Metabolism of very-low-density lipoprotein and chylomicrons by streptozotocin-induced diabetic rat heart: effects of diabetes and lipoprotein preference

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Niu YG, Evans RD. Metabolism of very-low-density lipoprotein and chylomicrons by streptozotocin-induced diabetic rat heart: effects of diabetes and lipoprotein preference. Am J Physiol Endocrinol Metab 295: E1106–E1116, 2008. First published September 9, 2008; doi:10.1152/ajpendo.90260.2008.—Very-low-density lipoprotein (VLDL) and chylomicrons (CM) are major sources of fatty acid supply to the heart, but little is known about their metabolism in diabetic myocardium. To investigate this, working hearts isolated from control rats and diabetic rats 2 wk following streptozotocin (STZ) injection were perfused with control and diabetic lipoproteins. Analysis of the diabetic lipoproteins showed that both VLDL and CM were altered compared with control lipoproteins; both were smaller and had different apolipoprotein composition. Heparin-releasable lipoprotein lipase (HR-LPL) activity was increased in STZ-induced diabetic hearts, but tissue residual LPL activity was decreased; moreover, diabetic lipoproteins stimulated HR-LPL activity in both diabetic and control hearts. Diabetic hearts oxidized lipoprotein-triacylglycerol (TAG) to a significantly greater extent than controls (>80% compared with deposition as tissue lipid), and the oxidation rate of exogenous lipoprotein-TAG was increased significantly in diabetic hearts regardless of TAG source. Significantly increased intracardiomycyte TAG accumulation was found in diabetic hearts, although cardiac mechanical function was not inhibited, suggesting that lipotoxicity precedes impaired cardiac performance. Glucose oxidation was significantly decreased in diabetic hearts; additionally, however, diabetic lipoproteins decreased glucose oxidation in diabetic and control hearts. These results demonstrate increased TAG-rich lipoprotein metabolism concomitant with decreased glucose oxidation in type 1 diabetic hearts, and the alterations in cardiac lipoprotein metabolism may be due to the properties of diabetic TAG-rich lipoproteins as well as the diabetic state of the myocardium. These changes were not related to cardiomyopathy at this early stage of diabetes.

Very-low-density lipoprotein (VLDL) and chylomicrons (CM) are the two major lipoprotein carriers of triacylglycerol (TAG) in plasma. Together with circulating nonesterified fatty acids (NEFA), they are important substrates for heart to utilize for energy requirements (33, 34, 65). Following hydrolysis by lipoprotein lipase (LPL; EC 3.1.1.34), which is synthesized in the cardiomycyte but translocated to its active site on the luminal surface of capillary endothelium and expressed in large amounts by myocardium, VLDL and CM can potentially release more fatty acids (FAs) than albumin-bound NEFA can provide (2, 60). By using liver perfusion and thoracic duct cannulation techniques to prepare radiolabeled rat VLDL and CM, respectively, we previously investigated the metabolism of these TAG-rich lipoproteins (TGRLP) by the “working” rat heart under physiological conditions (43); both of them are important myocardial substrates capable of maintaining efficient cardiac performance. Therefore, they are believed to be the principal source of FA supply to the heart and play an important role in ~70% of ATP production from circulating lipid in the heart under normal physiological workload conditions. There are two mechanisms for heart to take up TGRLP, LPL-mediated hydrolysis and lipoprotein receptor-mediated particle assimilation. It is possible that different uptake pathways selectively channel these TGRLPs to different metabolic fates, i.e., to oxidation or esterification (34, 43). Besides demonstrating that both are important myocardial substrates (43), our previous work also suggested that the roles of VLDL and CM in cardiac lipid metabolism are different.

In diabetes, inadequate glucose transport and metabolism as the consequence of insulin deficiency (type 1) and resistance (type 2) results in FA oxidation becoming almost the exclusive energy source for hearts (55, 57). This shift of cardiac substrate utilization from glucose to FAs may have deleterious effects on myocardial function. Recent evidence suggests that altered cardiac substrate (particularly lipid) metabolism could result in diabetic cardiomyopathy (1, 4, 32, 47, 54, 57, 68), constituting a cause of heart failure independent of other cardiac risk factors, such as coronary atherosclerosis. Furthermore, plasma TAG concentrations are increased in diabetes (dyslipidemia) (1, 3, 57), partly as a result of increased hepatic VLDL synthesis and secretion (24, 62) and partly due to tissue-specific impaired TAG uptake (59). TGRLP particles are abnormal in composition in diabetes (24, 35, 62), and this may affect their cardiac uptake and subsequent metabolism. However, to date, most studies examining how diabetes alters cardiac metabolism were conducted mostly by perfusing isolated hearts with NEFA (4, 31–33, 50, 55). Although observations demonstrated that free FA oxidation was increased in both type 1 and type 2 diabetic hearts, little is known about cardiac lipoprotein metabolism in diabetes. TGRLPs, especially VLDL, are rarely used to examine metabolism in diabetes, and this has hampered the study of cardiac lipid metabolism in this condition to date (1).

Therefore, the present study was designed to investigate myocardial TAG-rich lipoprotein metabolism in STZ-induced...
diabetic heart. We used rat liver perfusion to prepare species-specific diabetic VLDL containing radiolabeled TAG in sufficient quantities for use in rat heart perfusion experiments. Also, a thoracic duct cannulation technique was used to prepare radiolabeled diabetic rat CM. By perfusing diabetic rat hearts in working mode with these TAG-rich substrates, we investigated different lipoprotein utilization and metabolism by heart in diabetes. The experimental protocol was designed to permit independent evaluation of both the effect of the diabetic state on the heart and on the lipoproteins themselves.

METHODS

The investigation was performed in accordance with the Home Office Guidance On The Operation Of The Animals (Scientific Procedures) Act of 1986 published by Her Majesty’s Stationery Office, London, UK, and conducted under Home Office Project Licence PPL 30/2139. The study was approved by the Home Office following review by the Oxford University Ethics Review committee.

Animals

Male Wistar rats were fed ad libitum on a chow diet [comprising by weight 52% carbohydrate, 21% protein, and 4% fat; the residue was nondigestible material (Special Diet Services, Witham, Essex, UK)], with free access to drinking water, and were maintained at an ambient temperature of 20 ± 2°C with a 12:12-h light-dark cycle (light from 0730).

Diabetic Rat Model

Streptozotocin (STZ), which produces selective necrosis of pancreatic β-cells and results in an insulin deficiency state, was administered to rats to induce type 1 diabetes. STZ was prepared in 100 mM citrate buffer (pH 4.5). Rats at 8 wk of age were randomly divided into diabetic and nondiabetic (control) groups and injected with STZ (60 mg/kg iv) or an equivalent volume (1 ml/kg) of saline. Blood glucose was tested at 48 h by glucometer. All STZ-treated rats displayed hyperglycemia (>13 mmol/l). Rats were kept for 2 wk following injection, at which time they were used for preparation of radiolabeled lipoproteins and working heart perfusions.

Chemicals

[9,10(N)-3H]oleic acid, glycerol tri-[9,10(N)-3H] oleate, and [U-14C] glucose were obtained from Amersham Biosciences (Amersham, Bucks, UK); Waymouth’s medium was purchased from Gibco BRL (Life Technologies, Paisley, UK); and streptozotocin and other biochemicals were obtained from Sigma Chemical (Poole, Dorset, UK).

Experimental Protocol

To investigate the effects of type 1 diabetes on cardiac lipoprotein metabolism, nondiabetic (control) VLDL, diabetic VLDL, nondiabetic (control) CM, and diabetic CM were prepared and used to perfuse nondiabetic (control) and diabetic hearts.

Preparation of Lipid Substrates

Preparation of VLDL. VLDLs containing 3H-labeled triolein were prepared by rat liver perfusion. 3H-labeled sodium oleate (specific activity 485 mCi/mmol) was prebound to FA- and endotoxin-free bovine serum albumin (5% wt/vol) and added to liver perfusate for production of [3H]triolein-VLDL as described (7). Rats (diabetic or control) were anesthetized with intraperitoneal pentobarbitone sodium (60 mg/kg body wt), and the portal vein and thoracic inferior vena cava were rapidly cannulated; the abdominal inferior vena cava was ligated. Heparin was not used. The liver was perfused in situ for 8 h, with a recirculating solution comprising Waymouth’s synthetic tissue culture medium supplemented with amino acids (glutamine, serine, alanine) and glucose. Washed red cells were added to give a final hematocrit of 10% (vol/vol), and the perfusate was gassed with 95% O2-5% CO2 (vol/vol) at 37°C; [3H]oleate (1.0 mM final concentration) preload to FA-free albumin was added to the perfusate prior to liver perfusion and subsequently also infused into the perfusate for the first 4 h of the perfusion to maintain the circulating NEFA concentration at 0.4 mM. After the perfusion, the perfusate was filtered through an ultrafilter, with molecular weight cutoff at 30,000 Da (Amicon, Stonehouse, Gloucestershire, UK), and then ultracentrifuged at 144,500 g to separate the <1.006 g/ml layer. Thin-layer chromatography of radiolabeled VLDL showed that >95% of the 3H label was in the TAG band. VLDLs were suspended in FA-free bovine serum albumin (5% wt/vol), and TAG content was assayed with an enzymatic colorimetric test kit (Randox Laboratories, Crumlin, Antrim, UK). The 3H-labeled VLDL was added to the heart perfuse reservoir to give a final concentration of 0.4 mM TAG.

Preparation of CM. CM containing 3H-labeled triolein were prepared using a rat thoracic duct cannulation technique (10). Briefly, anesthetized rats (diabetic or control) had a polyethylene catheter inserted into the lower thoracic duct via an extraperitoneal loin incision and externalized to continuously collect chyle; a gastrostomy was also performed. Heparin was not used. The animals were maintained in a restraining cage, with free access to food and water, but were given additional intragastric fluid replacement. Triolein-[3H] triolein (1.0 g, 2 mcI) was administered into the stomach, and chyle was collected for the subsequent 12 h. Radiolabeled CM were isolated by washing with bovine serum albumin solution and centrifugation. Thin-layer chromatography of the radiolabeled CM showed that >95% of the 3H label was in the TAG band. CM were suspended in fatty-acid free bovine serum albumin (5% wt/vol), and TAG was assayed with an enzymatic colorimetric test kit (as described above).

Analysis of TAG-Rich Lipoprotein Composition

Lipoprotein samples were delipidated as described by Scanu and Edelstein (53). Apolipoprotein components of both VLDL and CM were determined by electrophoresis on gradient sodium dodecyl sulphate-polyacrylamide slab gels as described (8, 25). A molecular weight marker ladder and albumin standard were co-run in the same gel. Following electrophoresis, the gel was stained with Coomassie blue solution; gels were scanned using commercially available software (GeneSnap; Synoptics, Cambridge, UK). By referring to the molecular weight marker ladder, bands of different apolipoproteins were identified. The optical density of each band was calculated by a commercially available software for gel analysis (GeneTools; Synoptics). By calibrating with albumin standard bands, the amount of protein in each sample band was calculated.

Analysis of Myocardial Tissue Glycogen and Lipid Content

In separate experiments, diabetic (STZ 60 mg/kg iv) and control (sham injected) rats were examined 2 wk after treatment. Blood glucose was measured [diabetic rats: 18.9 ± 0.6 mM (n = 5); control rats: 5.8 ± 0.4 mM (n = 5) P < 0.01] and the heart rapidly removed and freeze-clamped in liquid nitrogen. Hearts were subsequently ground and a portion analyzed for glycogen content (18); the remainder was subjected to Folch extraction, separation of lipid classes by thin-layer chromatography (see below), and quantitation of the lipid bands by enzymatic colorimetric test kits (TAG and cholesterol; Randox Laboratories, Crumlin, Antrim, UK, and phospholipid and NEFA; Wako Chemicals).
Isolated Perfused Working Heart Preparation

Hearts were perfused through the left atrium (anterograde) in working mode by the method of Taegtmeyer et al. (56). Fed rats were anaesthetized with intraperitoneal pentobarbitone sodium (60 mg/kg body wt). The heart was rapidly excised and briefly placed in ice-cold Krebs-Henseleit bicarbonate saline; it was then cannulated via the aorta (<2 min from excision) and perfused retrogradely through the coronary arteries in “Langendorff” mode, whereas lung, mediastinal, and pericardiac brown adipose tissue were excised, right pulmonary arteriometry performed, and the left atrium separately cannulated, after which the apparatus was switched to working mode and cardiac perfusion maintained through the left atrium. A recirculating Krebs-Henseleit bicarbonate buffer solution containing 1.3 mM CaCl₂, 11 mM glucose, and endotoxin- and 1% FA-free bovine serum albumin (wt/vol) was filtered through a 5-μm cellulose nitrate filter (Millipore, Bedford, MA) and gassed with 95% O₂-5% CO₂ at 37°C. The first 50 ml of coronary effluent was discarded to free the circuit of blood cells; final perfusate volume was 100 ml. Afterload was maintained at 100 cmH₂O and preloaded (atrial filling pressure) at 15 cmH₂O. After an initial 15-min stabilization period, lipoprotein was added slowly (2 min) to the reservoir (time 0). [U-¹⁴C]glucose in aqueous solution was also added to the perfusate (see below). Peak systolic pressure and heart rate were measured by calibrated pressure transducer (Druck, Groby, Leicestershire, UK) connected to a side arm of the aortic cannula. Aortic flow rate was measured by a timed collection of perfusate ejected through the aortic line, and coronary flow rate was measured by a timed collection of perfusate effluent dripping from the heart. Measurements were made at time 0 and at 10-min intervals for 60 min. Cardiac output was calculated as (coronary flow rate + aortic flow rate). Hydraulic work was calculated as cardiac output × mean aortic pressure/heart wt/min). After the final measurements at 60 min, 5 U/ml heparin (Leo Laboratories, Princess Risborough, Bucks, UK) was added to the perfusate, and after a further 2 min the heart was rapidly excised, freeze-clamped in light alloy tongs cooled in liquid nitrogen, and weighed. A duplicate sample of the postheparin perfusate was also frozen in liquid nitrogen.

Measurement of Lipoprotein-TAG Oxidation Rate

TAG oxidation rate was estimated by measuring ³H₂O production from [¹⁴H]triolein in the perfusate as described (19); at 10-min intervals, aliquots of perfusate (1.0 ml) were removed and subjected to Folch lipid extraction with chloroform-methanol (2:1, vol/vol) and water. An aliquot of the water phase was removed and counted for radioactivity.

Incorporation of Exogenous Lipoprotein-TAG Into Myocardial Lipid

Myocardial ³H-lipid content was estimated by grinding frozen myocardium to powder under liquid N₂ and extracting the lipids from an aliquot with chloroform-methanol (Folch). After repeated washing, the lipids were resolubilized in chloroform and separated by thin-layer chromatography using a hexane-diethyl ether-acetic acid system with standards co-run. ³H radioactivity was measured in the various lipid bands after visualization with rhodamine 6G under ultraviolet light.

Lipoprotein-TAG utilization was calculated as the sum of TAG oxidation and tissue lipid incorporation.

Measurement of Glucose Oxidation Rate

Glucose oxidation rate was determined by measuring ¹⁴CO₂/H¹³CO₃ production as described (5). [U-¹⁴C]glucose (3.23 mCi/mol) in aqueous solution was added to the perfusate. Hearts were then perfused in a closed system that allowed quantitative collection of gaseous and perfusate ¹⁴CO₂. Perfusate and gaseous samples were collected at 10-min intervals throughout the perfusion. The ¹⁴CO₂ liberated in the gaseous state was trapped in Optisorb “I” carbon dioxide absorbent-liquid scintillant (Wallac UK, Milton Keynes, Bucks, UK) in the gas outlet line. Samples of this solution were counted directly for radioactivity. Perfusate samples were immediately injected into the middle well of sealed Ehrenmeyer flasks containing H₂SO₄, with Optisorb CO₂ absorbent/scintillant in the outer well. The flasks were gently agitated for 1 h and the absorbent removed and counted for radioactivity (H¹⁴CO₃⁻ production).

LPL Activity

LPL activity was estimated in duplicate samples by using a [¹⁴H]triolein substrate emulsion containing fasted rat serum as a source of apo lipoprotein (apo)C-II to maximize LPL detection (42); the serum was pretreated by heating to 56°C to inactivate nonspecific plasma lipases. Radioactivity in evolved FAs was counted following extraction in methanol-chloroform-heptane. Heparin-releasable LPL (HR-LPL) activity was measured by adding postheparin perfusate taken at 62 min directly in the above assay system without modification (expressed as nmol FA released/min⁻¹ g wet wt⁻¹ heart). Tissue residual LPL activity was measured in tissue powders ground from the hearts frozen in liquid nitrogen; a duplicate sample of frozen heart tissue was weighed, dried down with acetone-diethyl ether, and reweighed to correct expression of activity (from nmol of FA released/min⁻¹ mg⁻¹ aceton-dried powder to nmol of FA released/min⁻¹ g wet wt⁻¹ heart).

Statistics

Results are expressed as means ± SE. Statistical analysis was performed by one-way ANOVA for repeated measurements and Tukey’s test or by Student’s t-test with Bonferroni correction for multiple comparisons where appropriate. Statistical significance was set at P < 0.05.

RESULTS

General Characteristics of Control and STZ-Treated Rats

Induction of type 1 diabetes with STZ (60 mg/kg iv) resulted in hyperglycemia; the dose was chosen to reliably induce hypoinsulinemia (26). STZ causes initial (<24 h) hyperinsulinemia followed by establishment of the diabetic state; systemic side effects occur and are well documented in this model (63). Body weight gain over 2 wk of treatment was decreased significantly in diabetic rats; heart weight was also decreased in STZ-induced diabetes (Table 1).

Lipoprotein Composition Analysis in Control and Diabetes

Lipoprotein particles synthesized during diabetes differed in composition from control lipoproteins. Lipoprotein core-to-surface ratio provides a valid approximation of particle size (27); therefore, VLDL and CM size were estimated by calculating TAG/PL ratios (Table 2). On this basis, control CM particles were larger than control VLDL particles, as expected.

Table 1. General characteristics of diabetic rats at 2 wk after STZ administration

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>STZ-Induced Diabetes</th>
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<tbody>
<tr>
<td>Body weight, g</td>
<td>322.8 ± 3.3</td>
<td>262.5 ± 5.1*</td>
</tr>
<tr>
<td>Heart weight, g</td>
<td>1.32 ± 0.04</td>
<td>1.18 ± 0.03**</td>
</tr>
<tr>
<td>Blood glucose, mmol/l</td>
<td>6.2 ± 0.2</td>
<td>19.5 ± 0.8**</td>
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</table>

Values are means ± SE (n = 30). STZ, streptozotocin. Diabetes was induced by administration of STZ (60 mg/kg iv). Body weight, heart wet weight, and blood glucose concentration were measured in control and STZ-treated rats. Statistically significant differences between diabetic and control rats are indicated: *P < 0.05, **P < 0.01.
Table 2. TAG/PL ratio of VLDL and CM particles prepared from control and diabetic rats

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>STZ Treated</th>
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<tbody>
<tr>
<td>VLDL</td>
<td>9.44±1.13</td>
<td>3.62±0.79**</td>
</tr>
<tr>
<td>CM</td>
<td>19.95±3.75#</td>
<td>4.85±1.19*</td>
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</table>

Results are expressed as means ± SE (n = 4–5/group). TAG, triacylglycerol; PL, phospholipid; VLDL, very-low-density lipoprotein; CM, chylomicrons. TAG and PL contents were determined using enzymatic colorimetric assay kits. For further details, see text. Statistically significant differences between STZ-treated diabetic rats and controls are indicated: *P < 0.05; **P < 0.01; significant differences between VLDL and CM particles are indicated: #P < 0.05.

“Diabetic” lipoproteins (both VLDL and CM) were significantly smaller than corresponding control lipoproteins, with no significant difference in size between diabetic CM and diabetic VLDL (Table 2). Because each lipoprotein particle contains one copy of apoB, the sample content of apoB represents the number of lipoprotein particles (17). Therefore, the ratio of other major apolipoproteins to apoB content was used to represent the relative proportion of each apolipoprotein in the VLDL and CM particle. Insulin deficiency had significant effects on apolipoprotein constituents of lipoprotein particles. Increased ratios of apoE and apoC to apoB were found in STZ-treated rat VLDL 2 wk following injection, and diabetic CM particles also had significantly increased proportions of apoE, apoC, and apoA-I to apoB (Table 3).

**VLDL-TAG and CM-TAG Metabolism by Working Diabetic Rat Hearts**

Myocardial tissue glycogen and TAG contents were increased significantly in diabetic hearts prior to perfusion (Table 4). Metabolism of assimilated lipoprotein-[³H]TAG was examined by measuring oxidation rate and deposition into tissue ³H-labeled lipids. Control hearts oxidized more (about twice) control CM-TAG than control VLDL-TAG, confirming previous observations (43); these control hearts oxidized diabetic VLDL-TAG to an extent similar to control VLDL-TAG but oxidized diabetic CM-TAG to an even greater extent than control CM-TAG (Fig. 1). Diabetic hearts oxidized all types of lipoprotein-TAG to a greater extent than control hearts. Oxidation rate of CM-TAG in diabetic hearts was again significantly greater than that of VLDL-TAG; diabetic CM-TAG was oxidized at 3.6-fold higher rate than diabetic VLDL-TAG. In addition, diabetic VLDL was oxidized to an extent similar to control VLDL, whereas diabetic CM was oxidized to a significantly greater extent than control CM, regardless of the diabetic state of the hearts (Fig. 1).

The incorporation of exogenous [³H]TAG into tissue lipids was also measured. Control CM-TAG and VLDL-TAG were esterified into tissue lipids to a similar extent (Fig. 2), in contrast to oxidation rates. In control hearts, diabetic lipoproteins were esterified into tissue lipids to a significantly lesser extent than that of control lipoproteins (less than one-half); however, diabetic hearts incorporated diabetic lipoproteins into tissue lipids to the same extent as control lipoproteins. Both diabetic VLDL-TAG and diabetic CM-TAG were esterified into tissue lipids to a greater extent by diabetic hearts than control hearts (Fig. 2). Therefore, diabetic lipoproteins were poor esterification substrates for control hearts but better substrates for diabetic hearts. Diabetic hearts showed significantly decreased accumulation of diabetic CM-TAG compared with

Table 3. Apolipoprotein composition of VLDL and CM particles prepared from control and diabetic rats

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>STZ Treated</th>
</tr>
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<tbody>
<tr>
<td>VLDL</td>
<td>apoE/apoB</td>
<td>2.03±0.23</td>
</tr>
<tr>
<td></td>
<td>apoC/apoB</td>
<td>1.86±0.31</td>
</tr>
<tr>
<td>CM</td>
<td>apoE/apoB</td>
<td>0.67±0.11</td>
</tr>
<tr>
<td></td>
<td>apoC/apoB</td>
<td>0.51±0.04</td>
</tr>
<tr>
<td></td>
<td>apoA-I/apoB</td>
<td>0.54±0.16</td>
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Results are expressed as means ± SE (n = 4–5/group). apo, Apolipoprotein. Apolipoprotein contents were determined by SDS-PAGE. Apolipoprotein content was expressed as a proportion of apoB content to correct for particle numbers. For further details, see text. Statistically significant differences between STZ-treated diabetic rats and controls are indicated: *P < 0.05; **P < 0.01.

Table 4. Myocardial tissue glycogen and lipid content

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>STZ Treated</th>
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<tbody>
<tr>
<td>Glycogen, µmol glucose/g wet wt</td>
<td>15.8±2.2</td>
<td>33.0±3.3**</td>
</tr>
<tr>
<td>TAG, µmol FA/g wet wt</td>
<td>4.0±0.5</td>
<td>6.8±0.7*</td>
</tr>
<tr>
<td>Cholesterol (total), µmol/g wet wt</td>
<td>10.7±1.0</td>
<td>12.2±1.3</td>
</tr>
<tr>
<td>PL, µmol/g wet wt</td>
<td>39.5±3.0</td>
<td>40.3±5.2</td>
</tr>
<tr>
<td>Nonesterified FA, µmol/g wet wt</td>
<td>0.32±0.07</td>
<td>0.40±0.06</td>
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</table>

Results are expressed as means ± SE (n = 5/group). FA, fatty acid. Diabetes was induced by administration of STZ (60 mg/kg iv). Animals were maintained for 2 wk after injection, and then the heart was removed, immediately freeze-clamped, and analyzed for glycogen and lipid content. For further details, see text. Statistically significant differences between diabetic and control rat hearts are indicated: *P < 0.05; **P < 0.01.
As previously noted (43), a greater proportion of CM-TAG was oxidized (relative to tissue lipid deposition) compared with VLDL-TAG in normal rat hearts (Fig. 5). The proportion of diabetic CM-TAG oxidized was again significantly greater than that of diabetic VLDL-TAG in control hearts, but the proportion of diabetic lipoproteins (both VLDL and CM) oxidized was significantly greater than that of control lipoproteins (Fig. 5), consistent with decreased tissue $[^{3}H]$lipid deposition (Fig. 2). Diabetic hearts oxidized control lipoprotein-TAG to a significantly greater extent (relative to tissue deposition) than control hearts; diabetic lipoprotein-TAGs were all oxidized to a high degree (>80%) by working myocardium. Interestingly, although control VLDL-TAG was partitioned equally between oxidation and deposition as myocardial tissue lipids in control hearts, the proportion of control VLDL-TAG oxidized in diabetic hearts reached ~85%. Therefore, diabetic hearts perfused with diabetic lipoproteins (both VLDL and CM) had proportionately greater oxidation compared with control hearts perfused with control lipoproteins (Fig. 5).

TAG utilization was expressed as TAG oxidation plus total $[^{3}H]$lipid accumulation (assuming no other metabolic fate of assimilated lipoprotein lipid; Fig. 6). Since most of the TAG was oxidized, a similar pattern to TAG oxidation (Fig. 1) was found.

Glucose Oxidation Rate

Glucose oxidation rate was significantly decreased in STZ-treated hearts (Fig. 7). In control hearts, a significantly lower glucose oxidation rate was found when hearts were perfused with either diabetic lipoprotein; the effect was significantly greater with VLDL than CM. This inhibitory effect of diabetic

cardiovascular disease (CVD) and diabetic cardiomyopathy, a condition believed to be associated with cardiomyopathy (1).
LPL Activity

LPL activity was measured in the perfusate at the end of the 60-min perfusion period following heparin administration (heparin-releasable portion, corresponding to the physiologically active endothelial enzyme). Diabetic hearts had higher HR-LPL activity than control hearts (Fig. 8A). HR-LPL activity was relatively low in control hearts perfused with control lipoproteins. Interestingly, however, control hearts showed markedly increased HR-LPL activity when perfused with diabetic lipoproteins. A similar, although less dramatic, effect was seen in diabetic hearts (Fig. 8A); this stimulation may have been maximal, since diabetic CM failed to increase HR-LPL activity further in diabetic hearts more than the fivefold increase seen in control hearts. Therefore, in STZ-induced diabetic hearts perfused with diabetic lipoproteins, HR-LPL activity was significantly greater than in control hearts perfused with control lipoproteins (Fig. 8A). The pattern of tissue residual LPL activity (Fig. 8B) in control hearts was similar to that of HR-LPL (Fig. 8A); diabetic lipoproteins increased residual LPL activity significantly. By contrast, in diabetic rat hearts the tissue residual LPL activity was markedly lower in hearts perfused with diabetic lipoproteins compared with those perfused with control lipoproteins (Fig. 8B); hence, diabetic hearts perfused with diabetic lipoproteins had lower tissue LPL activity than control hearts perfused with control lipoproteins (Fig. 8B), suggesting that more LPL synthesized by cardiomyocytes was translocated to its physiologically active endothelial (heparin-releasable) site.

Cardiac Mechanical Function

Control CM tended to support better cardiac performance in control hearts, measured as hydraulic work, than control VLDL, although this was not statistically significant (Fig. 9). Although impaired cardiac function has been demonstrated in both type 1 and type 2 chronic diabetic animals, in the present study hydraulic work in STZ-treated rat hearts was not decreased over a relatively short (2 wk) period of insulin deficiency. Indeed, performance was generally increased in diabetic hearts at this time scale. Diabetic lipoproteins tended to be worse substrates for control hearts but were significantly better substrates for diabetic hearts (Fig. 9). Interestingly, diabetic VLDL was a highly effective substrate for contractile function in diabetic hearts, compared with control VLDL in control hearts, whereas diabetic CM did not improve the contractile function of the diabetic heart compared with control CM in the control heart (Fig. 9).
major limitation because FAs supplied to the heart in vivo are derived from two sources, NEFA and TAG in the core of TAG-rich lipoproteins. Recent studies demonstrated that lipoproteins are a major source of FA for the myocardium (2, 23, 60). Therefore, the present study was designed to investigate the metabolism of VLDL and CM by type 1 diabetic heart; to investigate lipoprotein preference as well as effects of diabetes on cardiac lipoprotein metabolism, we used nondiabetic (control) and diabetic lipoproteins to perfuse control and STZ-induced diabetic rat hearts.

**LPL Activity and TAG Uptake in STZ-Induced Diabetic Rat Heart**

LPL-mediated hydrolysis is the initial step for the “bulk” uptake of lipoprotein-TAG by heart (2, 22, 36); alterations in LPL activity change FA delivery and subsequent oxidation.
endothelial cells. We found that diabetic VLDL and CM particles had proportionately higher apoE contents than control particles. This could explain the stimulating effects of diabetic lipoproteins on HR-LPL since apoE is a strong heparan-binding protein and can anchor apoE-rich lipoproteins to the cellular heparan sulfate proteoglycan site, inducing HR-LPL activity. Tissue residual LPL activity was also measured in hearts following perfusion. The stimulation of residual LPL by diabetic lipoproteins was also found in control hearts but not in diabetic hearts; these hearts had lower tissue residual LPL activity compared with control hearts when perfused with diabetic lipoproteins. It is not possible with the current data to establish whether this is because more tissue residual LPL has been translocated to the endothelium, resulting in enhanced HR-LPL activity in diabetes. O’Looney et al. (44) reported changes in rat VLDL composition 48 h after STZ treatment. These changes are difficult to compare with the current data since correction for particle number was not made, but decreased levels of apoE and apoC were noted, and this was offered as an explanation for the decreased VLDL-TAG lipolysis observed in relation to diabetic lipoproteins and diabetic heart. These authors reasonably suggest this effect as a mechanism for the hypertriglyceridemia seen in diabetic animals, but this does not explain the intracellular TAG accumulation consistently observed in this state and noted in the present study. The reason for the difference between LPL and TAG hydrolysis/uptake seen in the studies by O’Looney et al. (44) and the present work may reflect the different time course of STZ exposure and the use of modified VLDL.

Lipoprotein-TAG uptake was estimated from its metabolic fate (“utilization”). Results broadly agree with the HR-LPL data; increased LPL activity was associated with increased lipoprotein-TAG utilization. However, lipoprotein-TAG uptake via LPL will depend not only on the LPL activity but also on the particle composition. Diabetic lipoproteins were found to be small, suggesting that they are inefficient LPL substrates, but by contrast they had a higher apoE content, suggesting improved ligand binding for lipoprotein receptor-mediated uptake (43). Changes in the structure of diabetic LPL (e.g., by glycosylation) could make catabolism of the TAG core less efficient, and an LPL inhibitor could be present in diabetic rat, as reported in diabetic mice (29). Lipoprotein receptor-mediated TGLLP uptake was not specifically examined in the current study but may be relevant in insulin deficiency; since tissue distribution of VLDL receptor and LPL are similar (12, 58, 61, 69), an interaction between them could exist, which could regulate lipoprotein metabolism. Smaller particles may also facilitate receptor-mediated uptake.

**Metabolism of VLDL and CM by Diabetic Rat Hearts**

TAG-FAs assimilated into cardiomyocytes have two principal metabolic fates (23, 43, 49): 1) oxidation, providing ATP for energetic needs; and 2) esterification, forming structural lipids (such as phospholipids) and providing a limited energy storage resource (e.g., cellular TAG). In addition, there are putative minor fates for TAG-FAs, such as lipoprotein synthesis (38, 39, 67). In the present study, most TAG-FAs were oxidized. Diabetes increased the proportion of TAG-FAs oxidized relative to esterification (consistent with decreased glucose oxidation, but also regarding the effect of diabetes on LPL metabolism.)
activity). In the diabetic heart this was due to increased oxidation rate, since tissue lipid accumulation was unchanged; however, despite this, the diabetic heart tended to partition more of its unoxidized FA as TAG, and whereas the mechanism for this is uncertain, it explains the increased intramyocardial TAG concentrations consistently observed in diabetes (64) and confirmed here in unperfused hearts (Table 4). Furthermore, since FA oxidation consumes more oxygen than glucose oxidation, cardiac efficiency (ratio of cardiac work to myocardial oxygen consumption) in diabetes decreases in line with increased FA utilization, and this makes the heart especially vulnerable to damage following increased workload or ischemia (1). Reduced cardiac efficiency due to increased FA oxidation in diabetes is one type of lipotoxicity contributing to diabetic cardiomyopathy (1, 64). The present study confirmed raised glycogen content in diabetic hearts and the shift in substrate preference away from carbohydrate (glucose) utilization and further toward lipid oxidation (55, 57). However, we also demonstrate that suppression of glucose oxidation occurs with TAG as lipid substrate, not solely with fatty acids. Furthermore, we show that diabetic lipoproteins further suppress glucose oxidation, regardless of the diabetic state of the myocardium. The mechanism for this effect is not clear but is not obviously related to TAG-FA oxidation rate. The pattern of tissue lipid deposition, however, suggests that this compartment may be critical in linking TAG-FA metabolic fate to glucose utilization and oxidation.

Mechanisms responsible for increased lipoprotein-TAG oxidation in STZ-treated hearts are likely multifactorial. Whereas increased HR-LPL activity suggests that augmented TAG-FA uptake drives the increased FA oxidation in diabetes, increased NEFA oxidation is found in perfused diabetic hearts (33), suggesting additional involvement of an intracellular mechanism of FA disposal independent of TAG uptake mechanisms. Carnitine palmitoyltransferase I, malonyl-CoA, and AMP kinase may all be involved. We found increased cardiomycyte [3H]TAG accumulation in diabetic hearts, a marker of lipotoxicity (1), and it is noteworthy that diabetic lipoproteins only contributed to increased cellular TAG accumulation in diabetic hearts, i.e., that myocardial TAG accumulation is a function of the diabetic heart rather than of diabetic lipoproteins. The role of cellular TAG accumulation in diabetic cardiomyopathy is still unknown (TAG may be a surrogate for accumulation of a more detrimental lipid, e.g., fatty acyl-CoA). Interestingly, cardiac mechanical performance in diabetic heart was moderately increased despite the increased myocardial [3H]TAG accumulation. Strikingly, diabetic lipoproteins were better substrates for diabetic heart function. Cardiac performance could be enhanced due to increased FA oxidation at an early stage of insulin deficiency, and in type 1 diabetes, changes in cardiac metabolism occur early and precede the development of cardiac dysfunction. We found significantly altered cardiac metabolism 2 wk following STZ administration, and others observed changes as early as 4 days following induction of diabetes (21). Unchanged or even improved mechanical function despite moderately increased TAG accumulation argues against cellular TAG as a cause of heart failure in diabetes. However, diabetic cardiac dysfunction (cardiomyopathy) may only be apparent only after 4–6 wk (1).

**Lipoprotein Preference in Diabetic Rat Hearts**

Previously, we found that nondiabetic hearts assimilated and oxidized more CM-TAG than VLDL-TAG (43), suggesting that CM is a preferred lipoprotein substrate; the present study confirmed this result. CM have a greater affinity for LPL than VLDL, probably because of their greater size (66). However, even diabetic CM was a preferred substrate for control hearts to assimilate compared with VLDL, despite their relatively small size, so it is possible that another mechanism is responsible for the high uptake of diabetic CM, including a receptor-mediated route. This could be explained by the altered structure and composition of diabetic lipoproteins (24, 35, 62). We have examined different roles of VLDL and CM in cardiac energy metabolism under normal physiological conditions (23, 43). These studies suggest that CM, from which most derived FAs gain access through LPL, are the major cardiac FA supplier. However, because the metabolic fate of VLDL is split evenly between oxidation and deposition as myocardial tissue lipid, VLDL may play an alternative role in cardiac lipid metabolism, possibly through a lipoprotein receptor-mediated mechanism. In diabetes, the role of VLDL may change, a greatly increased proportion being oxidized by diabetic heart, suggesting that, in this energy stress condition, VLDL becomes a more important cardiac energy source. However, CM, especially diabetic CM, remained the preferred oxidative substrate for diabetic hearts. The mechanisms that control cardiac lipoprotein utilization during diabetes are complex, and their relative metabolic fates through uptake or intracellular channeling mechanisms have yet to be completely resolved. We chose 0.4 mM TAG for both control and diabetic perfusion protocols, being a typical (and comparable) concentration; however, in vivo the diabetic heart would be exposed to a higher TAG concentration than the nondiabetic heart due to the hypertriglyceridemia (1, 3, 57). Further studies are required to investigate whether this would influence TAG uptake and metabolism by the diabetic myocardium.

Both VLDL and CM particles analyzed in the present study were newly synthesized and secreted. These particles had not been exposed to LPL action; therefore, these studies represent examination of the fully mature, unmodified TGRLP particle. However, to our knowledge, other studies on lipoprotein composition in diabetes were performed on particles isolated from plasma by ultracentrifugation. For example, O’Looney et al. (44) found increased apoA-I and apoA-IV in VLDL from STZ-treated rats; we found no such effect in freshly secreted particles. Clearly, there may be differences between mature particles and those partly metabolized in vivo.

In conclusion, the present study in STZ-treated rats demonstrates evidence of early lipotoxicity in hearts without affecting cardiac mechanical performance. Changes observed were due to differences in the diabetic states of both the heart and the lipoproteins themselves. Further work to define the role of VLDL and CM in energy provision in diabetes and in the causation of the abnormalities in lipid metabolism that accompany this condition is required. Subsequently, manipulation of lipoproteins and their utilization in diabetes may be important therapeutically.
REFERENCES


