Chylomicron and palmitate metabolism by perfused hearts from diabetic mice

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Metabolism of exogenous substrates is altered markedly in diabetic hearts (26, 38). As a consequence of decreased glucose utilization and increased oxidation of albumin-bound FA by hearts from insulin-deficient (type 1) diabetic rats (35), FA oxidation becomes almost the exclusive energy source for the diabetic hearts. Similar results showing elevated FA oxidation have been reported recently for perfused working hearts from diabetic db/db mice (2), an animal model of type 2 diabetes with obesity and insulin resistance (9, 22). However, observations that FA oxidation was increased in type 1 and type 2 diabetic hearts have been obtained from perfusions with albumin-bound FA (palmitate) only (2, 35, 38). In the case of chylomicron metabolism, the supply of FA for the diabetic heart could be altered if endothelium-bound LPL activity is changed, in addition to potential alterations in the metabolic fate of LPL-derived FA. Endothelium-bound (functional) LPL can be displaced into the perfusate of ex vivo hearts by heparin (6). Therefore, the first objective of this investigation was to determine whether heparin-releasable LPL (HR-LPL) activity was changed in hearts from diabetic db/db mice by use of genetic type 2 diabetic db/db mice as well as streptozotocin-induced insulin-deficient type 1 diabetic mice. The second objective was to assess the metabolic fate of LPL-derived FA in diabetic hearts (oxidation, esterification to intracellular TG) from perfusions with radiolabeled chylomicrons (29). Comparative experiments on the metabolism of an albumin-bound FA (palmitate) were conducted with perfused hearts from diabetic mice.

METHODS

Diabetic mouse models. Genetically diabetic C57BL/KsJ-lepr^db^-lepr^- (db/db) type 2 diabetic mice and lean control heterozygotes (db/+ ) were purchased from Jackson Laboratories (Bar Harbor, ME). All experiments were conducted on male animals at 12 wk of age, when plasma insulin levels in db/db mice are at peak values with concomitant hyperglycemia (2, 9).

A chemically induced murine model of insulin-deficient type 1 diabetes was also utilized. Swiss Webster (SW) male mice (30–40 g) were acquired from local breeding sources provided by the University of Calgary. Mice (10 wk of age) were made diabetic by injection of streptozotocin (65 mg/kg, intraperitoneal) or left untreated (SW). Diabetes was defined by plasma glucose concentrations >350 mg/dl at 1 wk after injection. Type 2 diabetes was induced in C57BL/KsJ-db/db-H11001 (db/db) mice with streptozotocin (65 mg/kg, intraperitoneal) to produce type 2 diabetes. After 1 wk, mice were treated with streptozotocin (65 mg/kg) for 1 wk. Only those mice whose plasma glucose concentration was >400 mg/dl 2 wk after initial injection were included in the study. All animals had free access to food and water. Blood was obtained from the retroorbital plexus of anesthetized mice, and plasma was collected and stored at −80°C until analysis.

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Neitzel, Andrew S., Andrew N. Carley, and David L. Severson. Chylomicron and palmitate metabolism by perfused hearts from diabetic mice. Am J Physiol Endocrinol Metab 284: E357–E365, 2003.—Hydrolysis of triacylglycerols (TG) in circulating chylomicrons by endothelium-bound lipoprotein lipase (LPL) provides a source of fatty acids (FA) for cardiac metabolism. The effect of diabetes on the metabolism of chylomicrons by perfused mouse hearts was investigated with db/db (type 2) and streptozotocin (STZ)-treated (type 1) diabetic mice. Endothelium-bound heparin-releasable LPL activity was unchanged in both type 1 and type 2 diabetic hearts. The metabolism of LPL-derived FA was examined by perfusing hearts with chylomicrons containing radiolabeled TG and by measuring 3H2O accumulation in the perfusate (oxidation) and incorporation of radioactivity into tissue TG (esterification). Rates of LPL-derived FA oxidation and esterification were increased 3.4-fold and 2.5-fold, respectively, in perfused hearts from STZ-treated mice. The oxidation and esterification of [3H]palmitate complexed to albumin were also increased in type 1 and type 2 diabetic hearts. The metabolism of LPL-derived FA in diabetic hearts (oxidation, esterification) was enhanced.

FATTY ACIDS (FA) are generally considered to be the preferred oxidative substrate for the heart (41). The two sources of FA for cardiac metabolism are 1) circulating FA bound to plasma albumin that can be taken up directly by the heart, and 2) hydrolysis of triacylglycerols (TG) in circulating lipoproteins (chylomicrons and very-low-density lipoproteins) by an endothelium-bound enzyme, lipoprotein lipase (LPL), to also yield FA for cardiac uptake and metabolism (6).

Chylomicrons are the preferred lipoprotein substrate for LPL (14). Recently, chylomicron metabolism has been measured with isolated perfused working hearts from rats (15, 42) and mice (29), so that the metabolic fate of LPL-derived FA could be compared with the utilization of albumin-bound FA.

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were treated with streptozotocin (STZ), which produces selective necrosis of pancreatic β-cells, resulting in a well-characterized model of insulin deficiency that has been used to evaluate changes in cardiac function (39). STZ was prepared in 100 mM citrate buffer (pH 4.5) at a concentration of 30 mg/ml and was administered to animals by intraperitoneal injection over a 3-day protocol (18). On the 1st day, mice received 85 mg/kg STZ, on the second day 70 mg/kg, and on the third day 55 mg/kg, for a total cumulative dose of 210 mg STZ/kg mouse body weight. Control animals received only the citrate buffer. Metabolic studies were conducted 2 wk after the 3-day STZ injection protocol (at 12 wk of age). Insulin-deficient (type 1) diabetes was also produced by administration of STZ to C57BL/KsJ-lepr<sup>db/db</sup> mice, for direct comparison with the type 2 (db/db) model of murine diabetes.

Control and diabetic mice were housed in the animal facility at the University of Calgary, maintained under a 12:12-h light-dark cycle, and fed standard laboratory chow and water ad libitum. Diabetes was confirmed by measurements of blood glucose levels. Whole blood for analysis of glucose concentration was obtained by tail tip slice. Blood glucose was measured with a One Touch Ultra blood glucose meter. All experiments were approved by the University of Calgary Health Sciences Animal Welfare Committee in accordance with the regulations of the Canadian Council on Animal Care.

**Mouse heart LPL activity.** Functional endothelium-bound HR-LPL activity was measured with the use of retrogradely perfused Langendorff hearts, essentially as described by Mardy et al. (29). The perfusion buffer was a modified Krebs-Henseleit bicarbonate (KHB) buffer consisting of (in mM): 118.5 NaCl, 25 NaHCO<sub>3</sub>, 4.7 KCl, 1.2 MgSO<sub>4</sub>, 1.2 KH<sub>2</sub>PO<sub>4</sub>, 2.5 CaCl<sub>2</sub>, 0.5 EDTA, and 11 glucose, plus 3% (wt/vol) BSA, gassed with 95% O<sub>2</sub>-5% CO<sub>2</sub> (pH 7.4). The glucose concentration of 11 mM in the KHB buffer is very near plasma glucose concentrations (9–12 mM) measured in control mice (1, 2).

At time 0, after a 10-min stabilization period, the KHB perfusion buffer was switched to one that contained 5 U/ml of heparin. Hearts were perfused with this nonrecirculating buffer (45 ml) for the duration of the 20-min experiment. At given time points, 0.5-ml samples of perfusate were collected and flash frozen for subsequent determinations of HR-LPL activity. At the end of the experiment, the heart was removed from the perfusion apparatus and was flash frozen so that residual tissue LPL activity could be measured. Frozen tissue was homogenized as described by Mardy et al. (29). LPL activity in preheparin and postheparin (HR-LPL) perfusate samples and the residual LPL activity in postheparin heart homogenates were determined from the hydrolysis of a [14C]-triolein substrate emulsion (7, 29). All assays were performed in duplicate, under conditions in which the LPL assay was linear with respect to time of incubation and quantity of LPL enzyme. LPL activity is routinely expressed as nanomoles of FFA released per hour per milligram of tissue homogenate protein, measured using the Coomassie Protein Assay Reagent kit (Pierce, Rockford, IL).

**Chylomicron metabolism by perfused working mouse hearts.** Radiolabeled chylomicrons were collected by feeding rats with [3H]palmitate after intestinal lymphatic duct cannulation, as described by Mardy et al. (29). The orally administered [3H]palmitate is taken up by the intestine, esterified into [3H]TG, and incorporated into the core of chylomicron particles (CM-[3H]TG). After purification by a series of centrifugations (29), the total TG concentration of the stock CM-[3H]TG preparation (55 mM) was determined by a TG assay kit (GPO-Trinder; Sigma Diagnostics). Lipid extraction and thin-layer chromatography (29) revealed that >90% of incorporated radioactivity was in [3H]TG. A specific activity of LPL-derived FA was calculated ([3H]TG radioactivity/TG concentration × 3; 1,590 dpm/nmol), with the assumption that all three fatty acids in the [3H]TG were radiolabeled and subjected to hydrolysis by LPL.

The ex vivo working mouse heart preparation provides an experimental system with precise control of physiological factors (afterload, preload) and the supply of exogenous substrates in the perfusate (3, 21). Isolated working mouse hearts were perfused with recirculating KHB buffer (total volume of 40 ml) containing 3% BSA, 0.4 mM CM-[3H]TG, and 1% heat-inactivated rat serum as an additional source of apolipoprotein CII, an essential cofactor for LPL activity (29). The chylomicron preparation was preincubated with 1% serum for 10 min at 37°C before addition to the working heart perfusion buffer. Increasing the serum concentration to 3% did not increase chylomicron metabolism further. Mouse hearts were perfused (preload pressure of 15 mmHg; afterload pressure of 50 mmHg) for 90 min. At 15-min intervals, a total of 1 ml of perfusate was removed for determination of very low density lipoprotein (VLDL) and high density lipoprotein (HDL) cholesterol and triglycerides (29). The lipid content of the perfusate was measured by a TG assay kit (GPO-Trinder; Sigma Diagnostics). Lipid extraction and thin-layer chromatography (29) revealed that >90% of incorporated radioactivity was in [3H]TG. A specific activity of LPL-derived FA was calculated ([3H]TG radioactivity/TG concentration × 3; 1,590 dpm/nmol), with the assumption that all three fatty acids in the [3H]TG were radiolabeled and subjected to hydrolysis by LPL.

At the end of the 90-min perfusion with CM-[3H]TG, the esterification of LPL-derived FA into tissue lipids was measured (29). Briefly, hearts were flash frozen, and the incorporation of radioactivity into tissue lipids (TG, diacylglycerol, monoacylglycerol, FA, and phospholipids) was determined after lipid extraction and thin-layer chromatography. Very little radiolabel (<5% of total) was present as unmetabolized FA; the predominant metabolic fates were oxidation and esterification to TG. A portion of the frozen heart tissue was dried so that metabolic rates (oxidation and esterification of LPL-derived FA) could be normalized as micromoles per minute per gram dry weight to correct for any differences in heart size between diabetic and control mice. It should be noted that incorporation of radioactivity into tissue lipids was measured only at the end of the 90-min perfusion, and so the calculation of an esterification rate is based on the assumption that this rate is linear without an appreciable lag.

**Palmitate metabolism by perfused working mouse hearts.** The metabolism of 0.4 mM [3H]palmitate (specific activity 800 dpm/nmol), complexed to 3% BSA in KHB buffer, was determined as described by Mardy et al. (29). Perfusate samples were removed at time 0 and after 30, 60, and 90 min of working heart perfusion, and the generation of [3H]2O was measured as an index of palmitate oxidation. The incorporation of radioactivity into tissue lipids (esterification) at the end of the 90-min perfusion was measured, as described in the preceding section on CM-[3H]TG metabolism. Parameters of contractile function were measured every 15 min.

**Statistics.** Results are presented as means ± SE. An unpaired t-test was used to test for significant differences be-
between control and diabetic hearts; a $P$ value <0.05 was considered significant.

RESULTS

Characteristics of control and diabetic mice. Type 2 diabetic $db/db$ mice were markedly obese and hyperglycemic (Fig. 1) relative to control heterozygotes ($db/+), consistent with previous studies (2). Induction of insulin-deficient (type 1) diabetes by administration of STZ to SW and $db/+$ mice produced an equivalent degree of hyperglycemia (Fig. 1; 30.1 ± 0.8 and 28.5 ± 1.4 mM glucose, respectively), but these diabetic mice lost weight (−5.1 ± 1.5 g and −8.5 ± 0.7 g, respectively) over the 2-wk treatment

Fig. 1. Characteristics of control and diabetic mice. Body weight (g), plasma glucose (mM), and heart dry weight (mg) were measured with control $db/+ (n = 10)$, type 2 diabetic $db/db (n = 10)$, control Swiss-Webster (SW; $n = 10$), streptozotocin-induced type 1 diabetic Swiss-Webster [SW(STZ), $n = 10$], and STZ-induced type 1 diabetic $db/+ [db/+ (STZ); $n = 6]$ mice. Values are means ± SE. *Significantly different from respective control mice.
period. Consequently, the final body weights of STZ-treated SW (31.9 ± 0.7 g) and STZ-treated db/db (19.8 ± 0.6 g) mice were significantly lower compared with control SW (39.2 ± 1.3 g) and db/db (30.0 ± 0.7 g) mice. Heart weights were also reduced significantly in type 1 diabetic mice (Fig. 1); in contrast, heart weight was unchanged in type 2 db/db mice.

**Mouse heart LPL activity.** Perfusion of mouse hearts with heparin resulted in the rapid release of LPL activity into the perfusate, which then declined to preheparin levels by the end of the perfusion (Fig. 2). HR-LPL activity in the perfusate is the result of heparin displacing the enzyme from heparan sulfate proteoglycan-binding sites on the endothelial surface of the coronary vasculature (6, 14). The peak HR-LPL activity (measured 30 s after heparin) from db/db hearts (1,913 ± 146 nmol·h^{-1}·ml^{-1}) was not different from control db/db + (1,908 ± 126 nmol·h^{-1}·ml^{-1}) activity (Fig. 2A). Similarly, total HR-LPL activities (sum of activities from all perfusion times) from db/db and db/db + hearts was not significantly different (9,085 ± 829 and 10,522 ± 653 nmol·h^{-1}·ml^{-1}, respectively). Although peak HR-LPL in SW(STZ) hearts (2,463 ± 232 nmol·h^{-1}·ml^{-1}) was significantly higher (P < 0.05) than the peak activity from control SW hearts (1,882 ± 362 nmol·h^{-1}·ml^{-1}), HR-LPL activity from SW(STZ) hearts was not different from control enzyme activity at all other time points (Fig. 2B). As a result, total HR-LPL activity for SW(STZ) and SW control hearts was not different (9,650 ± 1,102 and 10,477 ± 832 nmol·h^{-1}·ml^{-1}, respectively). Similar results were obtained with STZ-treated db/db + hearts (Fig. 2B). Peak HR-LPL activity from db/db + (STZ) hearts (2,469 ± 287 nmol·h^{-1}·ml^{-1}) was significantly higher (P < 0.05) than peak activity from control db/db + hearts (1,908 ± 126 nmol·h^{-1}·ml^{-1}), but total activity was unchanged (9,954 ± 1,250 and 9,858 ± 829 nmol·h^{-1}·ml^{-1}, respectively). Residual LPL activity remaining in the hearts after heparin perfusion was also measured, with no significant differences between control db/db + (2,572 ± 109 nmol·h^{-1}·mg^{-1}; n = 7), db/db (2,588 ± 115 nmol·h^{-1}·mg^{-1}; n = 9), control SW (2,246 ± 120 nmol·h^{-1}·mg^{-1}; n = 5), diabetic SW (STZ) (2,392 ± 261 nmol·h^{-1}·mg^{-1}; n = 5), and diabetic db/db + (STZ) hearts (2,452 ± 177 nmol·h^{-1}·mg^{-1}; n = 6).

**Chylomicron metabolism by perfused working mouse hearts.** Working mouse hearts were perfused with [3H]chylomicron with a TG concentration of 0.4 mM, chosen as representative of a postprandial concentration in vivo (29, 42). The fate of LPL-derived FA from CM-[3H]TG hydrolysis was determined by measuring the accumulation of [3H]2O in the perfusate (FA oxidation) and the incorporation of radioactivity into tissue lipids (esterification) at the end of the 90-min perfusion (11, 29). The time course of [3H]2O accumulation in the perfusate of db/db + and db/db hearts was reasonably linear (Fig. 3A); oxidation of LPL-derived FA by db/db hearts was significantly higher than by control hearts at all time points. When steady-state rates were calculated (mean of the three perfusion times), the oxidation of LPL-derived FA by db/db hearts (0.32 ± 0.04 μmol·min^{-1}·g dry wt^{-1}) was 2.3-fold higher than the rate of oxidation (0.14 ± 0.02 μmol·min^{-1}·g dry wt^{-1}) by control db/db hearts (Fig. 3B).

The incorporation of radioactivity into tissue lipids was also measured (11, 29). Esterification to tissue TG was increased from 0.10 ± 0.02 μmol·min^{-1}·g dry wt^{-1} in db/db + hearts to 0.17 ± 0.03 μmol·min^{-1}·g dry wt^{-1} in db/db hearts (Fig. 3B). The percentage of incorporation of radioactivity into tissue TG relative to total lipid incorporation was 74 ± 1 and 80 ± 2% for db/db + and db/db hearts, respectively. There was also no difference between db/db + and db/db hearts for the low incorporation of radioactivity into other lipid classes.

When the fate of LPL-derived FA was calculated from the sum of oxidation and esterification rates (Fig. 3B), FA oxidation represented 58 ± 4 and 66 ± 2% of total FA utilization by control db/db + and diabetic db/db hearts. Thus, although total utilization (oxidation plus esterification) of LPL-derived FA was elevated in db/db hearts (0.49 μmol·min^{-1}·g dry wt^{-1}) compared with db/db + hearts (0.24 μmol·min^{-1}·g dry wt^{-1}), the relative proportion undergoing oxidation vs. esterification was unchanged.

Chylomicron metabolism by perfused working hearts from type 1 diabetic mice is shown in Fig. 4. The oxidation of LPL-derived FA by control SW and db/db +
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Fig. 3. Chylomicron metabolism by perfused working hearts from control db/+ (n = 4) and type 2 diabetic db/db (n = 4) mice. Hearts were perfused with a 0.4 mM [3H]chylomicron (CM) preparation. A: oxidation of lipoprotein lipase (LPL)-derived fatty acids (FA) was determined by measuring the perfusate content of 3H2O for hearts from db/+ (○) and db/db (●) mice at the indicated perfusion times. B: steady-state rates of LPL-derived FA oxidation and esterification were measured for control and db/db hearts. Values are means ± SE. *P < 0.05 relative to db/+ control hearts.

hearts was identical (Fig. 4A), consistent with similar HR-LPL activities (Fig. 2). The accumulation of 3H2O in the perfusate of SW(STZ) hearts was higher than respective control rates at all perfusion times (Fig. 4A). Consequently, steady-state oxidation rates were increased from 0.13 ± 0.03 to 0.44 ± 0.09 μmol·min⁻¹·g dry wt⁻¹ by control SW and diabetic SW(STZ) hearts, respectively (Fig. 4B). A very similar rate of oxidation was observed for diabetic db/+ (STZ) hearts (0.44 ± 0.06 μmol·min⁻¹·g dry wt⁻¹), which was elevated significantly compared with control db/+ hearts (0.14 ± 0.02 μmol·min⁻¹·g dry wt⁻¹).

Esterification into tissue TG (Fig. 4B) was also elevated in SW(STZ) and db/+ (STZ) hearts (0.20 ± 0.04 and 0.18 ± 0.02 μmol·min⁻¹·g dry wt⁻¹, respectively) compared with control SW and db/+ hearts (0.08 ± 0.01 and 0.10 ± 0.02 μmol·min⁻¹·g dry wt⁻¹, respectively). Incorporation into tissue TG as a percentage of total lipid incorporation was 73 ± 2 and 77 ± 2% for SW(STZ) and db/+ (STZ) hearts, which was not different compared with SW (73 ± 2%) and db/+ (74 ± 1%) control hearts. The proportional fate of LPL-derived FA between oxidation and esterification was also calculated. FA oxidation was 69 ± 4 and 71 ± 4% of total FA utilization (oxidation + esterification) for SW(STZ) and db/+ (STZ) hearts, respectively, with 63 ± 7% for control SW and 58 ± 4% for control db/+ hearts. Thus, as noted for db/db hearts, the enhanced total utilization (oxidation plus esterification) of LPL-derived FA by type 1 diabetic SW(STZ) hearts (0.64 μmol·min⁻¹·g dry wt⁻¹) compared with control SW hearts (0.21 μmol·min⁻¹·g dry wt⁻¹) was not associated with any change in the relative proportion undergoing oxidation vs. esterification. Finally, it is evident that there are no significant strain differences for chy-
lomicron metabolism by perfused hearts from SW and db/db mice (±STZ).

Palmitate metabolism by perfused working mouse hearts. Previous publications have reported that palmitate oxidation was increased in perfused hearts from db/db mice (1, 2), but esterification was not measured. Therefore, in this study, db/db and db/+ hearts were perfused with albumin-bound [3H]palmitate. Palmitate oxidation by db/db hearts increased 3.9-fold, as observed previously (1, 2). Incorporation of radiolabeled palmitate into tissue TG was 0.10 ± 0.02 μmol·min⁻¹·g dry wt⁻¹ (n = 5) and 0.18 ± 0.03 μmol·min⁻¹·g dry wt⁻¹ (n = 4; P < 0.05) for db/+ and db/db hearts, respectively. Thus, both oxidation and esterification of palmitate were increased in db/db hearts, consistent with results for LPL-derived FA (Fig. 3). Palmitate metabolism was also studied with perfused hearts from type 1 diabetic mice. Palmitate oxidation was reasonably linear with perfusion time for control SW and db/+ hearts (Fig. 5A), with steady-state rates of 0.54 ± 0.11 and 0.47 ± 0.17 μmol·min⁻¹·g dry wt⁻¹ (Fig. 5B) that are very similar to previous results (2, 29). Rates of palmitate oxidation for diabetic SW(STZ) and db/+ (STZ) hearts were more than doubled, to 1.12 ± 0.09 and 1.01 ± 0.09 μmol·min⁻¹·g dry wt⁻¹, respectively. Esterification to tissue TG was increased significantly in SW(STZ) hearts (0.25 ± 0.01 μmol·min⁻¹·g dry wt⁻¹) compared with control SW hearts (0.17 ± 0.03 μmol·min⁻¹·g dry wt⁻¹; Fig. 5B). In contrast, esterification to TG in db/+ (STZ) hearts was not significantly different from that in control db/+ hearts. As observed for hearts perfused with CM-[3H]TG, the incorporation of radioactivity into TG after [3H]palmitate complexed to 3% BSA was the predominant fate, with 71 ± 1, 75 ± 4, 77 ± 1, and 81 ± 1% of total lipid incorporation for SW(STZ), db/+ (STZ), SW, and db/+ hearts, respectively. Similarly, there were no differences between control and type 2 diabetic hearts for the low level of radiolabel incorporation into other lipid classes. As noted previously for chylomicron metabolism, there were no significant differences in palmitate metabolism by perfused hearts from different mouse strains (db/+ and SW, ±STZ).

Mardy et al. (29) observed previously with control SW heart perfusions that a greater proportion of [3H]palmitate-albumin underwent oxidation relative to esterification compared with LPL-derived FA. Similar results were obtained in this study. Palmitate oxidation as a percentage of total FA utilization was 76 ± 5%, significantly greater than the percent oxidation of LPL-derived FA (58 ± 4%). The percentage of [3H]palmitate oxidation for diabetic SW(STZ) hearts was 82 ± 2 and 81 ± 5%, not significantly different from that for control SW hearts. Thus total [3H]palmitate utilization (oxidation plus esterification) was increased in type 1 diabetic hearts without a change in relative intracellular fate, as observed for metabolism of LPL-derived FA by both type 2 db/db and type 1 SW(STZ) and db/+ (STZ) hearts.

Contractile function in perfused working mouse hearts. A number of contractile parameters were monitored at 15-min intervals during working heart perfusions. In general, cardiac function was stable over the 90-min perfusion for both control and diabetic hearts, with the exception that there was a progressive decline in heart rates for the type 1 SW(STZ) and db/+ (STZ) hearts perfused with [3H]palmitate. Average values calculated from all time points are presented in Table 1 for hearts from db/+ and db/db (perfusions with chylomicrons) and SW and SW(STZ) mice (perfusions with both chylomicrons and palmitate). Heart rates for db/db and SW(STZ) hearts were significantly lower than for their respective controls, but all other parameters of contractile function were unchanged. Consequently, the rate-pressure product (HR × PSP), an accepted index of cardiac performance (42), was significantly reduced in db/db hearts (17.2 ± 0.4 mmHg/
Table 1. Cardiac function in perfused working hearts from control db/+ , diabetic db/db, control SW, and STZ-induced diabetic SW mice

<table>
<thead>
<tr>
<th>Mouse Hearts</th>
<th>Perfusion</th>
<th>n</th>
<th>HR, beats/min</th>
<th>PSP, mmHg</th>
<th>AF, ml/min</th>
<th>CF, ml/min</th>
<th>CO, ml/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control db/+</td>
<td>CM</td>
<td>4</td>
<td>357 ± 9g</td>
<td>55 ± 1</td>
<td>6.7 ± 0.1</td>
<td>1.6 ± 0.01</td>
<td>8.3 ± 0.1</td>
</tr>
<tr>
<td>Diabetic db/db</td>
<td>CM</td>
<td>4</td>
<td>302 ± 23g</td>
<td>57 ± 1</td>
<td>6.6 ± 0.1</td>
<td>1.3 ± 0.02</td>
<td>7.9 ± 0.2</td>
</tr>
<tr>
<td>Control SW</td>
<td>CM</td>
<td>6</td>
<td>370 ± 22</td>
<td>54 ± 1</td>
<td>7.5 ± 0.1</td>
<td>2.0 ± 0.03</td>
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<tr>
<td></td>
<td>P</td>
<td>8</td>
<td>317 ± 23</td>
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<tr>
<td>Diabetic SW(STZ)</td>
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<td>7.0 ± 0.6</td>
<td>1.6 ± 0.1</td>
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Values are means ± SE for the no. of heart perfusions. Hearts were perfused with either 0.4 mM chylomicrons (CM) or 0.4 mM palmitate (P). HR, heart rate; PSP, peak systolic pressure; AF, aortic flow; CF, coronary flow; CO, cardiac output; SW, Swiss Webster; STZ, streptozotocin induced. *P < 0.05 vs. appropriate control hearts.

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.min × 10 ⁻³; n = 4) and in SW(STZ) hearts (17.5 ± 0.4 mmHg/min × 10 ⁻³; n = 8) relative to respective control hearts (db/+ , 19.7 ± 0.1 mmHg/min × 10 ⁻³; n = 4; SW, 19.4 ± 0.7 mmHg/min × 10 ⁻³; n = 6). Similar results were obtained with perfusions of db/+ (STZ) hearts with chylomicrons, except that cardiac output (6.4 ± 0.1 ml/min) was reduced significantly compared with control db/+ hearts (8.3 ± 0.1 ml/min; Table 1). However, the dry weight of db/+ (STZ) hearts was lower than that of control hearts (Fig. 1). When cardiac output was corrected for this difference in dry weight, cardiac output for diabetic db/+ (STZ) hearts (284 ± 5 ml/min·g dry wt⁻¹) was no longer different from the cardiac output calculated for control db/+ hearts (296 ± 3 ml/min·g dry wt⁻¹).

DISCUSSION

Effect of diabetes on mouse heart LPL activity. LPL is an endothelium-bound enzyme in the heart that hydrolyzes the TG core of circulating lipoproteins like chylomicrons, providing FA for uptake and metabolism by cardiomyocytes. Thus LPL has an essential role in the utilization of TG-rich lipoproteins as an energy source for the heart (6, 14). Therefore, the first objective of this investigation was to determine whether endothelial-bound HR-LPL activity was changed in perfused hearts from diabetic mice.

Possible mechanisms that could change HR-LPL activity in diabetic hearts include 1) altered synthesis and/or processing of LPL in cardiomyocytes (6), 2) altered translocation from cardiomyocytes to functional sites on endothelial cells in the coronary vasculature (32), and 3) altered binding of LPL to the endothelium because of changes to heparan sulfate proteoglycans on the cell surface (10, 34). The influence of type 2 diabetes on cardiac LPL activity has not been investigated in detail. Although cardiac LPL activity in obese and insulin-resistant Zucker fatty rats has been reported to be either unchanged (27) or decreased (4), these studies did not measure functional endothelial-bound HR-LPL activity by a heparin perfusion protocol. Furthermore, HR-LPL activity has not been determined for hearts from Zucker diabetic fatty rats, another monogenic model of type 2 diabetes with obesity and insulin resistance (22). With perfused hearts from db/db mice, an accepted monogenic model of obesity and type 2 diabetes (9, 22), HR-LPL and residual tissue LPL activities were not different from activities in hearts from control db/+ heterozygotes or SW mice (Fig. 2). Although Kobayashi et al. (19) reported that LPL mRNA was reduced in hearts from db/db mice, this change in LPL gene expression must not be accompanied by any alteration in cardiac LPL activity. It should be noted that the absence of a change in HR-LPL activity in db/db hearts does not conflict with the observation that the hypertriglyceridemia in db/db mice (19) was due to reduced catabolism of TG-rich lipoproteins (23). First, endothelium-bound LPL could be reduced in other db/db tissues (adipose tissue, skeletal muscle) because of tissue-specific mechanisms that regulate LPL activity (6). Second, changes in the composition of the TG-rich lipoproteins (30) could reduce their catabolism in vivo without any change in LPL catalytic activity measured in vitro. And finally, an LPL inhibitor could be present in diabetic mice (20).

The effect of type 1 diabetes on cardiac LPL activity has been the subject of many more investigations, with discrepant results. Induction of insulin-deficient type 1 diabetes by STZ administration to rats has been associated with reduced (24, 29), unchanged (33), or increased (32, 36) cardiac HR-LPL activities. Differences in the duration and severity of STZ-induced diabetes and the influence of the rat strain (Sprague-Dawley, Wistar, Wistar Kyoto) contribute to this variability in responses of cardiac HR-LPL activity to type 1 diabetic conditions. In the present study, although there was a slight increase in peak HR-LPL activity (Fig. 2) in hearts from SW(STZ) and db/+ (STZ) mice, total HR-LPL activity, which will be the best reflection of endothelial-bound activity, was not different from total HR-LPL activities in control SW and db/+ hearts. It must be acknowledged that in vitro determinations of HR-LPL activity in postheparin perfusates may represent a maximal activity or capacity of LPL that may not necessarily reflect the in vivo activity of the endothelial-bound enzyme. Nevertheless, it is reasonable to conclude that neither type 2 diabetes (db/db mice) nor type 1 diabetes (STZ-treated SW and db/+ mice) has any effect on functional endothelial-bound cardiac HR-LPL or residual tissue LPL activities. In other words, the supply of LPL-derived FA from degradation of chylomicrons by perfused hearts from diabetic mice will be unchanged. Thus uptake of FA may be dependent on factors other than LPL activity, in particular...
the metabolic needs of the heart. Therefore, perfusions of diabetic hearts with chylomicrons allowed us to examine whether the fate of LPL-derived FA was altered, which was the second objective of this investigation.

Chylomicron and palmitate metabolism by diabetic mouse hearts. Perfusions of hearts from db/db, SW-(STZ), and db/+ (STZ) mice with radiolabeled chylomicrons revealed that both the oxidation and esterification of LPL-derived FA were enhanced compared with control SW and db/+ heart perfusions (Figs. 3 and 4). Therefore, both type 2 and type 1 diabetic models result in enhanced cardiac utilization of LPL-derived FA. This influence of diabetes on the fate of LPL-derived FA, with an increase in both oxidation and esterification to tissue TG, was very similar to the fate of [3H]palmitate complexed to albumin. Belke et al. (2) reported previously that rates of palmitate oxidation were increased in db/db hearts. The elevated TG content of db/db hearts (1) is consistent with the increased FA esterification observed in the present study. In addition, [3H]palmitate oxidation and esterification by type 1 SW(STZ) and db/+ (STZ) hearts were increased relative to control hearts (Fig. 5). Therefore, although the absolute rates of FA utilization were much higher for [3H]palmitate compared with LPL-derived FA from CM-[3H]TG catabolism, the stimulatory effect of diabetes on FA utilization was the same, irrespective of the source. It must be acknowledged that this comparison of chylomicron and palmitate metabolism by diabetic mouse hearts was conducted with concentrations that reflect a control postprandial (fed) condition, when chylomicrons (0.4 mM TG) will be present and FA concentrations will be low (0.4 mM; see Ref. 2), because adipose tissue lipolysis will be suppressed under fed conditions. Future experiments should determine the metabolism of diabetic hearts perfused with elevated concentrations of chylomicrons and FA that reflect diabetic conditions.

A number of biochemical mechanisms could account for the increase in FA utilization by diabetic hearts. Increased FA oxidation could be due to upregulation of mitochondrial uncoupling proteins (5, 16) in diabetic mouse hearts. The content of uncoupling protein 3 was increased in hearts from STZ-treated rats (43). It should be noted that the observation of increased rates of FA oxidation in diabetic mouse hearts (Figs. 3–5) without any change in contractile function (Table 1) suggests that cardiac efficiency was reduced in diabetic hearts, which could be due to uncoupling of oxidative phosphorylation and reduced ATP synthesis in mitochondria from diabetic hearts due to increased content of uncoupling protein(s). A second unknown aspect of efficiency is whether β-oxidation intermediates accumulated in cardiac tissue during the perfusion time course. Another mechanism resulting in increased FA oxidation in diabetic mouse hearts could be enhanced entry of FA into mitochondria because of a reduction in the content of malonyl-CoA, an inhibitor of carnitine palmitoyltransferase-1 (35, 38).

Because esterification to tissue TG was also increased in diabetic mouse hearts along with enhanced FA oxidation, additional mechanisms must also contribute to the overall increase in FA utilization by diabetic mouse hearts. Increased FA utilization could be due to increased rates of FA uptake by diabetic mouse hearts as the consequence of an increased abundance of putative FA transporters in the plasma membrane (28).

The plasma concentrations of both TG-rich lipoproteins and FA-albumin are increased in both type 1 (17) and type 2 (19, 23) diabetic conditions in mice. The chronic overutilization of plasma lipids by diabetic hearts could contribute to the reduced mechanical function that constitutes a diabetic cardiomyopathy (13, 25, 37), perhaps as a consequence of a cardiac lipotoxicity mechanism (40, 44). Consistent with this proposed lipotoxicity mechanism are recent observations of the use of genetically engineered mice with enhanced cardiac FA utilization, due to cardiac-specific overexpression of either long-chain fatty acyl-CoA synthetase (8) or peroxisome proliferator-activated receptor-α (12), which exhibited reduced cardiac contractile function. Therefore, interventions that can reduce FA utilization by diabetic hearts will be an important goal for future investigations.

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REFERENCES


