Increased Myocardial Oxygen Consumption Reduces Cardiac Efficiency in Diabetic Mice

Ole-Jakob How, Ellen Aasum, David L. Severson, W.Y. Anna Chan, M. Faadiel Essop, and Terje S. Larsen

Cardiac efficiency is the ratio between energy output (work) and energy input (myocardial oxygen consumption [MVO₂]) for the heart. Currently, the most accepted definition of total cardiac work is pressure-volume area (PVA), the sum of external mechanical work and the potential energy triangle (1). Importantly, MVO₂ is linearly related to PVA. Extrapolation of this linear relationship to 0 work gives unloaded (PVA independent) MVO₂, the oxygen cost of excitation-contraction coupling and basal metabolism.

Furthermore, the inverse slope of the MVO₂-PVA relationship defines the contractile efficiency.

Recently, How et al. (2) demonstrated that pressure-volume loops and resulting determinations of PVA can be obtained with ex vivo perfused working mouse hearts, using a combined micromanometer (pressure)-conductance (volume) catheter. A fiber-optic oxygen probe gave simultaneous measurements of MVO₂. An elevation in perfusate fatty acid concentration resulted in augmented fatty acid oxidation and reduced cardiac efficiency (increased MVO₂ with no change in work), manifested as increased unloaded MVO₂ (2).

Perfused hearts from db/db mice, a monogenic model of type 2 diabetes with obesity and insulin resistance, have been characterized as having an early increase in fatty acid oxidation that precedes the onset of contractile dysfunction (3). Because elevated rates of fatty acid oxidation produce a decrease in cardiac efficiency in control hearts (2), the objective of the current investigation was to test the hypothesis that cardiac efficiency will be reduced in diabetic db/db hearts because of enhanced rates of fatty acid oxidation (3). Accordingly, db/db hearts were perfused with both low and high concentrations of fatty acids (palmitate) in the perfusate. Moreover, comparative studies were performed with perfused hearts from a model of insulin-deficient type 1 diabetes, produced by the administration of streptozotocin (STZ) to control (db/+ ) mice (4). Both type 1 and type 2 diabetic hearts exhibited reduced cardiac efficiency, a characteristic that may have significant pathophysiological implications.

RESEARCH DESIGN AND METHODS
Male C57BL/KsJ-leprdb/leprdb and C57BL/KsJ-lepr+/lepr+ type 2 diabetic (db/db) mice and their nondiabetic heterozygote littermates (db/+ ) were purchased from M&B (Ry, Denmark) and used for investigations of cardiac efficiency and ventricular function at 12-13 weeks of age. The same mouse strains from Harlan (Oxon, U.K.) were used for measurements of myocardial metabolism at the University of Tromsø and mitochondrial respiration at the University of Cape Town. All animals were treated according to the guidelines on accommodation and care of animals formulated by the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes. Mice were housed at 23 ± 1°C on a 12-h light/dark cycle and given ad libitum access to food and water. Type 1 (insulin-deficient) diabetes was induced in 10-week-old db/db mice by injection of a total cumulative dose of 210 mg/kg i.p., which was administered as three individual doses delivered over 3 consecutive days (4, 5). The animals were killed and hearts perfused after 2 weeks, and only the animals that showed significantly elevated plasma glucose concentration were included.

Cannulation and instrumentation of the heart. After intraperitoneal injection of heparin (100 units), animals were anesthetized with sodium pentobarbital, after which the heart was quickly excised and cannulated for perfusion in the working mode, using Krebs-Henseleit buffer (KHB) buffer with 11 mmol/l glucose as well as albumin-bound fatty acids as energy substrates (2). A 1.4-F micromanometer-conductance catheter (Millar Instru-
Thereafter, new measurements of PVA-MVO₂ relationships were obtained over a wide range of workloads by stepwise changes in the hydrostatic pressure of preload (preload) was set to 8 mmHg; the afterload column was set to a height corresponding to 50 mmHg (2). The hearts were initially perfused with KHB (preload) for achieving an offset in the volume signal. The injections were performed as described above. Cardiac efficiency. Cardiac efficiency is the ratio between cardiac work (PVA) and MVO₂. PVA is derived from the sum of external stroke (mechanical) work and the potential energy triangle (1). Stroke work was calculated by integrating the pressure-volume loop, whereas the potential energy triangle was assessed by a temporary occlusion of the preload line at each steady state. This occlusion was performed to determine end-systolic and end-diastolic pressure-volume relationships. The volume intercept of these relationships is defined as \( V_{es} \). PVA was calculated according formula: \( PVA = SW + \left\{ V_{es} \times \left( V_{es} - V_{ed}/2\right) - \left[ V_{es} \times \left( V_{es} - V_{ed}/4\right)\right] \right\} \) in accordance with Korvald et al. (7), where SW is stroke work (the area in the pressure-volume loop), \( P_{es} \) is end-diastolic pressure, \( P_{ed} \) is end-systolic pressure, \( V_{es} \) is end-diastolic volume, and \( V_{ed} \) is end-systolic volume. MVO₂ was calculated by the following equation: \( MVO₂ = \left( P_{ed} \right) \times \left( \text{oxygenated perfusate} \right) \times \left( \text{Bunsen solubility coefficient of O₂} \right) \times \left( \text{coronary flow} \right) \). P_{ed} was measured by the use of a fiber-optic oxygen sensor (FOXY-AL300; Ocean Optics), which was connected to a spectrophotometer (USB2000-FL450; Ocean Optics) (2.8). Oxygen saturation of the coronary effluent was measured by placing the sensor in the opening of the pulmonary trunk.

Plasma analysis. After excision of the heart, a blood sample was taken from the chest cavity and quickly centrifuged; thereafter, the plasma was frozen at \(-70^\circ C\) for later analysis. The plasma concentrations of glucose, fatty acids, and triacylglycerol were measured using commercial kits from Boehringer Mannheim (no. 1442449; Mannheim, Germany), Wako Chemicals (no. 994-75409; Neuss, Germany), and ABX Diagnostics (Montpellier, France), respectively. Measurements of cardiac metabolism. In a separate set of experiments, fatty acid and glucose oxidation were measured as described in detail by Aasum et al. (9). Hearts were perfused in working mode for 40 min in the presence of 11 mmol/l glucose and either 0.3 or 1.4 mmol/l palmitate. Glucose and fatty acid oxidation was determined by measuring \(^{14}\text{CO}_2\) released by the metabolism of \([\text{U-}^{14}\text{C}]\text{glucose}\). Palmitate oxidation was determined by measuring the amount of \(\text{H}_2\text{O}_2\) released from \(9,10\)-H\text{palmitate}\. Metabolic rates were calculated based on \(\text{H}_2\text{O}^{14}\text{CO}_2\) production and the specific activities of the radiolabeled substrates in the perfusate.

Isolation of mitochondria and measurement of respiration. Mitochondria were isolated, using the method of Sordahl et al. (10) with slight modifications. Mitochondrial protein concentrations were determined, using the method of Lowry et al. (11). Mitochondrial respiration (state 2, 3, and 4) was measured polarographically at 25°C, using an oxigraph (Hansatech Instruments, London). Mitochondrial respiration analyses were performed only when the respiratory control ratio was \(\geq 2\). Two sets of experiments were performed, using either pyruvate or \(L\)-palmitoyl-carnitine/malate as substrates.

Statistics. Differences in cardiac function in response to increasing workloads were determined by repeated-measures ANOVA followed by unpaired Student’s t test for between-group analysis. Other data were assessed statistically by ANOVA followed by a paired (effect of elevated fatty acids within the same group) and/or unpaired (between groups) Student’s t test. Bonferroni’s method was applied in the case of multiple comparisons. \( P < 0.05 \) was considered statistically significant. All data are the means ± SE.

RESULTS

Characteristics of type 1 and type 2 diabetic mice. In accordance with previous results (9), db/db mice showed severe obesity and significantly elevated plasma concentrations of fatty acids and glucose, compared with nondiabetic controls (Table 1). Conversely, STZ-administered db/+ mice displayed reduced body weight and no elevation in plasma fatty acids compared with nondiabetic controls. Nevertheless, the diabetic state was confirmed by significantly elevated plasma glucose concentrations (Table 1) as well as the appearance of ketone bodies in the urine (not shown). In addition, heart weights were significantly lower in STZ-administered mice compared with controls, as noted previously (4). Thus, despite similarities regarding the degree of hyperglycemia, the type 1 and type 2 diabetic mouse models used in this study displayed distinct metabolic signatures.

Cardiac metabolism and mitochondrial respiration are altered in hearts from type 1 and type 2 diabetic mice. Glucose and fatty acid oxidation rates from db/+, db/db, and STZ-administered db/+ hearts are summarized in Fig. 2. Elevation of fatty acids in the perfusate (from 0.3 to 1.4 mmol/l) caused a marked shift in substrate utilization in control hearts. This can be seen by the eightfold increase in fatty acid oxidation combined with a marked reduction in glucose oxidation rates, reflecting metabolic control by the Randle cycle.
Substrate utilization by db/db hearts clearly differed from control db/+ hearts. At low fatty acid supply, glucose oxidation was markedly reduced, whereas fatty acid oxidation was fivefold elevated (Fig. 2). Perfusion of db/db hearts with an elevated perfusate fatty acid concentration produced a further increase in fatty acid oxidation and an additional decrease in glucose oxidation. The oxidation of fatty acids and glucose in hearts from STZ-administered mice was not different from control rates when perfused with low fatty acid supply (Fig. 2). However, there was a blunted response in the metabolic shift after elevation of fatty acids, as seen by only a 2.5-fold increase in fatty acid oxidation and a smaller reduction in glucose oxidation.

Respiration rates for mitochondria isolated from control, db/db, and STZ-administered hearts are shown in Table 2. State 3 respiration was elevated in db/db mitochondria incubated with palmitoyl-carnitine but not with pyruvate, consistent with the elevated rates of fatty acid oxidation observed with perfused db/db hearts (Fig. 2). In contrast, state 3 respiration was attenuated in type 1 (STZ-administered db/+ ) diabetic mouse mitochondria incubated with either palmitoyl-carnitine or pyruvate (Table 2).

Cardiac efficiency is reduced in type 1 and type 2 diabetic hearts. Exposure of perfused hearts to different loading conditions revealed a linear relationship between MVO2 and cardiac work (PVA), as observed previously (2). Figure 3 shows pooled data points relating MVO2 and PVA at increasing workloads in perfused working hearts from control, db/db, and STZ-administered mice. The data scatter includes 5–7 hearts from individual experiments listed in Table 3. Table 3 also gives the slope and y-intercept for the individual regression lines, as well as group means. The y-intercept (unloaded MVO2) was significantly higher in both db/db and STZ-administered hearts compared with control hearts, indicating reduced cardiac efficiency in unloaded type 1 and type 2 diabetic hearts. At low fatty acid supply (0.3 mmol/l), hearts from db/db and STZ-administered mice consumed 86 and 57% more oxygen for noncontractile purposes, respectively, compared with control hearts (indicated by the y-intercept of the MVO2-PVA regression lines in the upper panel of Fig. 3 as well as in Table 3).

At high fatty acid (1.4 mmol/l) supply, control hearts showed a relatively small (17%) but significant elevation in unloaded MVO2 (Table 3); in contrast, increased fatty acid supply had no effect on the already elevated unloaded MVO2 in db/db and STZ-administered hearts. However, unloaded MVO2 was still significantly higher in diabetic hearts (both models) than in control hearts during high fatty acid supply. Strikingly, hearts from db/db mice showed a significant increase in the slope of the MVO2-PVA regression line after the increase in perfusate fatty acids, which implies a reduction in contractile efficiency. The contractile efficiency of STZ hearts was unaffected by the changes in substrate supply.

The reduction in cardiac efficiency was most pronounced in db/db hearts (Table 3) and was consistent over a broad range of workloads (Fig. 3). In STZ-administered hearts, the more moderate decrease in cardiac efficiency diminished at increased workloads.

Ventricular function in perfused working hearts from type 1 and type 2 diabetic hearts. Stepwise changes in pre- and afterload settings revealed end-diastolic and end-systolic pressure-volume relationships (Fig. 4A). The ventricular function of db/db hearts differed substantially compared with the two other groups (Fig. 4B); at all steady states, the pressure-volume loop was shifted to the left in

**TABLE 1**

Characteristics of control (db/+), type 2 (db/db), and type 1 (STZ-administered db/+ ) diabetic mice; body weights and dry heart weights; and plasma levels of glucose, free fatty acids, triglycerides, and insulin at time of death

<table>
<thead>
<tr>
<th>Mice</th>
<th>Body weight (g)</th>
<th>Heart dry weight (mg)</th>
<th>Glucose (mmol/l)</th>
<th>Fatty acids (mmol/l)</th>
<th>Triglycerides (mmol/l)</th>
<th>Insulin (μg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>db/+</td>
<td>28.3 ± 0.7</td>
<td>30.5 ± 1.0</td>
<td>11.2 ± 0.4</td>
<td>0.51 ± 0.04</td>
<td>0.68 ± 0.07</td>
<td>1.20 ± 0.21</td>
</tr>
<tr>
<td>db/db</td>
<td>47.0 ± 0.8*</td>
<td>27.6 ± 0.4</td>
<td>32.2 ± 1.6*</td>
<td>1.03 ± 0.08*</td>
<td>0.89 ± 0.09</td>
<td>4.77 ± 0.83*</td>
</tr>
<tr>
<td>STZ</td>
<td>21.5 ± 0.6*</td>
<td>21.2 ± 0.4*</td>
<td>31.8 ± 1.8*</td>
<td>0.45 ± 0.06</td>
<td>0.57 ± 0.09</td>
<td>0.25 ± 0.04*</td>
</tr>
</tbody>
</table>

Data are means ± SE. n = 15–19, and n = 6–11 for the insulin values. *P < 0.05 vs. db/+.

**FIG. 2.** Glucose and fatty acid oxidation rates were measured in hearts from control (db/+ , n = 7), type 2 (db/db, n = 5), and type 1 (STZ-administered db/+, n = 9) diabetic mice, perfused with low and high fatty acid concentrations. *P < 0.05 vs. db/+ hearts; **P < 0.05 vs. low fatty acids. □, low fatty acid concentration; □, high fatty acid concentration.
whereas state 4 respiration was recorded after complete phosphorylation of added ADP. Respiration was measured in the absence of added ADP; rates of state 3 respiration were recorded after the addition of 300 

Respiration rates with different oxidative substrates for mitochondria isolated from control (db/+), type 2 (db/db), and type 1 (STZ-administered db/+ ) diabetic mice

<table>
<thead>
<tr>
<th></th>
<th>Pyruvate</th>
<th>Malate/carnitine- L-palmitoyl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>State 2</td>
<td>State 3</td>
</tr>
<tr>
<td></td>
<td>16.9 ± 2.1</td>
<td>14.3 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>68.7 ± 11.0</td>
<td>53.1 ± 4.7</td>
</tr>
<tr>
<td></td>
<td>13.2 ± 3.6</td>
<td>9.2 ± 2.7</td>
</tr>
<tr>
<td></td>
<td>39.1 ± 2.7</td>
<td>37.8 ± 2.7</td>
</tr>
<tr>
<td></td>
<td>103.7 ± 23.5</td>
<td>157.9 ± 13.7*</td>
</tr>
<tr>
<td></td>
<td>22.6 ± 6.4</td>
<td>23.3 ± 5.5</td>
</tr>
</tbody>
</table>

Data are means ± SE. Mitochondrial respiration was measured using either 7 mmol/l pyruvate or 25 μmol/l palmitoyl-carnitine/5 mmol/l malate as substrates (n = 5–8). The mitochondrial respiration data are expressed in nmol O₂ · min⁻¹ · mg protein⁻¹. Basal (state 2) respiration was measured in the absence of added ADP; rates of state 3 respiration were recorded after the addition of 300 μmol/l ADP, whereas state 4 respiration was recorded after complete phosphorylation of added ADP. *P < 0.05 vs. db/+.

db/db hearts. Intrinsic heart rates in both diabetic models (db/db and STZ) were significantly reduced at all workloads (Fig. 5 and Table 4). Only db/db hearts, however, showed reduced cardiac output. In all groups cardiac function was unaffected by the elevation of fatty acids in the perfusate, except for a minor reduction in contractility in db/db hearts, shown by the small reduction in E_max (the slope of time-varying maximal ventricular elastance) and the preload recruitable stroke work index (Table 4). However, all hearts responded with increased MVO₂ after elevation of fatty acids, which was most evident in control hearts.

**FIG. 3.** Pooled scatter plot showing the relationship between MVO₂ and PVA at increasing workloads in hearts from control (db/+, n = 5), type 2 (db/db, n = 7), and type 1 (STZ-administered db/+, n = 7) diabetic mice. A: Data obtained at low fatty acid concentrations. B: Data obtained at high fatty acid concentrations. Each heart was subjected to different workloads by varying the preload (3–12.5 mmHg) and afterload (35–65 mmHg) settings. The regression line for each group is based on the average y-intercepts and slopes given in Table 3.

**TABLE 2**

<table>
<thead>
<tr>
<th></th>
<th>db/+</th>
<th>db/db</th>
<th>STZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyruvate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>State 2</td>
<td>16.9</td>
<td>14.3</td>
<td>15.2</td>
</tr>
<tr>
<td>State 3</td>
<td>68.7</td>
<td>53.1</td>
<td>30.3</td>
</tr>
<tr>
<td>State 4</td>
<td>13.2</td>
<td>9.2</td>
<td>8.3</td>
</tr>
<tr>
<td>Malate/carnitine- L-palmitoyl</td>
<td>39.1</td>
<td>37.8</td>
<td>23.9</td>
</tr>
<tr>
<td>db/+</td>
<td>103.7</td>
<td>157.9</td>
<td>56.3</td>
</tr>
<tr>
<td>STZ</td>
<td>22.6</td>
<td>23.3</td>
<td>13.3</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Measurements of MVO₂ (including any use of oxygen that might not be tightly coupled to oxidative phosphorylation) and PVA over a wide range of workloads are essential for a proper evaluation of cardiac efficiency. Reduced cardiac efficiency may be caused by an increase in unloaded MVO₂ (the y-intercept of the regression line), reflecting the oxygen cost of excitation-contraction coupling and/or basal metabolism. In addition, a reduction in contractile efficiency (increased slope of the regression line) can contribute to overall cardiac inefficiency. The current investigation tested the hypothesis that diabetic hearts exhibit reduced cardiac efficiency, using perfused working hearts from both type 1 (STZ-induced) and type 2 (db/db) diabetic mice.

Cardiac efficiency was reduced in type 2 diabetic hearts, as evidenced by the 86% increase in unloaded MVO₂ (Table 3). A likely explanation for this reduction in cardiac efficiency is the altered metabolism of db/db hearts (Fig. 2) because other investigations have shown that elevated rates of fatty acid uptake/oxidation produced an increase in unloaded MVO₂ in nondiabetic hearts from dog (12), pig (13), rat (14), and mouse (2). The extra oxygen cost of increased fatty acid oxidation relative to glucose oxidation will, however, make a minor contribution because there is only a theoretical 11% decrease in efficiency in hearts shifting from 100% glucose oxidation to 100% palmitate oxidation (15), an extreme condition that does not apply to the metabolic rates measured in perfused db/db hearts (Fig. 2). We therefore propose that intracellular futile metabolic cycles may also contribute to the increase in unloaded MVO₂ in db/db hearts. For example, with high intracellular fatty acid levels, a triacylglycerol–fatty acid cycle has been shown to increase oxygen consumption by up to 30% (16). Finally, peroxisome proliferator–activated receptor-α–dependent upregulation of mitochondrial uncoupling proteins induced by elevated plasma fatty acids (17) could dissipate the proton gradient across the inner mitochondrial membrane; uncoupling of electron flow from oxidative phosphorylation will reduce ATP synthesis and increase O₂ consumption (18). Examination of these mechanisms will be an important objective for future investigations.

An elevation in perfusate fatty acids to 1.4 mmol/l did not produce a further increase in unloaded MVO₂ in db/db
vated MVO$_2$. The findings in the current study are in
contrast to a recent study by Mazumder et al. (19), where
reduced ventricular work and elevated MVO$_2$. Neverthe-
less, results from the current investigation and Mazumder
et al. (19) both support a common conclusion that cardiac
oxidation pattern as controls, but they still had a
57% increase in unloaded MVO$_2$ (Table 3). The increased
unloaded MVO$_2$ in these diabetic hearts could therefore not be
explained in terms of elevated fatty acid oxidation.

Unloaded MVO$_2$ consists of two components: basal
metabolism and the oxygen cost of excitation-contraction
coupling. Several studies have shown that high extracel-
nular Ca$^{2+}$ concentration or β-adrenergic stimuli increased
the oxygen cost for excitation-contraction coupling and
consequently elevated unloaded MVO$_2$ (21); conversely,
low Ca$^{2+}$ concentration or a calcium antagonist decreased
unloaded MVO$_2$ (22). It is reasonable to suggest, therefore,
that abnormal Ca$^{2+}$ homeostasis as shown in diabetic
hearts (23) could contribute to the increased unloaded
MVO$_2$ in hearts from both of the diabetic models.

The decreased cardiac efficiency in hearts from STZ-
administered $db/+$ mice, manifested by an increase in
unloaded MVO$_2$, contrasts with data obtained with diabetic
hearts from STZ-administered sheep (24), in which de-
creased cardiac efficiency was the result of impaired
contractile efficiency with no change in unloaded MVO$_2$. In
fact, the slope of the PVA-MVO$_2$ relationship for STZ-
administered mouse hearts was the lowest (Table 3). Diabetic
sheep had elevated plasma fatty acids and increased
cardiac fatty acid uptake (24), a metabolic profile that is more similar to $db/db$ mice.

The metabolic phenotype of diabetic hearts will reflect,
in part, an adaptation to chronic changes in substrate
supply in vivo (25). Type 2 diabetic $db/db$ mice have elevated plasma lipids (Table 1) (3). Thus, oversupply of

### Table 3

<table>
<thead>
<tr>
<th></th>
<th>Low fatty acids</th>
<th></th>
<th>High fatty acids</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$y$-intercept · 10$^{-2}$</td>
<td>Slope</td>
<td>$r^2$</td>
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<tr>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2.5</td>
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</tr>
<tr>
<td>2</td>
<td>2.0</td>
<td>2.4</td>
<td>0.95</td>
</tr>
<tr>
<td>3</td>
<td>1.8</td>
<td>3.2</td>
<td>0.97</td>
</tr>
<tr>
<td>4</td>
<td>1.4</td>
<td>2.8</td>
<td>0.91</td>
</tr>
<tr>
<td>5</td>
<td>2.9</td>
<td>2.1</td>
<td>0.86</td>
</tr>
<tr>
<td>Means ± SE</td>
<td>2.1 ± 0.3</td>
<td>2.5 ± 0.3</td>
<td>0.91 ± 0.03</td>
</tr>
<tr>
<td>$db/db$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>3.7</td>
<td>2.1</td>
<td>0.96</td>
</tr>
<tr>
<td>2</td>
<td>4.8</td>
<td>2.5</td>
<td>0.94</td>
</tr>
<tr>
<td>3</td>
<td>3.0</td>
<td>1.4</td>
<td>0.95</td>
</tr>
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<td>4.0</td>
<td>3.2</td>
<td>0.89</td>
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<tr>
<td>5</td>
<td>4.0</td>
<td>2.9</td>
<td>0.87</td>
</tr>
<tr>
<td>6</td>
<td>4.7</td>
<td>1.9</td>
<td>0.90</td>
</tr>
<tr>
<td>7</td>
<td>3.4</td>
<td>2.3</td>
<td>0.97</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>3.9 ± 0.3†</td>
<td>2.3 ± 0.3</td>
<td>0.92 ± 0.02</td>
</tr>
</tbody>
</table>

The $y$-intercept represents MVO$_2$ for unloaded hearts, expressed as Joules · beat$^{-1}$ · gram dry heart wt$^{-1}$. Slope is dimensionless, whereas $r^2$ is the square of the regression coefficient. Only experiments with a regression coefficient $>0.9$ ($r^2 ≥ 0.81$) were included. *$P < 0.05$ vs. low fatty acids; †$P < 0.05$ vs. $db/+$ hearts.

hearts (Table 3), even though fatty acid oxidation rates
were enhanced (Fig. 2), suggesting that the contribution of
fatty acid metabolism to cardiac inefficiency was already
maximal in $db/db$ hearts perfused at low fatty acid levels. On
the other hand, $db/db$ hearts perfused with 1.4 mmol/l
fatty acids did exhibit a significant increase in the slope of
the PVA-MVO$_2$ relationship (Table 3), indicating that a fatty
acid–induced decrease in contractile efficiency could also
contribute to the overall reduction in cardiac efficiency.

The decreased efficiency caused by elevated substrate
fatty acids in the control hearts was the result of increased
MVO$_2$, whereas ventricular work was unaffected (Tables 3
and 4). This suggests that the hearts were normoxic under
all perfusion conditions because cardiac performance at
high fatty acid supply is only impaired on insufficient
oxygen delivery to the myocardium (12,14). Similarly, the
decreased efficiency in $db/db$ hearts was caused by ele-
vated MVO$_2$. The findings in the current study are in
contrast to a recent study by Mazumder et al. (19), where
reduced cardiac efficiency in $ob/ob$ hearts was caused by
reduced ventricular work and elevated MVO$_2$. Neverthe-
less, results from the current investigation and Mazumder
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efficiency is reduced in diabetic $db/db$ and $ob/ob$ hearts. In
contrast, a study on type 2 diabetic ZDF rat hearts reported
that cardiac efficiency was normal, despite ele-
vated rates of fatty acid oxidation (20).

Alterations in metabolism related to hyperlipidemia (as
described above) are probably not the only factor causing
the pronounced oxygen waste in the unloaded $db/db$
hearts. This is supported by the fact that STZ-administered
hearts displayed a similar plasma fatty acid profile and
cardiac oxidation pattern as controls, but they still had a
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consequently elevated unloaded MVO$_2$ (21); conversely,
low Ca$^{2+}$ concentration or a calcium antagonist decreased
unloaded MVO$_2$ (22). It is reasonable to suggest, therefore,
that abnormal Ca$^{2+}$ homeostasis as shown in diabetic
hearts (23) could contribute to the increased unloaded
MVO$_2$ in hearts from both of the diabetic models.

The decreased cardiac efficiency in hearts from STZ-
administered $db/+ $ mice, manifested by an increase in
unloaded MVO$_2$, contrasts with data obtained with diabetic
hearts from STZ-administered sheep (24), in which de-
creased cardiac efficiency was the result of impaired
contractile efficiency with no change in unloaded MVO$_2$. In
fact, the slope of the PVA-MVO$_2$ relationship for STZ-
administered mouse hearts was the lowest (Table 3). Diabetic
sheep had elevated plasma fatty acids and increased
cardiac fatty acid uptake (24), a metabolic profile that is more similar to $db/db$ mice.

The metabolic phenotype of diabetic hearts will reflect,
in part, an adaptation to chronic changes in substrate
supply in vivo (25). Type 2 diabetic $db/db$ mice have elevated plasma lipids (Table 1) (3). Thus, oversupply of
fatty acids to db/db hearts will unquestionably be a factor in subsequent metabolic alterations, namely decreased glucose utilization and increased fatty acid oxidation (Fig. 2). It should be noted, however, that the absence of a functional leptin receptor in the db/db mouse could impair insulin sensitivity independently of hyperglycemia and hyperlipidemia, and extrapolation to type 2 diabetes in obese humans demands caution. Moreover, STZ treatment of db/+ mice produced an equivalent degree of hyperglycemia (confirming their insulin-deficient diabetic status), although plasma lipids were not elevated (Table 1). This somewhat surprising finding is most likely related to the duration and/or severity of the diabetic state; the animals showed a significant reduction in body weight, and visible fat was virtually absent at the time of death, which excludes mobilization of fatty acids from endogenous sources. Thus, the absence of any change in lipid substrate supply in vivo may explain the absence of any alteration in glucose and fatty acid oxidation (Fig. 2). Interestingly, the increase in fatty acid oxidation and suppression of glucose oxidation caused by the elevation in perfuse fatty acids in STZ-induced diabetic hearts was blunted compared with control or db/db hearts (Fig. 2), indicating that short-term (2 weeks) insulin deficiency had altered the metabolic phenotype of the mouse hearts. Finally, it must be acknowledged that results shown in Fig. 2 for hearts from STZ-administered db/+ mice are not consistent with a previous study by Neitzel et al. (4) that reported elevated palmitate oxidation by perfused hearts from STZ-administered db/+ mice. However, these authors did not measure glucose oxidation or plasma lipids; therefore, there may be differences in the type 1 diabetic model.

The observation that state 3 respiration by db/db mitochondria was elevated in the presence of palmitoyl-carnitine (Table 2) is consistent with enhanced rates of fatty acid oxidation measured with perfused db/db hearts. Respiration in mitochondria from STZ-administered hearts was generally impaired, with a significant reduced state 3 when pyruvate was used as substrate, indicating mitochondrial dysfunction (Table 2). STZ-administered db/+ mice also had decreased body weight and heart weight (Table 1), and this observation supports previous data by Lashin and Romani (26) that mitochondrial dysfunction in STZ-administered rat hearts required not only hyperglycemia but other signs of diabetes, such as weight loss. It cannot be excluded, however, that isolation of mitochondria from the more fragile STZ-administered hearts resulted in a lower fraction of intact mitochondria, which in turn could explain the impaired respiration in these preparations.

Previous studies have shown that db/db hearts exhibit a progressive age-dependent decline in contractile performance (3). The ability to obtain instantaneous pressure-volume loops in perfused mouse hearts has provided new mechanistic insights into alterations in ventricular function. Thus, the current study revealed that the end-diastolic, as well as the end-systolic, pressure-volume relationships for db/db hearts were markedly shifted to the left (Fig. 4) relative to control hearts. The interpretation of this finding is not obvious, but ventricular remodeling with concentric hypertrophy and/or reduced compliance because of myocardial fibrosis seems plausible. In accordance with the review by Cosson and Kevorkian (27), hearts from db/db mice also showed signs of diastolic dysfunction; the increased Tau value (Table 4) is indicative of an abnormal calcium reuptake into the sarcoplasmic reticulum (23), resulting in impaired relaxation in early diastole. In addition, the slope of the end-diastolic pressure-volume relationship and end-diastolic pressure was clearly increased for db/db hearts, indicating ventricular chamber stiffness and dysfunction also in late diastole (Figs. 4 and 5). The underlying mechanisms of these diastolic abnormalities and their potential contribution to the onset of heart failure, as reviewed by Kass et al. (28), require further investigations.

Elevation of perfusate fatty acids did not significantly affect cardiac performance in either control or diabetic hearts, except for a small decrease in contractility (reduced preload recruitable stroke work index and $E_{\text{max}}$) in db/db hearts (Table 4) associated with the reduction in contractile efficiency (increased slope of the PVA-MVo2
further investigations are required to reveal the impact of altered calcium homeostasis (23). However, hearts after elevation of fatty acids are probably a consequence of altered calcium homeostasis (23). However, further investigations are required to reveal the impact of fatty acids on contractility in the diabetic heart.

Intrinsic heart rates in both diabetic models (db/db and STZ) were significantly lower compared with control heart rates (Fig. 5 and Table 4). In STZ hearts, prolonged diastolic filling time resulted in elevation of stroke volume that compensated for reduced heart rates, so that cardiac output was similar to control. The db/db hearts, however, had no increase in stroke volume, despite the reduced heart rates and thus reduced cardiac output compared with controls, suggesting reduced compliance in the db/db left ventricle. This reduced compliance corresponded with an elevation in end-diastolic pressure (Fig. 5) in db/db

Table 4

<table>
<thead>
<tr>
<th></th>
<th>db/+ (n = 6)</th>
<th>db/db (n = 8)</th>
<th>STZ (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low fatty acids</td>
<td>High fatty acids</td>
<td>Low fatty acids</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>334 ± 11</td>
<td>320 ± 18</td>
<td>295 ± 12</td>
</tr>
<tr>
<td>End-systolic pressure (mmHg)</td>
<td>69 ± 2</td>
<td>70 ± 2</td>
<td>74 ± 3</td>
</tr>
<tr>
<td>End-diastolic pressure (mmHg)</td>
<td>7 ± 1</td>
<td>8 ± 1</td>
<td>9 ± 1</td>
</tr>
<tr>
<td>dP/dt max (mmHg/s)</td>
<td>4,915 ± 199</td>
<td>5,005 ± 248</td>
<td>5,483 ± 343</td>
</tr>
<tr>
<td>dP/dt min (mmHg/s)</td>
<td>-4,352 ± 203</td>
<td>-4,152 ± 257</td>
<td>-4,682 ± 264</td>
</tr>
<tr>
<td>Tau (ms)</td>
<td>15 ± 1</td>
<td>15 ± 1</td>
<td>16 ± 3</td>
</tr>
<tr>
<td>Cardiac output (ml/min)</td>
<td>11.4 ± 0.7</td>
<td>10.8 ± 0.7</td>
<td>9.4 ± 0.3</td>
</tr>
<tr>
<td>Coronary flow rate (ml/min)</td>
<td>2.5 ± 0.4</td>
<td>2.6 ± 0.5</td>
<td>2.1 ± 0.1</td>
</tr>
<tr>
<td>$E_{max}$</td>
<td>3.4 ± 0.3</td>
<td>3.7 ± 0.6</td>
<td>6.6 ± 0.9</td>
</tr>
<tr>
<td>PRSWI</td>
<td>51.5 ± 3.7</td>
<td>45.6 ± 6.2</td>
<td>57.3 ± 3.2</td>
</tr>
<tr>
<td>EDPVR × 10⁻²</td>
<td>6.5 ± 0.6</td>
<td>6.7 ± 0.9</td>
<td>9.2 ± 1.1</td>
</tr>
</tbody>
</table>

Parameters of cardiac function were measured with low and high fatty acid concentrations (before and after the buffer replacement) in working hearts at 8 mmHg preload and 50 mmHg afterload. The relaxation constant Tau (glanz) is the regression of dP/dt versus pressure. The time-varying maximal ventricular elastance ($E_{max}$), preload recruitable stroke work index (PRSWI), and exponential fit of the relationship between pressure and volume at end diastole (EDPVR) were assessed from a family of pressure-volume loops created by a temporary preload occlusion. *P < 0.05 vs. low fatty acids.
hearts. Thus, db/db hearts have impaired diastolic properties that are revealed at high workloads. Comparison of ventricular function at different heart rates is problematic because several functional parameters are heart rate–dependent. However, pacing electrodes markedly influence the conductance signal of the high-fidelity pressure-volume catheter.

In summary, this is the first study showing decreased cardiac efficiency in hearts from diabetic mice (both type 1 and type 2) assessed by the MVO2-PVA relationship. This inefficiency was revealed as a pronounced oxygen waste in the unloaded heart, which may compromise ventricular function when oxygen demand is high (elevated workloads) or when oxygen delivery is limited (ischemic insult).

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