, 458±86; for D100+I(6) rats, 1518±229; and for D100+I(24) rats, 1516±304; all values in nanomoles per milliliter per 10 minutes). To examine whether the enhanced coronary LPL activity at 24 hours was accompanied by a parallel increase in myocyte LPL, isolated myocytes were incubated in the presence of heparin to measure both surface-bound and secreted LPL. There was a significant reduction in heparin-releasable LPL from cardiac myocytes (Figure 6, inset) and a decrease in LPL activity in cell sonicates (for CON, 2000±125 nmol · 10^6 cells^-1 · h^-1; for the DIA group, 1200±75 nmol · 10^6 cells^-1 · h^-1) from 24-hour-hyperglycemic rats compared with CON. As with insulin depletion, release of enzyme into the interstitial fluid was low throughout the entire perfusion (data not shown).

Fasting

Fasting is known to increase cardiac heparin-releasable LPL activity without affecting mRNA levels or protein synthesis. In the present study, overnight fasting for 16 hours reduced plasma insulin to STZ levels (CON, 2.1±0.13 ng/mL; FAST, 0.5±0.03 ng/mL), with no effect on plasma glucose (CON, 7.0±0.1 mmol/L; FAST, 6.04±0.13 mmol/L). Fasting caused a 2-fold increase in heparin-releasable LPL activity at the coronary lumen (Figure 7A). However, unlike in acute diabetes, overnight fasting had no effect on the release of enzyme into the interstitial fluid (Figure 7B).

Discussion

Recently, using the conventional Langendorff perfused heart, we determined that peak heparin-releasable LPL activity was
augmented in the diabetic rat. This rapidly releasable LPL pool was believed to represent the endothelium-bound enzyme fraction. However, heparin can diffuse through the arterial wall. Thus, the conventional Langendorff heparin-perfused heart may release not only LPL bound to the luminal side of the endothelium but also enzyme present within the endothelial cells and at the subendothelial, interstitial, and myocyte surfaces. Indeed, with use of a modified Langendorff heart perfusion that separates coronary from interstitial fluid, heparin was found to release LPL from both compartments. In the present study, we used the modified Langendorff perfused heart to establish that in diabetes, the increase in LPL activity or protein originates mainly from the luminal surfaces of capillary blood vessels. Immunohistochemical studies of LPL confirmed this finding and verified that despite widespread labeling of the enzyme within CON and diabetic myocytes, there was a convincing increase in LPL immunoreactivity in the capillary blood vessels of the diabetic heart.

In the heart, capillary endothelial LPL is largely derived from cardiac myocytes that synthesize and continuously secrete LPL. Although we had hypothesized that the enhanced heparin-releasable LPL activity in diabetic rat hearts could be due to an increased synthesis of the enzyme, myocyte LPL in these animals was found to be dramatically reduced. In the present study, on continuous perfusion of CON hearts with heparin, there was a progressive increase in the discharge of LPL into the interstitial fluid, indicating that heparin could conceivably cross the capillary wall and release the enzyme from the extracellular space and myocyte surfaces. Interestingly, in diabetic hearts, there was a peak release of LPL into the interstitial fluid within 1 to 2 minutes after heparin perfusion, implying an accumulation of the enzyme at or near the endothelial cell, on the abluminal side. Moreover, release of LPL into the interstitial fluid of the diabetic hearts was depressed after prolonged heparin perfusion, an observation that is congruent with the previously reported reduction in myocyte LPL activity.

Insulin regulates LPL, but insulin’s effects vary, depending on the tissue being investigated. Thus, elevated levels of insulin in vivo (either postprandial or after a euglycemic clamp) or in vitro increase LPL activity in adipose tissue. In the heart, administration of insulin in vivo to control rats increases heparin-releasable LPL activity in isolated cardiac myocytes within 1 hour. However, incubation of control myocytes with insulin in vitro has no effect on LPL activity, indicating that in the heart, additional metabolic factors must be required for the regulation of LPL. Given these observations, a predicted outcome of insulin deficiency would be an attenuated LPL activity in cardiac cells. Indeed,
in this study, a brief duration of hyperglycemia (24 hours) reduced both heparin-releasable and intracellular LPL activity in cardiac myocytes. However, as demonstrated previously in 2-week-diabetic rats, even short-term diabetes (within hours) increased capillary luminal LPL stores. These results indicate that in the heart, an acute reduction in insulin has distinct and immediate regulatory effects on LPL at 2 levels: a decreased synthesis of LPL at the myocyte and an augmented association of LPL with the luminal surface of capillary endothelial cells.

Hypoinsulinemia can also be induced by fasting, which enhances but reduces LPL activity in the heart and adipose tissues, respectively. The fasting-induced changes in cardiac LPL activity were suggested to be posttranslational and did not involve altered mRNA levels, protein synthesis, or specific activity of the protein. As previously reported with the modified Langendorff perfused heart, the fasting-induced increase in heparin-releasable LPL activity occurred mainly at the coronary lumen. With the use of immunogold staining, other studies have also reported that the primary effect of fasting on the distribution of LPL occurred at the surface of endothelial luminal processes. Interestingly, enzyme activity in the interstitial fluid of fasted rats remained unchanged and is consistent with the finding that fasting does not influence myocyte LPL activity. Because the degree of hypoinsulinemia was comparable between fasted and diabetic rats, it appears that although hypoinsulinemia alone can enhance endothelial LPL activity, it may not entirely influence the synthesis of LPL. Hence, other short-term, diabetes-related factors may be necessary for a reduction in myocyte LPL production.

At present, the mechanism(s) by which insulin regulates LPL at the vascular endothelial cell is not known. Endothelial LPL is regulated by detachment from its HSPG binding sites into the circulation, followed by hepatic degradation. HSPGs associate with endothelial cells via their core proteins or a glycosylphosphatidylinositol linkage, and cleavage of the glycosylphosphatidylinositol anchor by insulin-sensitive phospholipases could release HSPGs and hence, LPL. Provided that this mechanism operates in vivo in the heart, hypoinsulinemia would enable the enzyme to remain attached to its endothelial binding site. In perfused guinea pig hearts, LPL moves from its site of synthesis in the parenchymal cells to the endothelial surface within 30 minutes. Thus, the enhanced capillary LPL pool in diabetic rats could involve an accelerated translocation of LPL from myocytes to the lumen. It should be noted that the augmented endothelial LPL could conceivably be derived from the circulation. However, we have reported that when the heparin-releasable LPL pool was allowed to recover for 1 hour after removal of the enzyme, diabetic hearts continued to demonstrate a higher peak LPL activity after a second heparin perfusion.
the amount of luminal LPL can be regulated by the endothelial cell. This process involves the internalization and recycling of LPL to the cell surface, thereby allowing endothelial cells to maintain an additional pool of the enzyme at the vascular endothelium.\textsuperscript{41} Alterations in pH can bring about dissociation of LPL from its binding site, with less detachment of cell surface–bound LPL at pH 5.5 compared with pH 7.4 and 8.5.\textsuperscript{40} Hence, the assumption is that inside the endothelial cell, an acidic pH would permit LPL to remain bound to proteoglycans and thus promote recycling of internalized LPL.\textsuperscript{40} Because diabetes results in an altered ability to regulate pH,\textsuperscript{42} it is possible that changes in pH within endothelial cells may augment this auxiliary pool of LPL.

In summary, capillary-bound and myocyte LPL are distinctly regulated during diabetes. Hence, unlike in the myocyte, acute hypoinsulinemia augments LPL in capillaries, presumably within or at the luminal and abluminal surfaces of the endothelial cell. Regulation at this location is essential, because it permits a rapid response to an acute demand for FFAs in the absence of glucose utilization. A caveat is that this abnormally high capillary LPL activity could provide excess FFAs to the diabetic heart, leading to a number of metabolic and morphological changes and eventually to cardiac disease.\textsuperscript{43,44} Indeed, in transgenic mouse lines overexpressing human LPL in skeletal and cardiac muscle, elevated FFA uptake induced a severe myopathy, characterized by muscle fiber degeneration, and extensive proliferation of mitochondria and peroxisomes.\textsuperscript{45} An oversupply of FFAs causes these cellular organelles to overproduce reactive oxygen species that can potentially contribute to the formation of lipid peroxidation products.\textsuperscript{46} Lipolysis products have also been shown to enhance endothelial permeability.\textsuperscript{47,48} Whereas enhanced FFA metabolism in the heart can inhibit glucose oxidation,\textsuperscript{49} the regulatory mechanisms governing changes in cardiac capillary–bound LPL in response to acute hypoinsulinemia are currently being investigated.

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