Echocardiographic assessment of cardiac function in diabetic db/db and transgenic db/db-hGLUT4 mice

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THE MOST PREVALENT FORM (90%) of diabetes mellitus is Type 2 (non-insulin-dependent) diabetes, resulting from the combination of insulin resistance and a β-cell secretory defect (6). Increased cardiovascular disease is the most common complication of diabetes (18, 19). Cardiac complications associated with type 2 diabetes are due to 1) increased coronary heart disease secondary to accelerated atherosclerosis because of associated risk factors (20) such as hypertension, obesity, and dyslipidemia (metabolic syndrome); and 2) a diabetic cardiomyopathy producing abnormalities in ventricular function in the absence of coronary heart disease or hypertension (31, 36).

Experimental studies with rodent models of diabetes allow assessment of direct deleterious effects of a diabetic cardiomyopathy without contributions from atherosclerotic coronary heart disease. However, most reports of diabetes-induced cardiac dysfunction have used insulin-deficient (Type 1) models (28, 35, 40). Relatively few studies on cardiac function have been conducted with type 2 diabetic animal models (29). Diabetic db/db mice provide an animal model of Type 2 diabetes (24). The natural history of the diabetic progression in db/db mice, with initial insulin resistance followed by an insulin secretion defect (37), is similar to the pathogenesis of Type 2 diabetes in humans (6).

Recently, we examined contractile function and metabolism using isolated perfused (ex vivo) working hearts from diabetic db/db mice (10–14 wk of age) compared with hearts from control (db/db) heterozygotes (2). Cardiac mechanical performance was significantly reduced with an increase in left ventricular (LV) end-diastolic pressure, decreased LV developed pressure, and reductions in both cardiac output and cardiac power. Perfused db/db hearts also had altered cardiac metabolism; glucose utilization was reduced, whereas fatty acid oxidation was increased (2). The hypothesis that changes in myocardial energy substrate use in the db/db heart could be a contributing mechanism for reduced contractile function (25) was tested using transgenic db/db mice expressing the human transgene for the insulin-regulatable glucose transporter (hGLUT4) (17). The altered metabolism of db/db hearts was entirely normalized in perfused working hearts from db/db-hGLUT4 transgenic mice, and contractile function was restored to normal (2).

Ex vivo perfusions to characterize the diabetic cardiomyopathy in db/db mice have a significant limitation, however, because contractile performance measured in control working hearts (2) is considerably less than cardiac function measured in vivo (15). Thus it could be argued that the reduced contractile function in ex vivo perfused db/db hearts relative to hearts from control db/+ and transgenic db/db-hGLUT4 mice (2) may merely represent a greater sensitivity of db/db hearts to trauma associated with isolation and perfusion rather than reflecting an intrinsic difference in contractility because of the in vivo diabetic state. Therefore, it is important to provide an in vivo validation of our observation that contractile performance...
was reduced in ex vivo perfused hearts from diabetic *db/db* mice (2) to substantiate that a diabetic cardiomyopathy is a feature of this murine model of Type 2 diabetes. Echocardiography is a noninvasive technique that can assess both systolic and diastolic function of the heart in vivo (22). Therefore, the objective of this study was to use echocardiography as a tool to assess cardiac contractile function in vivo with diabetic *db/db*, control *db/+*, and transgenic *db/db-hGLUT4* mice.

**METHODS**

**Animals.** Male C57BL/KsJ-lepr<sup>db/db</sup>/lepr<sup>db</sup> diabetic (*db/db*) mice and their nondiabetic C57BL/KsJ-lepr<sup>db/db</sup>/lepr<sup>+</sup> littermates (*db/+*) were obtained from Jackson Laboratories (Bar Harbor, ME). Cardiac function by echocardiography was assessed in *db/db* and *db/+* mice at 6 and 12 wk of age, reflecting early and advanced stages of diabetes (37). At 6 wk, *db/db* mice have a moderate increase in body weight and plasma glucose (Table 1). In comparison, *db/db* mice at 12 wk of age are markedly obese with extreme hyperglycemia (Table 1), hyperinsulinemia, and hyperlipidemia (2). Transgenic mice (12 wk old) with hGLUT4 overexpression (*db/db-hGLUT4* and *db/+−hGLUT4*) were bred at the University of Calgary (2, 9). General characteristics of these transgenic mice have been published previously (2, 3, 17). Global overexpression of the hGLUT4 transgene in *db/db* mice results in improved glucose homeostasis with reduced fasting hyperglycemia, but *db/db-hGLUT4* mice are still hyperglycemic and hyperlipidemic under fed conditions (2, 17). Mice were handled in accordance with guidelines of the Canadian Council on Animal Care, and all experiments were approved by the University of Calgary Health Sciences Animal Welfare Committee. The animals were housed in groups and given unrestricted access to water and food (9F, PMI Nutritional; Brentwood, MO).

**Echocardiography.** Echocardiograms to assess systolic function (M-mode measurements) were obtained from conscious mice to avoid any cardiodepression produced by anesthesia (33, 38). Conscious mice were restrained in a modified syringe case tubing, with an opening for the echocardiographic probe. The chest was shaved, and mice were allowed to condition for at least 15 min before echocardiography. Normal body temperature was maintained with a warming pad and monitored with a rectal probe (model BAT-12R, Physitemp Instruments).

Cardiac ultrasounds were performed using a Hewlett-Packard Sonus 4500 ultrasound machine (Agilent Technologies; Edmonton, Alberta, Canada) with a frame rate of 300 frames/s. A 15-MHz linear transducer was placed on the left hemithorax interfaced with a layer of ultrasonic transmission gel (Aquasonic 100, Parker Laboratories; Fairfield, NJ). Mice were imaged in a shallow left lateral decubitus position. The two-dimensional parasternal short-axis imaging plane was used as a guide for obtaining LV M-mode tracings close to the papillary muscle level. A minimum depth setting of 2 cm was used with a sweep speed of 100 mm/s. Doppler tracings of the mitral inflow and aortic outflow tract velocities were obtained in a modified parasternal long-axis view at a sweep speed of 100 mm/s, a sample volume length of 0.05 cm, and a gate of 0.6 cm. Tracings were recorded on VHS videotape (Maxell; Concord, Ontario, Canada) and printed on a Sony color printer (UP-5200, Sony).

Echocardiograms were completed in random order. Obtaining the echocardiograms in a blinded fashion was not possible because the diabetic *db/db* and transgenic *db/db-hGLUT4* mice were obviously obese compared with the lean controls. However, mice from each group were labeled with numbers only so that all analyses completed at least 2 wk later were blinded.

**M-mode measurements.** All data are the average of at least two separate scans, each scan representing the average of three selected beats. Septal wall thickness (SWT), posterior wall thickness (PWT), diastolic LV internal dimension (LVIDd), and systolic LV internal dimension (LVIDs) were determined using the leading-edge convention of the American Society of Echocardiography (30). End diastole was defined as the maximal LV diastolic dimension, and end systole was defined as the peak of posterior wall motion. SWT and PWT were measured at end diastole. LV mass was calculated using the following equation: LV mass (mg) = [(LVIDd + SWT + PWT<sup>2</sup> − LVIDs<sup>2</sup>) × 1.055, where 1.055 (mg/mm<sup>3</sup>) is the density of myocardium (13).

Systolic function was calculated from LV dimensions as fractional shortening (FS) and velocity of circumferential shortening (V<sub>cf</sub>) as follows: FS (%) = [(LVIDd − LVIDs)/LVIDd] × 100 and V<sub>cf</sub> (circ/s) = FS/ejection time (ET). Values for aortic ET and heart rate were obtained from Doppler measurements of LV outflow tract velocities (22).

**Doppler measurements.** Mitral inflow measurements included the peak E and A waves and the E-to-A ratio (E/A). Changes in the E/A provide a sensitive index of impairment in LV filling. Diastolic function could only be assessed in anesthetized mice, because transmitral E and A waves fuse at heart rates >500 beats/min (32). Mice were anesthetized (intramuscular injection) with xylazine (5 mg/kg; Bimeda-MTC Pharmaceuticals; Cambridge, Ontario, Canada) and ketamine (100 mg/kg; Bimeda-MTC Pharmaceuticals) and were allowed to breath spontaneously. A time study was performed initially to study the stability of echocardiographic parameters over time after anesthesia. Anesthetic injection time was taken as time 0, and echocardiographic traces were recorded approximately every 15 min for an hour. At each time, measurements were taken from three consecutive beats for each mouse (n = 6). There were no significant changes over time for any echocardiographic parameters. Heart rates were obtained from the LV outflow tract tracings (22).

**Interpretive variability and repeatability.** Mice selected at random from 6- and 12-wk-old *db/+* and *db/db* groups were reanalyzed for LV dimensions, wall thickness, and ET (44 mice) and transmural Doppler velocities (22 mice) by the same person for intraobserver variability and by a second

| Table 1. Body weight and plasma glucose levels for 6- and 12-wk-old control *db/+* and diabetic *db/db* mice |
|-----------------|-----------------|-----------------|-----------------|-----------------|
|                 |       |                 |                 |                 |
| *db/+*          |       | *db/db*         |                 |                 |
| Body weight, g  | 21.8 ± 0.9(6)  | 29.8 ± 1.0(6)*  | 28.1 ± 0.5(12)  | 47.8 ± 0.9(12)* |
| Plasma glucose, mM | 9.4 ± 0.6(5)  | 19.7 ± 1.9(4)*  | 14.1 ± 1.3(22)  | 53.0 ± 4.2(18)* |

Values are means ± SE; numbers in parentheses represent number of mice. *P < 0.05 vs. age-matched *db/+* mice (by Student’s t-test).
person for interobserver variability. Repeatability was determined by taking several separate scans of the measurements at the time of the initial study; 53 pairs of scans from 37 randomly selected mice were analyzed for LV dimensions, wall thickness, and ET, and 36 pairs of scans from 22 randomly selected mice were analyzed for transmitral Doppler velocities. All interpretive variability was also analyzed in a blinded fashion. Differences were calculated as both the difference between two observations divided by the mean of the observations expressed as percentages and by the limits of agreement (mean differences between observations ± 2SD) (4).

Statistical analysis. Mean control (db/+ values were compared with values obtained for 6-wk db/db values by Student's t-test and a Wilcoxon-Mann-Whitney test. Twelve-week-old db/db, db/db-hGLUT-4, and db/+ hGLUT-4 mice were compared with age-matched db/+ (control) mice by one-way ANOVA, Tukey test, and a one-way ANOVA on ranks with a Dunn's test. Data are expressed as means ± SE, with statistical significance accepted at the 95% confidence level (P < 0.05).

RESULTS

Comparison of db/+ and db/db mice at 6 wk of age. Heart rates were significantly reduced (672 ± 9 beats/min) in conscious 6-wk-old db/db mice compared with control db/+ (714 ± 8 beats/min) rates (Table 2). M-mode measurements of LV dimensions revealed no differences between db/+ and db/db mice. Consequently, calculated LV mass was unchanged in 6-wk-old diabetic mice. Systolic function (FS and Vcf) was not different in 6-wk-old db/db mice (Table 2). Parameters for diastolic function from Doppler-derived mitral inflow measurements were not different for diabetic db/db mice except for an increased E wave (Table 3). Nevertheless, the E/A was unchanged in 6-wk-old db/db mice.

Comparison of db/+, db/db, db/db-hGLUT4, and db/+ hGLUT4 mice at 12 wk of age. Heart rate was significantly lower in db/db mice (642 ± 9 beats/min) compared with the control (702 ± 9 beats/min) rate (Table 2). Calculated LV mass from LV dimensions was unchanged in db/db mice.

In contrast to the results for 6-wk-old db/db mice, a significant reduction in systolic function was evident in db/db mice at 12 wk of age (Table 2). FS was reduced from 59.5 ± 2.3% in db/+ mice to 43.8 ± 2.1% in db/db mice; similarly, Vcf was lowered significantly (8.3 ± 0.5 circs/s) in db/db mice compared with controls (11.8 ± 0.4 circs/s). For parameters of diastolic function, the A wave was significantly increased in db/db mice (Table 3). As a consequence, the E/A was reduced significantly from 3.56 ± 0.29 in db/+ mice to 2.40 ± 0.20 in db/db mice.

Although heart rate was still significantly reduced in transgenic db/db-hGLUT4 mice, systolic function (FS and Vcf) was normalized (Table 2) compared with db/+ controls. Similarly, the A wave velocity and E/A for transgenic db/db-hGLUT4 mice were not significantly different from those of control db/+ mice (Table 3). Parameters for systolic and diastolic function in transgenic db/+ hGLUT4 mice were measured as a control for hGLUT4 overexpression in db/+ mice. Systolic function (FS = 59.4 ± 3.2% and Vcf = 13.3 ± 0.8 circs/s) in db/+ hGLUT4 mice was not significantly different from values obtained with either control db/+ or db/db-hGLUT4 mice (Table 2), consistent with results from ex vivo perfusions showing no difference in cardiac power for db/+ and db/+ hGLUT4 hearts (3). Expression of the hGLUT4 transgene in control db/+ mice had no effect on E and A waves; therefore, the E/A (3.34 ± 0.28) was not different from the ratios for either control db/+ or db/db-hGLUT4 mice (Table 3).

Interpretative variability and repeatability. Intraobserver variability, interobserver variability, and repeatability (reproducibility) of M-mode dimensions, calculated LV mass, systolic function (FS and Vcf), and Doppler measurements are shown in Table 4. Interobserver variability was higher than the intraobserver variability; repeatability error tended to be higher for most parameters. Calculated values exhibited greater variability than measured parameters. The highest variability (11.7%) was for LV mass interobserver vari-

Table 2. Echocardiographic parameters of systolic function for 6-wk-old db/+ (control) and diabetic db/db mice and for 12-wk-old db/+, db/db, and transgenic db/+ hGLUT4 and db/db-hGLUT4 mice

<table>
<thead>
<tr>
<th></th>
<th>6 wk</th>
<th>12 wk</th>
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<tbody>
<tr>
<td></td>
<td>db/+</td>
<td>db/db</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>714 ± 8</td>
<td>672 ± 9*</td>
</tr>
<tr>
<td>SWT, mm</td>
<td>0.6 ± 0.01</td>
<td>0.6 ± 0.01</td>
</tr>
<tr>
<td>PWT, mm</td>
<td>0.6 ± 0.01</td>
<td>0.6 ± 0.01</td>
</tr>
<tr>
<td>LVIDd, mm</td>
<td>3.0 ± 0.05</td>
<td>3.0 ± 0.06</td>
</tr>
<tr>
<td>LVIDs, mm</td>
<td>1.3 ± 0.08</td>
<td>1.1 ± 0.05</td>
</tr>
<tr>
<td>LV mass, mg</td>
<td>49.2 ± 1.4</td>
<td>50.3 ± 1.6</td>
</tr>
<tr>
<td>FS, %</td>
<td>60.8 ± 1.4</td>
<td>63.7 ± 1.4</td>
</tr>
<tr>
<td>Vcf, circs/s</td>
<td>13.6 ± 0.4</td>
<td>13.4 ± 0.04</td>
</tr>
<tr>
<td>ET, sc</td>
<td>0.05 ± 0.002</td>
<td>0.05 ± 0.001</td>
</tr>
</tbody>
</table>

Values are means ± SE; numbers in parentheses represent numbers of mice. hGLUT4, human glucose transporter 4; HR, heart rate; SWT, septal wall thickness; PWT, posterior wall thickness; LVIDd, diastolic left ventricular (LV) internal dimension; LVIDs, systolic LV internal dimension; FS, fractional shortening; Vcf, velocity of circumferential shortening; ET, ejection time. *P < 0.05 compared with age-matched control db/+ mice.
ability; interobserver, intraobserver, and repeatability errors for all other measured parameters was <10%.

DISCUSSION

Echocardiography is a noninvasive method that can assess cardiac function in both conscious and anesthetized mice (22, 38). M-mode measurements of LV dimensions in end systole (LVIDs) and end diastole (LVIDd) provide information on morphology (LV mass) and systolic function; LV diastolic function can be assessed by Doppler measurements. Therefore, echocardiography has become a very useful tool to assess alterations in cardiac phenotype in vivo with genetic and transgenic mouse models of cardiac disease (22). Errors for interpretive variability and reproducibility of echocardiographic parameters in this study were generally <10% (Table 4). Therefore, it was feasible to compare echocardiographic parameters of cardiac systolic and diastolic function in diabetic db/db and transgenic db/+–hGLUT4 and db/db-hGLUT4 mice with control db/+ mice.

Systolic dysfunction in diabetic db/db mice. Heart rates of 714 and 702 beats/min at 6 and 12 wk of age for conscious control db/+ mice (Table 2) were somewhat higher than rates (658 beats/min) obtained with conscious 129 SvEv/Tac mice (38). Similarly, systolic function in db/+ mice (FS of 60–61%; Table 2) was also slightly higher than the value (51.4%) observed by Yang et al. (38). Strain differences between mice and differing degrees of training associated with conscious measurements likely account for these small differences.

At 12 wk of age, systolic function (FS and V\textsubscript{cf}) was reduced in db/db mice (Table 2). Ventricular systolic function in vivo is determined not only by contractility but by heart rate and loading conditions (afterload and preload). Given that there were no significant changes in SWT, PWT, or LV mass, a change in afterload due to elevated arterial blood pressure in db/db mice is unlikely. Jones et al. (23) reported that mean arterial pressure was not elevated in db/db mice, but blood measurements were obtained from anesthetized db/+ and db/db mice at a single age. The absence of a change in LVIDd suggests that preload is unchanged. Admittedly, it is difficult to define a change in contractility when heart rate is not constant. However, because contractility decreases at heart rates >600 beats/min (14), the reduced heart rate observed in db/db mice should augment contractility. Although the observed reductions in FS and V\textsubscript{cf} in 12-wk-old conscious db/db mice (Table 2) provide evidence for in vivo systolic dysfunction, a result that is consistent with results from perfused working hearts from db/db mice at 10–14 wk of age (2), any conclusion concerning echocardiographic evidence for systolic dysfunction must remain tentative until measurements of blood pressure can definitely establish that there is no elevation of arterial blood pressure in conscious db/db mice at 12 wk of age. This is an important issue because cardiac work in db/db mice could be similar to control if blood pressure was elevated. There was no apparent reduction in systolic function in 6-wk-old db/db mice (Table 2), although the reduction in heart rate may have compensated for any systolic dysfunction because of the nega-

### Table 3. Echocardiographic parameters of diastolic function for 6-wk-old db/+ and diabetic db/db mice and for 12-wk-old db/+ , db/db, and transgenic db/+–hGLUT4 and db/db–hGLUT4 mice

<table>
<thead>
<tr>
<th></th>
<th>db/+ (12)</th>
<th>db/db (14)</th>
<th>db/+ (11)</th>
<th>db/db (12)</th>
<th>db/db–hGLUT4 (7)</th>
<th>db/+–hGLUT4 (7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E, m/s</td>
<td>1.22 ± 0.03</td>
<td>1.48 ± 0.11*</td>
<td>1.26 ± 0.08</td>
<td>1.05 ± 0.08</td>
<td>1.10 ± 0.10</td>
<td>1.11 ± 0.06</td>
</tr>
<tr>
<td>A, m/s</td>
<td>0.45 ± 0.02</td>
<td>0.51 ± 0.03</td>
<td>0.35 ± 0.03</td>
<td>0.45 ± 0.03*</td>
<td>0.28 ± 0.02</td>
<td>0.35 ± 0.03</td>
</tr>
<tr>
<td>E/A</td>
<td>2.88 ± 0.15</td>
<td>2.78 ± 0.11</td>
<td>3.56 ± 0.29</td>
<td>2.40 ± 0.20*</td>
<td>3.91 ± 0.25</td>
<td>3.34 ± 0.28</td>
</tr>
</tbody>
</table>

Values are means ± SE; numbers in parentheses represent numbers of mice. E, peak E velocity; A, peak A velocity; E/A, E-to-A ratio. *P < 0.05 compared with age-matched control db/+ mice.

### Table 4. Interpretive variability and reproducibility of echocardiographic measurements

<table>
<thead>
<tr>
<th></th>
<th>Intraobserver Error Percentage, absolute values</th>
<th>Interobserver Error Percentage, absolute values</th>
<th>Repeatability Error Percentage, absolute values</th>
</tr>
</thead>
<tbody>
<tr>
<td>SWT</td>
<td>6.5 ± 4.9 (0.1 ± 0.1 mm)</td>
<td>9.9 ± 7.4 (0.02 ± 0.2 mm)</td>
<td>4.9 ± 6.3 (0.01 ± 0.1 mm)</td>
</tr>
<tr>
<td>PWT</td>
<td>4.8 ± 4.9 (0.1 ± 0.1 mm)</td>
<td>6.9 ± 5.6 (0.03 ± 0.1 mm)</td>
<td>7.2 ± 6.6 (0.01 ± 0.1 mm)</td>
</tr>
<tr>
<td>LVIDd</td>
<td>2.1 ± 1.8 (0.02 ± 0.2 mm)</td>
<td>2.6 ± 2.7 (0.03 ± 0.3 mm)</td>
<td>4.5 ± 4.1 (0.004 ± 0.4 mm)</td>
</tr>
<tr>
<td>LVIDs</td>
<td>5.8 ± 13.4 (0.1 ± 0.6 mm)</td>
<td>5.5 ± 5.3 (0.03 ± 0.5 mm)</td>
<td>7.0 ± 6.1 (0.02 ± 0.3 mm)</td>
</tr>
<tr>
<td>LV mass</td>
<td>10.2 ± 10.7 (0.6 ± 18.7 mg)</td>
<td>11.7 ± 8.2 (4.6 ± 17.2 mg)</td>
<td>10.0 ± 7.9 (0.6 ± 18.8 mg)</td>
</tr>
<tr>
<td>FS</td>
<td>7.2 ± 10.6 (0.3 ± 12.4%)</td>
<td>7.4 ± 8.3 (0.9 ± 10.1%)</td>
<td>8.9 ± 7.6 (0.9 ± 9.0%)</td>
</tr>
<tr>
<td>V\textsubscript{cf}</td>
<td>6.9 ± 7.8 (0.2 ± 1.5 circs/s)</td>
<td>7.3 ± 5.4 (0.1 ± 1.5 circs/s)</td>
<td>9.0 ± 7.4 (0.1 ± 1.6 circs/s)</td>
</tr>
<tr>
<td>ET</td>
<td>2.9 ± 2.8 (0.001 ± 0.004 s)</td>
<td>4.9 ± 3.4 (0.0001 ± 0.001 s)</td>
<td>6.0 ± 5.2 (0.01 ± 0.01 s)</td>
</tr>
<tr>
<td>E</td>
<td>1.70 ± 2.21 (0.002 ± 0.007 m/s)</td>
<td>2.30 ± 1.97 (0.0002 ± 0.09 m/s)</td>
<td>7.59 ± 5.70 (0.02 ± 0.23 m/s)</td>
</tr>
<tr>
<td>A</td>
<td>3.94 ± 3.19 (0.03 ± 0.05 m/s)</td>
<td>4.09 ± 3.77 (0.02 ± 0.48 m/s)</td>
<td>9.07 ± 8.65 (0.03 ± 0.10 m/s)</td>
</tr>
<tr>
<td>E/A</td>
<td>6.45 ± 7.49 (0.01 ± 0.57)</td>
<td>4.88 ± 6.66 (0.09 ± 0.36)</td>
<td>8.69 ± 7.53 (0.06 ± 0.82)</td>
</tr>
</tbody>
</table>

Values are means ± SD of the differences between two observations divided by the mean of the observations expressed as percentages. The mean of the differences between the observations (±2SD) is shown in parentheses.
tive force-frequency relationship observed with mouse hearts (14). The mechanical performance of ex vivo perfused working hearts from 6-wk-old db/db mice has not been studied to date.

Assessment of systolic function by echocardiography in other Type 2 diabetic animal models has been limited. Zucker diabetic fatty (ZDF) rats showed a modest decrease in FS (from 43% to 36%) with unchanged LV mass at 20 wk of age (42), similar to results for db/db mice (Table 2). On the other hand, echocardiography in obese mice with insulin resistance due to reduced brown fat revealed a very different cardiac phenotype, consisting of LV dilation and hypertrophy with increased cardiac output (9).

M-mode echocardiography has detected variable changes in LV mass and systolic function in humans with Type 2 diabetes. In women, LV mass was increased with either enhanced (36) or unchanged (12) systolic function. Celentano et al. (7) reported that LV mass and systolic function was unchanged in Type 2 diabetics, whereas Frustaci et al. (11) observed increased LV mass with decreased ventricular performance (decompensated eccentric hypertrophy).

Diastolic dysfunction in db/db mice. At heart rates >500 beats/min, E and A waves fuse (32); therefore, diastolic function had to be assessed in anesthetized mice (Table 3). Control values for E (1.22 and 1.26 m/s) and A wave (0.35 and 0.45 m/s) peak velocities for db/+ mice are somewhat higher than measurements (E = 0.5–0.7 m/s and A = 0.2–0.4 m/s) from other studies (21, 34). Doppler echocardiography revealed abnormal diastolic filling in 12-wk-old db/db mice with increased A wave velocity and decreased E/A (Table 3), typical of abnormal relaxation. LV diastolic filling is influenced by loading conditions and heart rate. As noted above, the absence of changes in LV dimensions (SWT, PWT, and LVIDd) in db/db hearts suggests that loading conditions are not a factor. In addition, transmitral flows were measured in anesthetized db/+ and db/db mice with no significant differences in heart rates (240 ± 10 and 235 ± 18 beats/min, respectively) due to the cardiopressant effect of anesthesia with ketamine and xylazine (33, 38). Thus it is reasonable to conclude that 12-wk-old db/db mice do exhibit diastolic dysfunction, consistent with observations of elevated LV end-diastolic pressure in ex vivo perfused hearts from db/db mice (2). Diastolic function was unchanged in 6-wk-old db/db mice (Table 3).

There have been very few investigations of diastolic function in other Type 2 diabetic animal models. Echocardiographic parameters of diastolic function were not reported for diabetic ZDF rats (42). Results with Osaka Long-Evans Tokushima fatty rats at an early prediabetic stage have been inconsistent. The observation by Mizushige et al. (27) that peak transmитral velocity was reduced with prolonged deceleration time was not confirmed in a subsequent study (39), in agreement with recent results showing no reduction in LV relaxation rates (1). In humans, diastolic filling abnormalities are an early manifestation of LV dysfunction in diabetes, typically preceding reductions in systolic function (36). Type 2 diabetics had reduced E and increased A waves producing a reduction in the E/A (10, 41), similar to the diastolic dysfunction in db/db mice.

Cardiac function in transgenic db/db-hGLUT4 mice. Echocardiographic parameters of systolic and diastolic function in transgenic db/db-hGLUT4 mice at 12 wk of age were not different from either control db/+ or db/+ -hGLUT4 mice (Tables 2 and 3), although the same qualification regarding the requirement for conscious blood pressure measurements also applies to assessment of systolic function in vivo with transgenic db/db-hGLUT4 mice. Nevertheless, these results are consistent with cardiac power measurements from ex vivo perfusions of db/db-hGLUT4 hearts (2), which were restored to a normal nondiabetic value. Therefore, the observations that metabolism was normalized in ex vivo perfused db/db-hGLUT4 hearts and that both in vivo (echocardiography) and ex vivo contractile function was also normalized (2) support the contention (25) that altered substrate utilization in db/db hearts can make a major contribution to reduced contractile function, perhaps by a lipotoxicity mechanism involving accumulation of intracellular lipids (triacylglycerol and ceramide) and enhanced apoptosis (42). Interestingly, the overutilization of fatty acids in transgenic mouse hearts with cardiac-specific overexpression of long-chain fatty-acyl CoA synthetase also resulted in reduced contractile function by echocardiography (8). However, other mechanisms such as interstitial fibrosis may also contribute to the contractile dysfunction in db/db hearts. Giacomelli and Wiener (16) observed increased interstitial deposits of collagen in hearts from female db/db mice (5 wk–4 mo of age), but detailed age-dependent changes were not reported. No information is available on changes in specific collagen types or whether advanced glycation end products of collagen accumulate in db/db hearts, mechanisms that could increase myocardial stiffness and reduce diastolic function (23, 31).

In conclusion, echocardiographic assessment of cardiac function in db/db mice supports the existence of a diabetic cardiomyopathy in this animal model of type 2 diabetes. Reduced systolic function and abnormal diastolic function were evident in 12-wk-old diabetic db/db mice but not at 6 wk of age, indicating that cardiomyopathic changes require a chronic diabetic environment. The age-dependent development of cardiac dysfunction in db/db mice has not been examined with ex vivo perfused working hearts. Importantly, systolic and diastolic dysfunction in db/db hearts was not associated with cardiac hypertrophy because calculated LV mass and heart dry weights (2) were unchanged. However, it must be acknowledged that hypertrophy with increased interstitial fibrosis could be balanced by loss of myocytes through apoptosis and/or necrosis, and so calculated or measured heart mass may not always be a good indicator of hypertrophy in situations of cardiomyopathy. Results from transgenic db/db-hGLUT4 hearts showing normalized systolic and diastolic function suggest that pharmacological interventions to im-
prove cardiac metabolism (enhanced glucose utilization and reduced fatty acid oxidation) may reduce diabetes-induced cardiac dysfunction.

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