Altered metabolism causes cardiac dysfunction in perfused hearts from diabetic (db/db) mice

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1Department of Pharmacology and Therapeutics, University of Calgary, Calgary, Alberta, Canada T2N 4N1; 2Institute of Medical Biology, University of Tromsø, N-9037 Tromsø, Norway; and 3Department of Metabolic Diseases, Central Research Division, Pfizer, Groton, Connecticut 06340

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Belke, Darrell D., Terje S. Larsen, E. Michael Gibbs, and David L. Severson. Altered metabolism causes cardiac dysfunction in perfused hearts from diabetic (db/db) mice. Am J Physiol Endocrinol Metab 279: E1104–E1113, 2000.—Contractile function and substrate metabolism were characterized in perfused hearts from genetically diabetic C57BL/KsJ-lepr<sup>db</sup>/lepr<sup>db</sup> (db/db) mice and their non-diabetic lean littermates. Contractility was assessed in working hearts by measuring left ventricular pressures and cardiac power. Rates of glycolysis, glucose oxidation, and fatty acid oxidation were measured using radiolabeled substrates ([5-3H]glucose, [U-14C]glucose, and [9,10-3H]palmitate) in the perfusate. Contractile dysfunction in db/db hearts was evident, with increased left ventricular end diastolic pressure and decreased left ventricular developed pressure, cardiac output, and cardiac power. The rate of glycolysis from exogenous glucose in diabetic hearts was 48% of control, whereas glucose oxidation was depressed to only 16% of control. In contrast, palmitate oxidation was increased twofold in db/db hearts. The hypothesis that altered metabolism plays a causative role in diabetes-induced contractile dysfunction was tested using perfused hearts from transgenic db/db mice that overexpress GLUT-4 glucose transporters. Both glucose metabolism and palmitate metabolism were normalized in hearts from db/db-human insulin-regulatable glucose transporter (hGLUT-4) hearts, as was contractile function. These findings strongly support a causative role of impaired metabolism in the cardiomyopathy observed in db/db diabetic hearts.

Diabetic cardiomyopathy

NON-INSULIN-DEPENDENT (type 2) diabetes mellitus (NIDDM) accounts for >90% of all cases of diabetes. An increased incidence of cardiovascular diseases is the most common complication of NIDDM (19, 25). The cardiac complications associated with NIDDM are due to both increased coronary heart disease secondary to atherosclerosis (37) and a specific diabetic cardiomyopathy resulting in ventricular dysfunction (8, 34).

Experimental studies with animal models of diabetes allow assessment of the direct deleterious effects of a diabetic cardiomyopathy in the absence of atherosclerotic coronary artery disease. However, most reports of diabetes-induced cardiac dysfunction have used insulin-deficient (type 1) diabetic animals (39). For example, after induction of insulin deficiency in rats with streptozotocin, decreased contractility was evident in experiments with isolated working hearts (17, 28, 40) and papillary muscles (9). Comparable cardiac contractile dysfunction was observed in spontaneously diabetic BB rats (32), a genetic model of insulin-dependent diabetes. Similarly, metabolic alterations in hearts from insulin-deficient animals have been well characterized (31, 35), with decreased glucose utilization resulting in the almost exclusive utilization of fatty acids (FA) as an energy source. By comparison, relatively few studies of cardiac contractility and metabolism have been conducted with NIDDM animal models exhibiting insulin resistance (29).

Type 2 diabetes (NIDDM) in humans is characterized initially by insulin resistance, resulting in enhanced secretion of insulin (hyperinsulinemia) to maintain normoglycemia (3, 27). A subsequent defect in β-cell insulin secretion then leads to overt signs of diabetes such as hyperglycemia and hyperlipidemia. The diabetic db/db mouse exhibits a similar pathophysiology (6). An increase in plasma insulin precedes development of hyperglycemia; peak levels of insulin are observed at 8–12 wk of age and then decline rapidly. Thus the db/db mouse exhibits the essential characteristics of NIDDM, namely concomitant hyperinsulinemia, insulin resistance, and hyperglycemia.

Previous studies of cardiac defects in db/db mice have been limited to histological and biochemical assays (13, 18), mainly because the small size of the mouse heart makes it technically difficult to assess parameters of mechanical performance. However, the advent of genetically engineered mice as experimental models to study cardiac function has led to the development of techniques to examine contractile function in isolated perfused working mouse hearts (7, 11, 16, 20). Furthermore, we have recently established that the perfused working mouse heart is a good model for studying cardiac metabolism (4), notwithstanding the high intrinsic metabolic rate inherent in hearts from very small mammals.

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CARDIAC DYSFUNCTION IN DIABETIC (db/db) MICE

The first objective of our study was to evaluate contractile performance and metabolism of exogenous substrates (glucose and palmitate) in isolated perfused db/db hearts, to determine if decreased contractile function (diabetic cardiomyopathy) could be detected in this NIDDM model and to examine if cardiac metabolism was altered. Because diabetic (db/db) hearts exhibited a reduction in cardiac contractility with decreased exogenous glucose utilization (glycolysis and glucose oxidation) but enhanced FA (palmitate) oxidation, our second objective was to test the hypothesis (31) that alterations in cardiac metabolism play a causative role in diabetes-induced contractile dysfunction, using perfused hearts from transgenic db/db mice that overexpress insulin-regulatable glucose (GLUT-4) transporters (14) as the experimental model system. Metabolism of glucose and palmitate was normalized in isolated perfused hearts from db/db-GLUT-4 mice, and contractility was increased to control nondiabetic values.

METHODS

Animals

All experiments were approved by the University of Calgary Health Sciences Animal Welfare Committee and followed the guidelines of the Canadian Council on Animal Care. The control and diabetic mice used in this study were the C57BL/KsJ strain, obtained from Jackson Laboratories (Bar Harbor, ME). All animals used in this study were males between 10–14 wk of age, when plasma levels of insulin are at peak values with concomitant hyperglycemia (6). The C57BL/KsJ-lept/db-db mice exhibiting the obese-diabetic (NIDDM) phenotype (db/db) have two mutant copies of the leptin receptor gene, while lean littermates (heterozygotes) possessing one mutant and one normal copy of the leptin receptor gene (db/+ ) were used as controls. The animals were housed in groups and given ad libitum access to food and water (mouse diet 9 F; PMI Nutrition International, Brentwood, MO).

Transgenic mice overexpressing the human insulin-regulatable glucose transporter (hGLUT-4) in C57BL/KsJ-db/db diabetic mice (homozygous for the hGLUT-4 transgene; Line 8) were obtained from Pfizer (Groton, CT). The genotype and phenotype of db/db-hGLUT-4 mice have been described previously (14). These transgenic animals overexpress hGLUT-4 in the same tissues (adipose, skeletal, and cardiac) that express the murine GLUT-4 transporter and show improved contractile function from aortic pressures and coronary plus aortic flows and metabolism of exogenous substrates (protocol B). The hGLUT-4 transgene was introduced into the genome of C57BL/KsJ-Leprdb/db/db mice by homologous recombination (4). The Leprdb/db/db line was selected because of the obese-diabetic phenotype of the Leprdb/db/db mice, while the C57BL/KsJ line was selected for its normal glucose tolerance. These transgenic mice overexpress GLUT-4 in the same tissues (adipose, skeletal, and cardiac) that express the murine GLUT-4 transporter and show improved contractile function from aortic pressures and coronary plus aortic flows and metabolism of exogenous substrates (protocol B).

Heart Perfusion Conditions

Mice (fed dietary status) were heparinized with 100 U heparin (ip) 15 min before anesthesia with 10 mg sodium pentobarbital (ip). The heart was excised and placed in ice-cold Krebs-Henseleit bicarbonate (KHB) buffer. Extraneous tissues (pericardium, lung, trachea, etc.) were removed. The aorta was cannulated with an 18-gauge plastic cannula, and the heart underwent a Langendorff perfusion (60 mmHg perfusion pressure) with KHB buffer for ~20 min to wash blood out of the heart. During this time, the left atrium was cannulated with a 16-gauge steel cannula that was connected to the preload reservoir (20). Initially, when the heart was switched from Langendorff to working mode, the left atrium was perfused at a preload pressure of 15 mmHg (height of preload column above the left ventricle), and the output from the left ventricle was ejected into an afterload column with a height corresponding to a pressure of 50 mmHg. Hearts were divided into two groups: left ventricular (LV) function was measured in the first group (20), and both contractile function and energy substrate metabolism (4) were measured in the second group (see Experimental Protocol). In both groups, pressure measurements were made using a 2.5 F miniature pressure transducer (Millar Micro-Tip, Millar Instruments, Houston, TX) and recorded on-line (10-s duration for each recording) at 500 samples per second using CYSOFT 2.0 (Odessa Computer Systems, Calgary, Alberta, Canada) for data acquisition and analysis (20).

Perfusate Solution for the Working Heart

The KHB buffer used for the initial Langendorff perfusion (pH 7.4) consisted of (in mM): 118.5 NaCl, 25 NaHCO3, 1.2 MgSO4, 1.2 KH2PO4, 2.5 CaCl2, 0.5 EDTA, and 11 glucose and was gassed with 95% O2-5% CO2. The working heart buffer used in these experiments was a modified KHB buffer containing 0.4 mM palmitate bound to 3% BSA as a substrate in addition to 11 mM glucose (4). The perfusate solution was diazoxide overnight to remove low molecular weight contaminants in BSA (4). The BSA (Sigma Chemical, St. Louis, MO) was not essentially FA free; the FA content in the perfusate from BSA alone was 0.25–0.33 mM. Therefore, 0.4 mM palmitate added to the perfusate is in addition to this endogenous FA content, to give a total FA concentration of 0.7 mM. The same KHB buffer was always used for both control and diabetic db/db heart perfusions, so metabolic rates would be comparable. The buffer reservoirs and the preload line were water-jacketed and heated to maintain heart temperature at 37°C. The total recirculating perfusion volume was 35 ml.

Experimental Protocol

Isolated mouse hearts were subjected to two separate perfusion protocols: 1) measurement of contractile function from LV pressures (protocol A), or 2) simultaneous measurements of contractile function from aortic pressure and coronary plus aortic flows and metabolism of exogenous substrates (protocol B). The perfusate solution was diazoxide overnight to remove low molecular weight contaminants in BSA (4). The BSA (Sigma Chemical, St. Louis, MO) was not essentially FA free; the FA content in the perfusate from BSA alone was 0.25–0.33 mM. Therefore, 0.4 mM palmitate added to the perfusate is in addition to this endogenous FA content, to give a total FA concentration of 0.7 mM. The same KHB buffer was always used for both control and diabetic db/db heart perfusions, so metabolic rates would be comparable. The buffer reservoirs and the preload line were water-jacketed and heated to maintain heart temperature at 37°C. The total recirculating perfusion volume was 35 ml.

Perfusion protocol A. To assess LV contractile function in hearts from diabetic (db/db) and control (db/+ ) mice, LV pressure development was measured through a 23-gauge steel cannula inserted into the left ventricle through the apex of the heart as described by Larsen et al. (20). During this portion of the protocol, heart rate was controlled at 390 beats/min by means of a pacing electrode attached to the right atrium. Larsen et al. had reported previously that pacing to give heart rates >400 beats/min reduced contractile function in perfused working mouse hearts. The heart was allowed to stabilize for 10 min at a preload pressure of 15 mmHg, and then baseline measurements of LV pressure and flows (aortic and coronary) were recorded. After this, preload pressure was first decreased to 7 mmHg and later increased to 20 mmHg. LV pressure and flow measurements were recorded at each preload setting once the heart had stabilized. The pressure traces were examined for peak LV systolic pressure (LVSP), LV end diastolic pressure (LVEDP), and positive and negative dP/dt. LV developed pressure (LVDevP) was calculated from LVSP – LVEDP.
**Perfusion protocol B.** For metabolic determinations, rates of glycolysis for exogenous glucose, glucose oxidation, and palmitate oxidation were measured over a 60-min period in working hearts from control (db/+), diabetic (db/db), and transgenic (db/db-hGLUT-4) mice and perfused at a preflush of 15 mmHg according to the methods outlined in Belke et al. (4). Because the working heart apparatus must be made airtight by sealing the heart within the perfusion apparatus (to allow for collection of $^{14}$CO$_2$ as a result of glucose oxidation), pressure development was measured in the aortic line (9 cm above the tip of the aortic cannula) instead of directly from the left ventricle as described in protocol A. Hearts were not paced during this metabolic protocol, because pacing electrodes could not be attached to the right atrium because the perfusion apparatus was sealed. Heart rate was determined from the pressure traces by measuring the time interval between peak systolic values (average heart rate: diabetic db/db = 210 beats/min; control db/+ = 290 beats/min; transgenic db/db-hGLUT-4 = 275 beats/min). To ensure continuity between pressure measured in the left ventricle and the aortic line for the calculation of cardiac power output, a pilot study was performed on a series of hearts in which LV and aortic pressures were measured simultaneously. Aortic pressure development plotted against LVDevP yielded a straight line described by a linear equation: aortic pressure development = 0.61 (LVDevP) + 0.77, with $r^2 = 0.97$ for $n = 77$ measurements made with 11 hearts over a wide range of LVDevP (65–124 mmHg). Aortic pressure development can be used as a surrogate for LVDevP when calculating cardiac power output in this model (cardiac power = developed pressure × cardiac output). Cardiac power values were converted to milliwatts per gram of dry heart weight by multiplying the product of developed pressure and cardiac output (mmHg × ml·s$^{-1}$·g dry wt$^{-1}$) by the conversion factor 1.33 × 10$^{-3}$ (1 mmHg = 0.133 kPa; Ref. 36). Aortic and coronary flow measurements were made, as described previously, with graduated cylinders sealed within the perfusion apparatus that could be filled at one end and drained at the other (4). Cardiac output was determined as the sum of the aortic and coronary flow rates.

Throughout the 60-min perfusion for the metabolic protocol, pressure and flow measurements were obtained every 10 min. At 20-min intervals (starting at 0 min), a 2.5-ml sample of buffer was withdrawn through an injection port mounted between the buffer reservoir and the oxygenator for determinations of metabolite content (4). At the end of the experiment, the hearts were quickly frozen between metal blocks cooled to −80°C, weighed, and stored at −80°C. A sample of heart tissue (~20 mg) was cut from the heart, weighed (wet wt), and then dried to constant weight (dry wt). The ratio of this sample (dry-to-wet wt = 0.18) was used to calculate the total dry mass of the heart.

The metabolism of exogenous glucose and palmitate was measured according to the methods described in detail by Belke et al. (4). All determinations of substrate metabolism for each time point were made in duplicate. Glycolysis and glucose oxidation were measured simultaneously in one set of hearts, while palmitate oxidation was measured in a separate set of hearts. Glycolytic flux was determined by measuring the amount of $^3$H$_2$O released from the metabolism of exogenous [5-$^3$H]glucose (specific activity = 400 M bq/mol). Glucose oxidation was determined by trapping and measuring $^{14}$CO$_2$ released by the metabolism of [U-$^{14}$C]glucose (specific activity = 400 M bq/mol). Palmitate oxidation was determined in separate perfused hearts by measuring the amount of $^3$H$_2$O released from [9,10-$^3$H]palmitate (specific activity = 18.5 G bq/mol); calculation of palmitate oxidation rates took into consideration the endogenous FA content of the BSA in the perfusate. Metabolic rates were calculated using the total dry mass of the heart to correct for variations in heart size. Metabolic rates were also compared after normalization for differences in contractile performance (cardiac power output). The accumulation of lactate in the perfusate of control db/+ hearts was also measured after depolarization (Lactate Assay Kit 826-B; Sigma Chemical).

The yield of ATP that could be expected from glucose and palmitate metabolism was calculated by use of a stoichiometric ratio of 2 mol ATP per mole of glucose passing through glycolysis, 30 mol ATP per mole glucose being oxidized, and 105 mol ATP per mole palmitate being oxidized (26). This calculation assumes 100% coupling of mitochondrial oxidative phosphorylation.

**Analysis of Serum Metabolites**

Serum levels of glucose, triglyceride (TG), free FA, and insulin were determined for diabetic (db/db), control (db/+), and transgenic (db/db-hGLUT-4) mice, under fed dietary conditions only. Due to the need to heparinize mice before the working heart experiments, levels of metabolites were assessed in blood samples taken from separate groups of mice that were not heparinized. Blood samples were collected from these mice via cardiac puncture. The blood was centrifuged in an Eppendorf centrifuge at 14,000 rpm for 10 min. The resulting serum sample was stored at −20°C before analysis. Serum glucose and TG levels were measured with kits from Sigma Chemical; nonesterified free FA were measured with a Wako kit (Wako Chemicals GmbH, Neuss, Germany). Insulin levels were determined using a radioimmunoassay kit (Pharimacis) with human insulin standards.

**Statistics**

Differences in function and substrate metabolism between diabetic (db/db) and control (db/+), or transgenic (db/db-hGLUT-4) hearts were determined by means of an unpaired t-test or a Mann-Whitney test. Differences between means were regarded statistically significant when the P values were <0.05.

**RESULTS**

**Do Isolated Perfused db/db Hearts Exhibit Decreased Contractile Function?**

At 10–14 wk of age, diabetic (db/db) mice weighed significantly more than their lean nondiabetic (db+)/ or transgenic hearts were determined by means of an unpaired t-test or a Mann-Whitney test. Differences between means were regarded statistically significant when the P values were <0.05.

Diabetic hearts had a much
higher LVEDP compared with non-diabetic hearts. The major difference between control and diabetic perfused hearts was observed in overall cardiac output, which was 6.9 ml/min in control hearts and only 3.9 ml/min in diabetic hearts (Table 2). The greatest contribution to this difference occurred in aortic output, where flow in control hearts was more than twice the value observed in diabetic hearts (4.6 vs. 2.1 ml/min, respectively). In contrast, coronary flow was not different in control and diabetic hearts (2.3 vs. 1.7 ml/min, respectively).

To test the response of the heart to different preload pressures, LV pressure was also measured at 7 and 20 mmHg preloads. LVSP increased significantly in control hearts when the preload pressure was raised from 7 to 20 mmHg (69 ± 4 vs. 84 ± 3 mmHg, respectively). In contrast, systolic pressure did not increase significantly in diabetic hearts in response to the same change in preload (70 ± 7 vs. 75 ± 8 mmHg, respectively); as a consequence, LVSP in diabetic hearts at 20 mmHg preload was significantly lower (P < 0.05) than LVSP in control hearts. As expected, LVEDP increased in both groups with increased preload; however, LVEDP was higher in diabetic hearts than control hearts at both preloads. LVDevP decreased in diabetic hearts as preload was increased from 7 to 20 mmHg (from 53 ± 7 to 45 ± 6 mmHg), whereas pressure development in control hearts remained unchanged (63 ± 5 and 64 ± 5 mmHg).

In addition to the testing of the contractile response of the heart to changes in preload pressure, the stability of the heart perfused under standard conditions was examined over a 60-min period (Fig. 1) using cardiac power calculated from pressure development measured in the aortic line times cardiac output as an index of contractile performance (see Perfusion protocol B). For both control and diabetic hearts, cardiac power output was relatively constant over the perfusion period, although mechanical performance of diabetic hearts declined slightly toward the end of the perfusion period. Average cardiac power output calculated for control hearts from 10 to 60 min of perfusion was significantly higher (2.4-fold) than that calculated for diabetic hearts (Table 2).

### Table 1. Characteristics of control (db/+), diabetic (db/db), and transgenic (db/db-hGLUT-4) mice

<table>
<thead>
<tr>
<th>Mouse Type</th>
<th>Body Weight, g</th>
<th>Ventricle Wet Weight, mg</th>
<th>Serum Glucose, mM</th>
<th>Serum FFA, mM</th>
<th>Serum TG, mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (db/+ )</td>
<td>31.8 ± 0.9</td>
<td>155 ± 5</td>
<td>12.6 ± 1.3</td>
<td>0.43 ± 0.04</td>
<td>0.31 ± 0.06</td>
</tr>
<tr>
<td>Diabetic (db/db)</td>
<td>48.7 ± 1.2</td>
<td>153 ± 7</td>
<td>59.7 ± 6.5*</td>
<td>0.98 ± 0.09*</td>
<td>1.05 ± 0.37*</td>
</tr>
<tr>
<td>Transgenic (db/db-hGLUT-4)</td>
<td>57.4 ± 1.2*</td>
<td>167 ± 5</td>
<td>42.1 ± 4.5*</td>
<td>1.01 ± 0.06*</td>
<td>1.77 ± 0.21*</td>
</tr>
</tbody>
</table>

Data are means ± SE for control (db/+ (n=10), diabetic (db/db (n=10), and transgenic (db/db-human insulin-regulatable glucose transporter (hGLUT-4) (n=5) mice; serum samples were collected from fed mice. FFA, free fatty acids; TG, triglycerides. *Significantly different from control (db/+).

Cumulative values of glycolysis from exogenous glucose, glucose oxidation, and palmitate oxidation were measured for control and diabetic hearts over 60 min of perfusion, as shown in Fig. 2. Values for glycolysis and glucose oxidation were greater in control hearts than in diabetic hearts. In contrast, the cumulative measure of palmitate oxidation was higher in diabetic hearts than in control hearts. Average rates of glycolysis, glucose oxidation, and palmitate oxidation are shown in Fig. 3. The rate of glycolysis from exogenous glucose in diabetic hearts (1.57 μmol/min−1·g dry wt−1) was only

### Table 2. Ventricular function in perfused hearts from control (db/+ ) and diabetic (db/db) mice

<table>
<thead>
<tr>
<th></th>
<th>Control (db/+ )</th>
<th>Diabetic (db/db)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVSP, mmHg</td>
<td>83 ± 4(7)</td>
<td>76 ± 7(9)</td>
</tr>
<tr>
<td>LVEDP, mmHg</td>
<td>14 ± 2(7)</td>
<td>22 ± 3(9)</td>
</tr>
<tr>
<td>LVDevP, mmHg</td>
<td>69 ± 5(7)</td>
<td>55 ± 7(9)</td>
</tr>
<tr>
<td>+dP/dt, mmHg/s</td>
<td>3,580 ± 310(7)</td>
<td>3,020 ± 470(9)</td>
</tr>
<tr>
<td>−dP/dt, mmHg/s</td>
<td>2,370 ± 160(7)</td>
<td>2,055 ± 230(9)</td>
</tr>
<tr>
<td>Coronary flow, ml/min</td>
<td>2.3 ± 0.3(12)</td>
<td>1.7 ± 0.3(10)</td>
</tr>
<tr>
<td>Aortic flow, ml/min</td>
<td>4.6 ± 0.6(12)</td>
<td>2.1 ± 0.7(10)</td>
</tr>
<tr>
<td>Cardiac output, ml/min</td>
<td>6.9 ± 0.8(12)</td>
<td>3.9 ± 0.9(10)</td>
</tr>
<tr>
<td>Cardiac power, mW/g</td>
<td>29.9 ± 4.8(11)</td>
<td>12.3 ± 4.7(11)</td>
</tr>
</tbody>
</table>

Data are means ± SE for perfused hearts from control (db/+ ) and diabetic (db/db) mice; number of heart perfusions is given in parentheses. LVSP, LV systolic pressure; LVEDP, LV end diastolic pressure; LVDevP, LV developed pressure. *Significantly different from control (db/+).
48% of the rate observed in control hearts (3.24 μmol·min⁻¹·g dry wt⁻¹). The rate of glucose oxidation in diabetic perfused hearts was markedly reduced to 16% of control (0.08 vs. 0.49 μmol·min⁻¹·g dry wt⁻¹, respectively). Diabetic hearts do not exhibit a general reduction in oxidative metabolism, because rates of FA oxidation (Fig. 3) were elevated in diabetic hearts relative to control hearts (0.74 vs. 0.35 μmol·min⁻¹·g dry wt⁻¹). When glucose and palmitate oxidation are normalized for cardiac power output (Fig. 4), palmitate oxidation was still significantly higher in the diabetic heart, indicating decreased metabolic efficiency.

When substrate metabolic rates are converted to ATP production (based on optimum ratios of substrate conversion to ATP), it becomes apparent that FA oxidation accounts for a much greater proportion of energy production in diabetic hearts than in control hearts (Fig. 5); FA oxidation accounts for ~93% of calculated ATP production in diabetic hearts compared with 64% in control hearts. ATP derived from glucose metabolism (glycolysis and glucose oxidation) is severely depressed in diabetic hearts; glycolysis accounts for only 4% of ATP production in diabetic hearts relative to 11% in control, and glucose oxidation accounts for only 3% compared with 25% in control hearts. This shift in ATP production toward increased reliance on FA underestimates the true contribution of FA in the diabetic state, due to the fact that plasma FA concentrations are higher in vivo (0.98 mM; Table 1) than the concentration (0.7 mM) used for our heart perfusions. The pattern of substrate utilization in control (db/+) hearts (Fig. 5) was nearly identical to results obtained with perfused hearts from Swiss-Webster mice (4).

Does Normalization of Cardiac Metabolism in db/db Hearts Overexpressing GLUT-4 Glucose Transporters Result in Improved Contractile Function?

Transgenic db/db-hGLUT-4 mice still exhibited pronounced hyperglycemia under fed conditions (Table 1), as observed previously by Gibbs et al. (14). Improved glucose homeostasis in db/db-hGLUT-4 mice was primarily evident as reduced fasting hyperglycemia and improved glucose disposal after an oral glucose challenge (14). Serum FFA and TG remained elevated in db/db-hGLUT-4 mice (Table 1).

The depressed rates of glycolysis and oxidation of exogenous glucose observed in perfused db/db hearts were entirely normalized in hearts from db/db-hGLUT-4 transgenic mice (Figs. 2 and 3). This observation indicates that the hGLUT-4 transporters expressed in cardiac cell membranes (13) are indeed functional. Elevated rates of palmitate oxidation in
**Fig. 4.** Glucose (A) and palmitate oxidation (B) normalized for cardiac power output for control (db/+; filled bars), diabetic (db/db; open bars) and transgenic (db/db-hGLUT-4; hatched bars) perfused hearts. *Significantly different (P < 0.05) from control (db/+).*

**Fig. 5.** Percent contribution of individual metabolic pathways to overall ATP production (calculated) in isolated working hearts from control (db/+), diabetic (db/db), and transgenic (db/db-hGLUT-4) mice, based on utilization of exogenous substrates only.

db/db hearts, expressed either per gram dry heart weight (Fig. 3) or after normalization for cardiac power output (Fig. 4) were reduced to control values obtained with perfused hearts from db/+ mice. Consequently, the proportion of ATP production from glycolysis, glucose oxidation, and palmitate oxidation in db/db-hGLUT-4 hearts was the same as for control db/+ hearts (Fig. 5). Cardiac power in perfused db/db-hGLUT-4 hearts was increased to 37.8 ± 3.4 mW/g dry wt (Fig. 1), which was not significantly different from contractile function in control db/+ hearts (29.9 ± 4.8 mW/g dry wt; Table 2).

**DISCUSSION**

Isolated perfused working mouse heart preparations (4, 7, 11, 16, 20) permit phenotypic analysis of cardiac contractile function and metabolism in an ex vivo model where preload, afterload, heart rate, and metabolic substrate supply can be carefully controlled without neurohumoral influences. Therefore, contractile performance and metabolism of exogenous substrates (glucose and palmitate) were examined in isolated perfused working hearts from diabetic db/db mice, an NIDDM model, and compared with hearts from lean control db/+ mice.

**Isolated Perfused Hearts from db/db Mice Exhibit a Diabetic Cardiomyopathy, with Decreased Contractile Function and Altered Metabolism**

Cardiac mechanical performance was significantly reduced in perfused db/db hearts compared with contractile function in hearts from lean control (db/+ heterozygotes; so db/db hearts do exhibit a diabetic cardiomyopathy. Specific contractile parameters altered in db/db hearts were decreased LVSP (at high preloads), increased LVEDP, and decreased cardiac output (aortic flow) and cardiac power (Table 2; Fig. 1). The increase in LVEDP and decrease in developed pressure in response to an increase in preload is indicative of an inability of the db/db heart to respond to increased workload.

It should be noted that, for the hearts to produce aortic flow, peak LVSP must be high enough to overcome the pressure represented by the afterload column plus the resistance offered by the aortic outflow tract. The maximum height of the afterload column against which diabetic hearts were able to eject was ~60 mmHg (determined in pilot experiments), whereas nondiabetic hearts could eject against considerably higher columns. Thus peak LVSP and dP/dt values observed for the diabetic hearts under standard conditions were close to their ex vivo contractile potential, whereas the nondiabetic hearts exhibited a contractile reserve that was masked by the prevailing perfusion conditions.

This decreased contractile function in db/db hearts is in marked contrast to other studies with animal models of insulin resistance and obesity. Langendorff hearts from obese Zucker rats showed increased (not decreased) LV pressure and +dP/dt (33). The corpulent JCR:LA-cp rat exhibits severe insulin resistance (29). Isolated working hearts from cp/cp rats showed a rapid decline in function after only 5 min of perfusion unless a large concentration of insulin (2 mU/ml) was added to
the perfusate and the calcium concentration was reduced to 1.75 mM (21). Under these special perfusion conditions, cp/cp hearts exhibited no reduction in contractile function. In contrast, nonworking (Langendorff) perfused cp/cp hearts did exhibit a modest depression of contractile function at 3 mo of age (22, 23) but not in older age groups. It should be noted that the contractile dysfunction in perfused working db/db hearts was stable over 60 min of perfusion (Fig. 1) without addition of insulin or a reduction in perfusate calcium; the addition of insulin (1 mU/ml) to the perfusate did not improve cardiac power in db/db hearts (Belke, D.D. and Severson, D.L., unpublished observations). The absence of significant contractile dysfunction in hearts from Zucker rats (33) suggests that insulin resistance without overt signs of diabetes (hyperglycemia and hyperlipidemia) does not produce a deleterious effect on contractile function. By comparison, db/db mice showed severe diabetic characteristics, with a 5-fold elevation in serum glucose and a 2.3-fold increase in serum FA (Table 1) and specific signs of contractile dysfunction. Patients with type 2 diabetes (19) exhibit signs of diastolic dysfunction with elevated LVEDP (30), which are similar to our observations with db/db hearts. Despite development of techniques to allow perfusion of mouse hearts under working conditions (7, 11, 16, 20), the contractile performance of isolated hearts from control db/+ mice is considerably less than cardiac function measured in mice in vivo. For example, Georgakopoulos et al. (12) reported heart rates of 634 beats/min and a +dP/dt value of almost 12,000 mmHg/s in anesthetized mice. Therefore, it will be important to confirm that contractile dysfunction is also evident in db/db mice in vivo. It is possible that the reduction in contractile function in perfused db/db hearts could reflect a greater sensitivity to the trauma associated with the isolation of hearts from diabetic mice compared with control db/+ hearts, rather than reflecting an intrinsic difference in contractility due to the diabetic state in vivo.

The metabolism of exogenous substrates by db/db hearts was altered substantially, with reductions in glycolysis from exogenous glucose and glucose oxidation to 48 and 16% of control, respectively, with a concomitant more than twofold increase in the rate of palmitate oxidation. The reduction in glycolytic rate in db/db hearts will likely be due to the reduced plasma membrane content of the GLUT-4 transporter (14), although decreased activity of key glycolytic enzymes may also contribute. The greater depression in rates of glucose oxidation in diabetic hearts relative to the decrease in glycolytic rates suggests a decrease in mitochondrial pyruvate metabolism beyond that which can be explained by the decreased glycolytic supply of pyruvate, consistent with inhibition of the pyruvate dehydrogenase complex (18) because of increased FA oxidation in db/db hearts. Belke et al. (4) reported that perfusion of control mouse hearts with an elevated concentration of palmitate markedly reduced glucose oxidation. Enhanced rates of FA oxidation in db/db hearts could reflect increased cellular FA uptake as well as accelerated mitochondrial uptake and oxidation (35).

In contrast to the altered metabolism of db/db hearts (Figs. 2–5), working hearts from insulin-resistant cp/cp rats perfused with insulin had increased rates of glucose oxidation but unchanged rates of palmitate oxidation compared with hearts from lean control rats (21). Consequently, the percent contribution of glucose and FA oxidation to calculated ATP production was not altered in cp/cp hearts; however, metabolic rates could not be measured in the absence of insulin. The changes in substrate utilization in db/db hearts (Fig. 5) closely resemble metabolic changes in insulin-deficient rat hearts (31, 35).

Altered Metabolism of Exogenous Substrates and Reduced Contractile Function Is Normalized in Perfused Hearts from Transgenic db/db-hGLUT-4 Mice

Glycolytic ATP production is important for the normal function of cardiac membrane ion channels and pumps (15, 41). Consequently, inhibition of glycolysis results in altered control of intracellular calcium concentrations (1, 24, 42). Thus the reduction in glycolysis observed in db/db hearts (Fig. 2A) could adversely affect contractility. In addition, the nearly exclusive utilization of FA as an energy source in db/db hearts (Fig. 5) could impose an increased oxygen requirement in relation to cardiac work, with the potential for intracellular accumulation of FA metabolites that can have adverse effects on membrane function (31). Therefore, an intervention that can restore the altered metabolism of db/db hearts to normal may increase contractility.

Decreased glucose utilization in hearts from diabetic db/db mice is likely due to reduced content of insulin-sensitive GLUT-4 transporters at their functional site on the plasma membrane (14). Therefore, it was anticipated that isolated perfused hearts from db/db-hGLUT-4 mice with increased cardiac cell membrane hGLUT-4 content should exhibit increased glucose utilization, with concomitant decreased FA oxidation, if flux through pyruvate dehydrogenase is increased (35). Consequently, these hearts provided an experimental test of the hypothesis that altered metabolism causes the contractile dysfunction in db/db hearts. Indeed, reductions in rates of glycolysis and glucose oxidation, together with the elevated rate of palmitate oxidation in db/db hearts, were restored to control nondiabetic values in perfused hearts from transgenic db/db-hGLUT-4 hearts (Figs. 2–5). Remarkably, the reduced contractile performance in db/db hearts was also completely normalized (Fig. 1) in the transgenic hearts overexpressing hGLUT-4 transporters. Therefore, altered substrate utilization in db/db hearts makes a major contribution to reduced contractile function. Increasing cardiac work will increase oxidative metabolism. The converse conclusion, that normalization of cardiac contractile function is the cause (rather than the effect) of the improvement in glucose metabolism,
is not consistent with the observed reduction in FA oxidation in db/db-hGLUT-4 hearts, expressed as both absolute rates (Fig. 3) and rates normalized for cardiac power (Fig. 4). The normalization of the pattern of exogenous substrate utilization (Fig. 5) is more likely to be a direct result of increased cardiac hGLUT-4 expression.

In vivo, transgenic db/db-hGLUT-4 mice demonstrated improved glucose tolerance with elevated fed plasma insulin concentration (14), suggesting that pancreatic function is preserved by hGLUT-4 expression. Overexpression of hGLUT-4 transporters in db/db mice also occurs in skeletal muscle and adipose tissue (14). Thus the possibility that the improved cardiac contractile function in perfused hearts from transgenic db/db-hGLUT-4 mice is secondary to systemic changes that produce improved glucose tolerance cannot be excluded as a contributing mechanism, however. Investigations with cardiac-specific hGLUT-4 overexpression will be required to address this issue.

Interestingly, Abel et al. (2) recently reported that contractile performance was not altered in perfused hearts from transgenic knockout mice that lack GLUT-4 only in cardiac tissue, possibly because compensatory upregulation of GLUT-1 expression in this model increased basal glucose uptake by the heart. Hearts lacking GLUT-4 developed diastolic dysfunction under conditions of stress such as low-flow ischemia, however (38), in support of the hypothesis that abnormalities in cardiac substrate utilization can result in contractile dysfunction.

Limitations of This Study

An important feature of our metabolic measurements was the use of palmitate in addition to glucose as exogenous substrates. Many working mouse heart models developed to date (7, 11, 16) have used only carbohydrates as an exogenous fuel source, whereas it is well established that FA are the preferred oxidative substrate for the myocardium (26). However, a limitation in the metabolic portion of this study is that turnover of endogenous glycogen and TG was not assessed. Thus calculated rates of glucose utilization and turnover of endogenous glycogen and TG was not assessed. Thus calculated rates of glucose utilization and turnover of endogenous glycogen and TG was not assessed. The significance of the absence of exogenous lactate as a substrate in the perfusate is accentuated by the observation by Chatham et al. (5) that insulin-deficient diabetes produced a greater reduction in lactate oxidation compared with glucose oxidation.

Although lactate was not added as an exogenous substrate, low levels of lactate did accumulate in the perfusate of control db/+ hearts (0.14, 0.20, and 0.33 mM after 20, 40, and 60 min of perfusion, respectively; n = 2). Therefore, radiolabeled lactate may be generated from glycolysis of exogenous glucose and be released into the recirculating perfusate solution for subsequent uptake and oxidation to yield $^{14}$CO$_2$.

Utilization of lactate could also contribute to the curvilinear rates of glucose oxidation (Fig. 2B), reflecting an increasing contribution of lactate oxidation to $^{14}$CO$_2$ production at longer perfusion times. Thus glucose oxidation by perfused mouse hearts may be more appropriately referred to as carbohydrate oxidation.

These metabolic limitations must be considered before concluding that our results from isolated heart perfusions can be extrapolated to the more complex in vivo situation for both control db/+ and diabetic db/db mice.

Summary of Results and Future Investigations

Perfused working hearts from db/db mice exhibit a distinct cardiomyopathy, with reduced contractile
function and a shift in substrate utilization toward increased reliance on FA oxidation as an energy source. Determining the biochemical mechanisms responsible for the altered metabolism and contractile dysfunction in db/db hearts will be an important area of investigation in the future. The marked cardiac dysfunction in perfused db/db hearts differs significantly from observations with models of insulin resistance only, such as the Zucker rat heart (33), suggesting that relatively severe diabetic conditions are required for the pathophysiological mechanism(s) in db/db mice similar to the cardiomyopathy seen previously with insulin-deficient rat hearts (8, 31, 35). The altered pattern of substrate metabolism was restored to normal in perfused hearts from transgenic db/db-hGLUT-4 mice, and contractile function was normalized even though signs of diabetes (hyperglycemia and hyperlipidemia) were still evident, indicating that altered cardiac metabolism is an important causative factor in the contractile dysfunction. This finding can be extended in the future, using pharmacological interventions to correct the altered cardiac metabolism in db/db mice.

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