Metabolism of VLDL is increased in streptozotocin-induced diabetic rat hearts

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Sambandam, Nandakumar, Mohammed A. Abrahani, Scott Craig, Osama Al-Atar, Esther J eon, and Brian Rodrigues. Metabolism of VLDL is increased in streptozotocin-induced diabetic rat hearts. Am J Physiol Heart Circ Physiol 278: H1874–H1882, 2000.—In streptozotocin (STZ)-induced diabetic rats, we previously showed an increased heparin-releasable (luminal) lipoprotein lipase (LPL) activity from perfused hearts. To study the effect of this enlarged LPL pool on triglyceride (TG)-rich lipoproteins, we examined the metabolism of very-low-density lipoprotein (VLDL) perfused through control and diabetic hearts. Diabetic rats had elevated TG levels compared with control. However, fasting for 16 h abolished this difference. When the plasma lipoprotein fraction of density <1.006 g/ml from fasted control and diabetic rats was incubated in vitro with purified bovine or rat LPL, VLDL from diabetic animals was hydrolyzed as proficiently as VLDL from control animals. Post-heparin plasma lipolytic activity was comparable in control and diabetic animals. However, perfusion of control and diabetic rats with heparin indicated that diabetic hearts had larger amounts of LPL bound to heparan sulfate proteoglycan-binding sites. [3H]VLDL obtained from control rats, when recirculated through the isolated heart, disappeared at a significantly faster rate from diabetic than from control rat hearts. This increased VLDL-TG hydrolysis was essentially abolished by prior perfusion of the diabetic heart with heparin, implicating LPL in this process. These findings suggest that the enlarged LPL pool in the diabetic heart is present at a functionally relevant location (at the capillary lumen) and is capable of hydrolyzing VLDL. This could increase the delivery of free fatty acid to the heart, and the resultant metabolic changes could induce the subsequent cardiomyopathy that is observed in the chronic diabetic rat.

Lipoprotein lipase; diabetes; very-low-density lipoprotein

CLINICAL AND EXPERIMENTAL studies have established that chronic diabetes mellitus can negatively alter myocardial function independent of vascular defects (14, 33). Although several etiological factors have been put forward to explain the development of diabetic cardiomyopathy, increasing evidence suggests that alterations in fuel supply and utilization by the heart may be the initiating factor for the development of this cardiac dysfunction (31, 34). Under normal aerobic conditions, cellular energy in the form of ATP is obtained via the oxidation of various substrates, with free fatty acids (FFAs) being the preferred energy substrate used by the heart muscle. In diabetes, because of inadequate glucose transport and oxidation, energy production in the heart is almost entirely via β-oxidation of FFA, a process that may have deleterious effects on myocardial function (31, 34).

Lipoprotein lipase (LPL) is an enzyme that is widely distributed in a variety of tissues (4, 11). In the adult heart, LPL is produced in myocytes, and the active dimeric enzyme is then translocated onto heparan sulfate proteoglycan (HSPG)-binding sites on the luminal surface of the capillary endothelium (5, 24). At this location, “functional” LPL plays a crucial role in the hydrolysis of triglyceride (TG)-rich lipoproteins [chylomicrons and very-low-density lipoprotein (VLDL)] to FFAs that are transported to the heart and used for energy production or the resynthesis of TG.

LPL localized at the capillary lumen is more sensitive to altered physiological and pathological states than is the total cellular pool (10, 40). Using conventional Langendorff retrograde perfusion of the heart with heparin, we reported significantly elevated LPL activity in streptozotocin (STZ)-induced diabetic rats after 2 or 12 wk of hypoinsulinemia (30). Inasmuch as heparin has been demonstrated to cross the endothelial barrier (20), we were uncertain as to whether this augmented LPL was bound exclusively to the luminal side of the capillary endothelium or was released from the abluminal surface, the interstitial space, and the myocyte cell surface. Recently, using a modified Langendorff isolated perfused heart (that separates the coronary from the interstitial effluent), we reported that the augmented LPL in the diabetic heart most likely represented the pool of enzyme at the coronary lumen, exclusive of the abluminal, interstitial, and myocyte pools (38). In all the above-mentioned studies (30, 38), LPL activity (determined by the in vitro hydrolysis of a synthetic substrate, triolein) and mass were measured subsequent to the release by heparin. However, HSPG-bound LPL exhibits a disparate rate of lipolysis compared with conventional lipolysis assays with LPL in solution (9). Hence, the present study was designed to investigate whether this enlarged LPL pool in the diabetic heart is metabolically relevant with regard to its efficiency in hydrolyzing native lipoproteins and, thereby, providing potentially toxic FFA to the heart.
MATERIALS AND 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. Male Wistar rats (280–290 g) were obtained from The University of British Columbia Animal Care Unit (Vancouver, BC). The rats were maintained under a 12:12-h light-dark cycle (lights on from 7 AM to 7 PM) 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 (35). A dose-dependent increase in severity of diabetes is produced by 25–100 mg/kg STZ. After an injection of STZ (55 mg/kg iv), stable hyperglycemia develops within 24–48 h and remains two to three times higher than normal, in concert with a ~50% reduction in plasma insulin levels. Although these animals are insulin deficient, they do not require insulin supplementation for survival and do not develop ketoacidosis. Rats were randomly divided into nondiabetic (control) and diabetic groups. Halothane-anesthetized rats were injected with STZ (55 mg/kg iv; Sigma Chemical) or an equivalent volume (1 ml/kg) of saline. Glicosuria was determined 24 h after STZ injection, and hyperglycemia was tested at 48 h via glucometer. All STZ-treated rats displayed glycosuria (=4) and hyperglycemia (~13 mmol/l). Diabetic rats were kept for 2 wk after the STZ injection, at which time they were euthanized and the hearts were removed.

Release of HSPG-bound LPL with heparinase. To reinforce our recent observation that the increase in cardiac LPL in the diabetic heart occurs specifically at its HSPG-binding site, we used the nonrecirculating Langendorff isolated heart perfusion technique with heparin (5 U/ml) or heparinase (10 U/ml). The reaction was stopped by addition of precooled 0.3 M Na2PO4 (pH 6.9), and the tubes were immediately immersed in ice.

In vitro lipolysis of VLDL by bovine and rat LPL. In vitro lipolysis of VLDL-TG was carried out on the basis of previously described methods (12, 37). Briefly, VLDL was isolated from control rat serum. To determine the optimal incubation conditions to study lipolysis of VLDL by LPL, TG in control VLDL was adjusted to various concentrations (0.05–0.35 mM) and incubated with bovine LPL (bLPL; 20–100 mU; Sigma Chemical) at 37°C for 30 min. VLDL and bLPL stock solutions were diluted with Tris buffer (pH 8.1) containing 0.5% essentially FFA-free BSA. From these experiments, it was determined that 0.3 mM VLDL-TG was a saturable concentration of substrate when incubated with 20 mU of bLPL (data not shown).

In vivo radiolabeling of VLDL. VLDL-TG was labeled in vivo with [3H]oleic acid by a previously described method (27). [3H]Oleic acid was complexed to BSA as follows: 20 µl (100 µCi) of [3H]oleic acid were mixed with 200 µl of 10% NaHCO3 and 25% BSA in distilled water was then added to produce an albumin-fat acid complex (23). The [3H]oleic acid-BSA complex was then injected intravenously (via the tail vein) into anesthetized control rats that were fasted for 16 h (6 PM – 10 AM). At 40 min after injection of [3H]oleic acid, rats were euthanized, blood was withdrawn from the inferior vena cava, and serum was subjected to ultracentrifugation to isolate [3H]VLDL. Incorporation of the radiolabel into VLDL-TG was confirmed by TLC analysis on the chloroform-methanol extract (2:1, vol/vol) with silica gel G plates and hexane-diethyl ether-methanol-acetic acid (90:20:3:2, vol/vol/vol/vol) solvent system. The spot corresponding to standard triolein (Sigma Chemical) was scraped off, counted for radioactivity, and compared with an equivalent volume of chloroform-methanol extract of VLDL. More than 97% of the VLDL-lipoprotein radioactivity was found in the TG moiety. [3H]VLDL obtained from six rats was pooled to perfuse one heart.

Langendorff isolated heart perfusion. To quantitate the magnitude of lipoprotein hydrolysis, a Langendorff retrograde perfusion technique was used to perfuse control and diabetic hearts with VLDL. The perfusate solution was made up of 1.25% albumin-fatty acid complex, 10.220.33 mg of [3H]oleic acid were mixed with 200 µl of 10% NaHCO3, and the tubes were immediately immersed in ice. From this reaction mixture, 50 µl were pipetted in triplicate to measure FFA released by hydrolysis of VLDL-TG with an NEFA-C kit (Wako Chemicals). The results were corrected for background with an unreacted blank containing various concentrations of VLDL-TG and an equivalent volume of BSA containing Tris buffer instead of bLPL. From these experiments, we determined that 0.3 mM VLDL-TG was a saturable concentration of substrate when incubated with 20 mU of bLPL (data not shown).

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diabetic hearts with radiolabeled VLDL. Rats were anesthe-
tized with pentobarbital sodium (50 mg/kg ip), the thoracic
cavity was opened, and the hearts were carefully excised.
Rats were not injected with heparin before they were 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-Henseleit 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 per-
fused retrogradely by the nonrecirculating Langendorff tech-
nique. At this time, most of the perfusate starts flowing
through the pulmonary artery and drips down to the apex of
the heart. 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 circulat-
ing water bath. A peristaltic pump controlled the rate of
coronary flow (7–8 ml/min). To measure hydrolysis of VLDL,
the perfusion solution was changed to Krebs buffer contain-
ing 1% BSA (fraction V, Boehringer-Mannheim) and
[³H]VLDL.

Hydrolysis of VLDL by the isolated heart. Using the recircu-
lating Langendorff retrograde perfusion (26), we compared
the rate of TG-rich lipoprotein clearance in control and
diabetic hearts. Twenty milliliters of Krebs-Henseleit perfu-
sion buffer (pH 7.4) were prepared containing 0.3 mM
[³H]VLDL-TG and 0.5% BSA. The perfusion apparatus was a
spacially designed closed recircurating system containing a
double-jacketed reservoir, heart chamber, coil condenser,
bubble trap, and injection port to collect samples of perfusate.
This miniature system permitted the use of small perfusion
volumes and diminished the loss of VLDL due to nonspecific
attachment (<10%, appropriate corrections were made for this
loss). After the heart was secured through the aorta, the basic
buffer was perfused for 3 min in a nonrecirculating mode to clear blood from the coronary blood vessels. Then
buffer containing [³H]VLDL was perfused through control
and diabetic hearts for 90 min at 5 ml/min in the recirculating
mode. Samples were collected through the injection port from
0 (just before perfusion through the heart) to 90 min, placed
on ice, and immediately extracted for TG (2). A decline in
perfuse TG radioactivity was used as a measure of VLDL-TG
clearance.

Post-heparin plasma lipolytic activity. At 2 wk after STZ,
plasma LPL activity in the basal (nonfasted) state and in
response to a heparin injection was determined in control and
diabetic rats. Heparin (0.5 U/g) was injected into the tail vein
of lightly anesthetized (pentobarbital sodium, 20 mg/kg ip)
rats, and blood samples were collected at 5 min. Plasma was
separated and stored at −70°C until assayed for LPL activity.
Total lipase (hepatic lipase + LPL) activity was determined
by measuring the in vitro hydrolysis of a sonicated [³H]tri-
olein substrate emulsion. The standard assay medium (400
µl) contained 0.6 mM glycerol tri[9,10-³H]oleate (1 mCi/mmol;
1 Ci = 37 GBq), 25 mM PIPES (pH 7.5), 0.05% (wt/vol) BSA,
50 mM MgCl₂, 10% (vol/vol) heat-inactivated chicken serum
(containing apolipoprotein C II), and 5 µl of plasma sample.
The release of [³H]oleate was measured after a 30-min
incubation period at 30°C. The presence of hydrolyzed [³H]ole-
ate in the medium was determined by addition of 3 ml of a
fatty acid extraction solution (30) and 100 µl of 0.1 M NaOH.
After vortex mixing and centrifugation, [³H]isodium oleate
in 0.5 ml of the upper phase was counted. In addition, hepatic
lipase activity was measured by incubating plasma with 1
M NaCl and conducting the assay in the absence of apoli-
poprotein C II to suppress LPL activity (13). Plasma LPL
was calculated as the difference between total and hepatic
lipase activity and is expressed in milliunits, which cor-
responds to 1 nmol of fatty acid released per minute. All LPL
assays were performed in duplicate in conditions where the
reaction rate was linear with respect to time and the volume
assayed.

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 RIA kit (Linco Research, St. Louis, MO).

Materials. [³H]oleic acid and [³H]triolein were purchased
from Amersham. Heparin sodium injection (Hapalean, 1,000
USP U/ml) was obtained from Oraganon Teknika. All other
chemicals were obtained from Sigma Chemical.

Statistical analysis. Values are means ± SE unless other-
wise 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 TG-rich
lipoprotein clearance over time were analyzed with a multivar-
iate ANOVA followed by the Newman-Keuls test with the
Number Cruncher statistical system. The level of statistical
significance was set at P < 0.05.

RESULTS

General characteristics. Induction of diabetes with STZ (55 mg/kg) resulted in glycosuria. Body weight
gain over 2 wk was reduced in diabetic animals relative to
controls. The STZ injection caused a reduction in plasma insulin levels that was accompanied by hyper-
glycemia (Table 1).

Plasma lipid profile and apolipoprotein analysis. In fed control rats, 75% of total TG was present in the
VLDL/chylomicron fraction whereas the remaining 25% of TG was present in low- and high-density lipoprotein
fractions. VLDL contributed the least to the total cholesterol pool, which was present predominantly in the
high-density lipoprotein (61% of total cholesterol) and low-density lipoprotein (30%) fractions (data not
shown). Figure 1 shows the distribution of TG and

Table 1. Characteristics of diabetes at 2 wk
after STZ injection

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>STZ Injected</th>
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<tbody>
<tr>
<td>Body wt, g</td>
<td>315 ± 5</td>
<td>289 ± 3*</td>
</tr>
<tr>
<td></td>
<td>(10)</td>
<td>(10)</td>
</tr>
<tr>
<td>Plasma insulin, ng/ml</td>
<td>2.04 ± 0.30</td>
<td>0.69 ± 0.11*</td>
</tr>
<tr>
<td></td>
<td>(10)</td>
<td>(5)</td>
</tr>
<tr>
<td>Plasma glucose, mmol/l</td>
<td>6 ± 0.3</td>
<td>18 ± 0.9*</td>
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<tr>
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<td>(5)</td>
<td>(2.0)</td>
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Values are means ± SE for number of animals in parentheses. Diabetes was induced by injection of streptozotocin (STZ, 55 mg/kg).
Values are those obtained before euthanasia. Plasma parameters were from fed rats. Blood was collected from tail vein in heparinized
tubes that were centrifuged for separation of plasma. Insulin was measured using rat insulin standards. *Significantly different from
control, P < 0.05.
cholesterol in the plasma lipoprotein fraction of density 1.006 g/ml. TG and cholesterol levels were higher in diabetic than in control animals. However, fasting for 16 h eliminated this increase in TG (0.37 ± 0.04 and 0.35 ± 0.003 mmol/l in control and diabetic animals, respectively), suggesting that diabetic hypertriglyceridemia is largely a result of an increase in the concentration of chylomicrons. Apolipoproteins in VLDL obtained from control and diabetic rats were analyzed by gel electrophoresis. There was no apparent difference (as measured by densitometry) in the apolipoprotein profile of VLDLs isolated from the two groups (data not shown).

Post-heparin plasma lipolytic activity. Inasmuch as LPL is the rate-limiting enzyme in TG clearance, a decrease or dysfunction in LPL activity often parallels a reduced catabolism of TG-rich lipoproteins that results in hypertriglyceridemia. To test whether the elevated plasma TG in diabetic rats result from a decrease in LPL activity, basal (nonstimulated, non-fasted) and post-heparin plasma was obtained from control and diabetic rats at 2 wk after diabetes induction. Basal and post-heparin plasma LPL activity was not adversely affected at 2 wk after diabetes induction (Fig. 2).

Effect of heparinase treatment. Retrograde perfusion of control hearts with heparin resulted in the release of LPL into the coronary perfusate (Fig. 3, inset). The heparin-induced LPL discharge was rapid, and peak activity, suggested to represent LPL located at or near the endothelial surface, was observed within 2 min. In contrast to the heparin discharge profile, release of LPL from control hearts with heparinase was slow and protracted (Fig. 3, inset). Comparison of the amount of LPL detached from HSPG-binding sites on 30 min of perfusion with heparinase illustrated that the amount of enzyme released from diabetic hearts was almost twofold higher than from control hearts. Thus the majority of augmented LPL in the diabetic heart is most likely bound to HSPGs.

In vitro hydrolysis of VLDL by bLPL and rLPL. Under our experimental conditions, FFA released due to lipolysis of VLDL by bLPL was rapid and could be detected within 1 min. Moreover, the rate of lipolysis was linear up to 60 min before it reached a maximum (data not shown). A double reciprocal plot of 1/V vs. 1/[S] (where V is rate of lipolysis and [S] is VLDL-TG concentration) is shown in Fig. 4. The intercept on the y-axis gives the maximum velocity of LPL activity ($V_{\text{max}}$), and the x-axis intercept gives the substrate (VLDL-TG) concentration at half-maximal velocity ($K_m$) for this lipolytic reaction. The apparent $K_m$ for control and diabetic VLDL was 0.15 and 0.26 mM, respectively.
whereas the $V_{\text{max}}$ was 20.4 and 31.3 mU of FFA released for control and diabetic VLDL, respectively, suggesting that diabetic VLDL was hydrolyzed as effectively as VLDL obtained from control animals.

Given the possibility that the interaction between VLDL and rLPL could be different from the interaction between VLDL and bLPL, we purified LPL from control and diabetic post-heparin plasma. Figure 5 (inset) is a representative elution profile of LPL and hepatic lipase (another major lipase released into plasma in response to intravenous heparin injection), indicating that hepatic lipase elutes at ~0.75 M NaCl while LPL elutes at 1.5 M NaCl. Fractions enriched with control and diabetic LPL (>90%) were adjusted to have equivalent activities and subsequently used for in vitro lipolysis of VLDL obtained from control and diabetic rats. Our results indicate that there is no decrease in lipolysis when diabetic VLDL was exposed to control and diabetic LPL (Fig. 5), suggesting that, at this duration of diabetes, there is no change in LPL or VLDL characteristics with respect to their enzyme-substrate interaction.

VLDL clearance by isolated control and diabetic hearts. We next attempted to assess whether the diabetic heart could clear [3H]VLDL faster than control hearts. Having established that control and diabetic VLDL were essentially similar, control and diabetic rats were perfused only with control [3H]VLDL. Figure 6 compares the rate of [3H]VLDL clearance from recirculating buffer perfused retrogradely through control and 2-wk diabetic hearts. Metabolism of [3H]VLDL by the isolated control heart was slow (net loss 17%, rate $0.50 \pm 0.17 \text{ nmol/min}$) but comparable to previously reported values (26). In contrast to control rat hearts, the rate of disappearance of VLDL-TG (over a perfusion period of 90 min) was more rapid from diabetic rat hearts (net loss 44%, rate $0.93 \pm 0.23 \text{ nmol/min}$). It has been suggested that LPL can detach from the endothelium during lipolysis (42). To determine whether LPL can be released by recirculating VLDL, enzyme activity was also determined in the buffer chamber after 30 min. Perfusion of control hearts for 90 min with unlabelled VLDL did not result in detachment of LPL from its binding sites (159 and 120 nmol·ml$^{-1}·h^{-1}$ for LPL activity in buffer without and with VLDL, respectively). The increased VLDL-TG hydrolysis was essentially abolished by prior perfusion of the diabetic heart with heparin, implicating LPL in this process (net loss of VLDL-TG in a heparin-preperfused diabetic heart at 10.2 ± 0.3 on June 29, 2017 http://ajpheart.physiology.org/ Downloaded from by 10.220.33.1
the end of 90 min was 16%). In all the above groups, heart rate remained unchanged (165 ± 8 and 167 ± 5 beats/min for control and diabetic rats, respectively) throughout the perfusion period.

**DISCUSSION**

We previously reported that peak heparin-releasable LPL activity (believed to represent the endothelium-bound enzyme fraction) was augmented in the isolated perfused diabetic rat heart (30). However, inasmuch as heparin can diffuse through the arterial wall (20), it could release LPL present within the endothelial cell and at the subendothelial, interstitial, and myocyte cell surfaces in addition to the enzyme bound to the luminal side of the endothelium. Recently, using a modified Langendorff heart perfusion (that separates coronary from interstitial fluid) and immunohistochemistry, we established that in diabetes the increase in LPL activity or protein originates mainly from the luminal surface of capillary blood vessels (38). Capillary luminal LPL can be regulated by way of translocation from the myocyte or through a process that involves internalization and recycling of LPL within the vascular endothelial cell (39). Given the latter, we were still undecided as to whether this augmented capillary LPL was located inside endothelial cells or was attached to binding sites in the lumen. In the present study we extend our earlier findings and report that inasmuch as heparinase was capable of releasing a two- to threefold greater quantity of LPL from diabetic than from control rat hearts, the augmented LPL is likely bound to HSPG-binding sites in the coronary lumen.

Hypertriglyceridemia is a common metabolic disorder associated with STZ-induced diabetes (32). In an insulin-deficient state, excessive circulating TG could be a consequence of increased production of lipoproteins or a reduced clearance of TG from the blood (due to a depleted post-heparin plasma lipolytic pool of LPL). However, TG secretion rates in control and

**Fig. 5.** In vitro lipolysis of control (A) and diabetic (B) VLDL-TG (0.3 mM) by rat LPL. Inset: fast-protein liquid chromatography elution profile of LPL and hepatic lipase (HL) from heparin-Sepharose affinity columns. LPL elutes at higher salt concentrations (~1.4 M NaCl) and hepatic lipase at ~0.8 M NaCl. LPL activity in each fraction was determined as difference between total lipase and hepatic lipase activities. Hepatic lipase activity was measured by inhibiting LPL in presence of 1 M NaCl and withholding apolipoprotein C II from assay. Fast-protein liquid chromatography fractions enriched in LPL (>90%) were adjusted to 20 nU before incubation with lipoproteins (0.3 mM TG). Values are averages of 2 separate experiments carried out in triplicate.

**Fig. 6.** In vitro lipolysis of [3H]VLDL (obtained from control rats) by isolated perfused hearts from control and diabetic animals. Recirculating Langendorff retrograde perfusion procedure was used to perfuse hearts (for 90 min) with Krebs buffer containing [3H]VLDL. Samples were collected at indicated times, placed on ice, and extracted for TG, and radioactivity was counted after addition of aqueous counting scintillant. A decline in perfusate TG radioactivity was used as a measure of VLDL-TG clearance. Values are means ± SE for 4 rats in each group. *Significantly different from control, P < 0.05.
diabetic rats have been reported to be comparable (8, 15), and post-heparin plasma lipolytic activity in this and other (7) studies was not significantly reduced in diabetic rats. The possibility remained that changes in structure of lipoproteins could explain this hypertriglyceridemia. Several in vitro and in vivo studies in clinical and experimental diabetes have shown that VLDL and chylomicrons undergo changes in structure and composition such that they may become poorer substrates for LPL (1, 15, 19, 21, 25). If VLDLs and chylomicrons are altered during diabetes, another question of overriding importance is whether the diabetic heart (with its augmented LPL activity) is, in fact, capable of metabolizing these TG-rich lipoproteins. In the present study we were unable to detect any difference in the composition of apolipoproteins between control and diabetic VLDL. More importantly, no significant difference in lipolysis rates or in the characteristics of LPL substrate kinetics (Km and Vmax) was observed between control and diabetic rats. Thus VLDL prepared from diabetic rats appears to be a normal substrate for LPL, and impaired lipolysis of these particles may not be a causal factor toward the development of hypertriglyceridemia.

Inasmuch as diabetic rats in the present study were hypertriglyceridemic in the fed but not the fasted state, it is likely that excessive accumulation of TG occurs as a result of changes in the characteristics of chylomicrons, the other major TG-containing lipoprotein. Mamo et al. (21) demonstrated that lipoproteins (plasma lipoprotein fraction of density <1.006 g/ml that includes chylomicrons and VLDL) of diabetic origin are hydrolyzed by LPL at a significantly slower rate than lipoproteins from normal rats. However, inasmuch as fasting for 16 h before recovery of lipoproteins eliminated this difference in rates of lipolysis, the authors concluded that during diabetes the lipolytic defect might be specific for chylomicrons. Interestingly, [14C]chylomicrons obtained from STZ-induced diabetic rats, when intravenously injected into control animals, disappeared from the circulation at a significantly slower rate than similarly prepared chylomicrons injected into control animals (19). The question of whether hearts from diabetic animals in our study are able to clear chylomicrons efficiently remains to be answered. However, an earlier study has reported that, despite poor peripheral clearance of chylomicrons, uptake of this lipoprotein is threefold higher in cardiac tissue from diabetic than from nondiabetic rats (19).

When radiolabeled VLDL-TG were perfused through the isolated heart and the decline in TG radioactivity was monitored with time, diabetic hearts metabolized VLDL at a faster rate than control rat hearts. This increased VLDL-TG clearance from the diabetic heart is in agreement with the enhanced LPL activity and mass reported previously (30, 38). Inasmuch as prior removal of LPL by perfusion with heparin prevented this increased clearance of VLDL, this argued against a receptor-mediated removal of this lipoprotein by the diabetic heart (41). Moreover, the increased clearance of VLDL from the diabetic heart was not a result of an increased lipolysis in the buffer chamber due to detachment of LPL. We (36) and others (9) demonstrated that VLDL does not result in detachment of LPL from its HSPG complex.

In conclusion, our results demonstrate that, during diabetes, rapid removal of VLDL from the diabetic heart could be one mechanism for the provision of FFAs for energy production, to compensate for the diminished contribution of glucose as an energy source. In an insulin-deficient state, adipose tissue lipolysis is enhanced, resulting in elevated circulating FFAs (29). In addition, an increased activity of myocardial enzymes that catalyze the synthesis of TG promotes the accumulation of intracellular TG stores during diabetes (22). Subsequent hydrolysis of this augmented TG store could also lead to high tissue FFA levels (17). In view of these mechanisms that enhance cardiac FFA levels, the relative significance of cardiac LPL activity in delivering FFA to the diabetic heart is unknown. Interestingly, when the uptake patterns of albumin-bound and VLDL-TG-derived FFA were compared in human placental cells (macrophages and trophoblasts), the uptake of

Fig. 7. Regulation of LPL in heart. After synthesis and activation of LPL in cardiac myocyte, enzyme is translocated onto heparan sulfate proteoglycan (HSPG)-binding sites on luminal surface of endothelial cells. At this location, LPL actively metabolizes TG core of chylomicrons and VLDL to FFAs, which are then transported to heart for numerous metabolic and structural tasks. Overall, our findings suggest that enlarged LPL pool in diabetic heart is present at a functionally relevant location (at capillary lumen) and is capable of hydrolyzing VLDL. This could increase delivery of FFA to heart to compensate for diminished contribution of glucose as an energy source. A caveat is that this abnormally high capillary endothelial LPL activity could provide excess FFA to diabetic heart, leading to a number of metabolic, morphological, and mechanical changes and, eventually, to cardiac disease (31, 34). G-6-P, glucose 6-phosphate; Chy, chylomicon; PDH, pyruvate dehydrogenase.
albumin-bound FFA after 24 h was 4–6% of the initial amount contained in the incubation medium. In contrast, when these cells were incubated with labeled VLDL-TG, cellular TG-FFA content was manyfold higher. Thus, at least in some cell types, TG-rich lipoproteins may be a more efficient means to deliver FFA than are albumin-bound FFAs, and this emphasizes the importance of LPL in the supply of FFA to the heart (3, 18). A caveat is that this abnormally high capillary endothelial LPL activity could provide excess FFA to the diabetic heart, leading to a number of metabolic, morphological, and mechanical changes and, eventually, to cardiac disease (31, 34) (Fig. 7).

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