

Age-Dependent Changes in Metabolism, Contractile Function, and Ischemic Sensitivity in Hearts From *db/db* Mice

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Glucose and palmitate metabolism and contractile function were measured with ex vivo perfused working hearts from control (*db/+*) and diabetic (*db/db*) female mice at 6, 10–12, and 16–18 weeks of age. Palmitate oxidation was increased by 2.2-fold in 6-week-old *db/db* hearts and remained elevated in 10- to 12- and 16- to 18-week-old hearts. Carbohydrate oxidation was normal at 6 weeks but was reduced to 27 and 23% of control at 10–12 and 16–18 weeks, respectively. At 6 weeks, *db/db* hearts exhibited a slight reduction in mechanical function, whereas marked signs of dysfunction were evident at 10–12 and 16–18 weeks. Mechanical function after ischemia-reperfusion was examined in hearts from male mice; at 6 weeks, *db/db* hearts showed normal recovery, whereas at 12 weeks it was markedly reduced. Fatty acid oxidation was the predominant substrate used after reperfusion. Thus, diabetic *db/db* hearts exhibit signs of a progressive cardiomyopathy; increased fatty acid oxidation preceded reductions in carbohydrate oxidation. Postischemic recovery of function was reduced in *db/db* hearts, in parallel with age-dependent changes in normoxic contractile performance. Finally, peroxisome proliferator-activated receptor- α treatment (3 weeks) did not affect sensitivity to ischemia-reperfusion, even though carbohydrate oxidation was increased and palmitate oxidation was decreased. *Diabetes* 52:434–441, 2003

Non-insulin-dependent (type 2) diabetes is a prevalent disease that results in a marked increase in cardiovascular complications (1) that are in part due to a specific cardiomyopathy, characterized by ventricular dysfunction in the absence of atherosclerotic coronary heart disease or hypertension (2,3).

Diabetic *db/db* mice provide an animal model of type 2 diabetes, with obesity and insulin resistance (4,5). Recently, we have reported that isolated perfused hearts from *db/db* mice at 10–14 weeks of age exhibited charac-

teristics of a diabetic cardiomyopathy, with decreased contractile performance and altered cardiac metabolism (6,7).

The natural history of *db/db* mice follows a distinct pattern (8,9). Initially, peripheral insulin resistance is overcome by increased insulin secretion, so hyperinsulinemia produces normoglycemia. Hyperglycemia develops when enhanced insulin secretion can no longer compensate for insulin resistance. The maximal extent of hyperinsulinemia occurs at 2–3 months of age. Subsequently, insulin levels fall rapidly as β -cells exhibit a severe secretory defect, resulting in a progressive increase in hyperglycemia. Thus, the metabolic features of *db/db* mice are similar to the pathogenesis of type 2 diabetes in humans (10).

The first objective of this investigation was to study age-dependent changes of cardiac function and metabolism in *db/db* mice assessed with ex vivo perfused hearts (7,11,12). Of particular interest was to establish whether the onset of metabolic alterations coincided with contractile dysfunction. Previously, evidence for contractile dysfunction in perfused *db/db* hearts has been obtained from normoxic perfusions (6,7); to date, no investigations have determined whether *db/db* hearts show an altered susceptibility to ischemic injury. Therefore, our second objective was to evaluate recovery of contractile performance in perfused *db/db* hearts at two different ages. Because treatment of *db/db* mice with BM 17.0744, an activator of peroxisome proliferator-activated receptor- α (PPAR- α), improves their diabetic status and normalizes cardiac metabolism (7), the effect of BM 17.0744 treatment on recovery of mechanical function of perfused *db/db* hearts after ischemia-reperfusion was also investigated.

RESEARCH DESIGN AND METHODS

Animals. Male and female C57BL/KsJ-lepr^{db}/lepr^{db} diabetic (*db/db*) mice and their nondiabetic heterozygote littermates (*db/+*) were purchased from Harland (Bicester, England) or M&B A/S (Ry, Denmark). Both male and female mice were studied to determine whether there were sex differences in the effect of type 2 diabetes on cardiac function. The animals were housed in groups of four to five in a room maintained at $23 \pm 1^\circ\text{C}$ and $55 \pm 5\%$ humidity with a 12-h light/dark cycle and were given ad libitum access to food and water. 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.

Isolated working heart perfusions. Heparin (100 units) was administered (intraperitoneally) 10 min before the mice were killed. Mice were anesthetized with an intraperitoneal injection (10 mg) of sodium pentobarbital. The hearts were quickly excised, and the aorta was cannulated for an initial Langendorff perfusion with Krebs-Henseleit bicarbonate (KHB) buffer. During this interval,

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CO, cardiac output; HR, heart rate; PPAR, peroxisome proliferator-activated receptor; PSP, peak systolic pressure; TG, triacylglycerol.

TABLE 1
Characteristics of nondiabetic (*db/+*) and diabetic (*db/db*) female mice, according to age

	6 weeks		10–12 weeks		16–18 weeks	
	<i>db/+</i>	<i>db/db</i>	<i>db/+</i>	<i>db/db</i>	<i>db/+</i>	<i>db/db</i>
Body weight (g)	17.6 ± 0.6 (8)	24.2 ± 0.5 (8)*	21.6 ± 0.3 (5)	42.3 ± 1.2 (9)*	26.3 ± 5.3 (4)	44.2 ± 1.1 (8)*
Heart dry weight (mg)	18.9 ± 0.6 (8)	18.5 ± 0.4 (8)	23.5 ± 0.6 (5)	24.5 ± 0.6 (9)	25.5 ± 1.1 (4)	26.7 ± 0.7 (8)
Plasma glucose (mmol/l)	13.2 ± 0.6 (8)	14.2 ± 1.0 (8)	14.1 ± 0.7 (5)	37.8 ± 3.0 (9)*	15.3 ± 1.5 (4)	45.0 ± 2.5 (8)*
Plasma insulin (μU/ml)	21 ± 8 (3)	85 ± 27 (5)*	9 ± 1 (3)	297 ± 51 (7)*	7 ± 2 (3)	257 ± 40 (9)*
Plasma FFA (mmol/l)	0.9 ± 0.2 (4)	1.9 ± 0.2 (3)*	1.2 ± 0.4 (4)	2.3 ± 0.3 (7)*	1.0 ± 0.2 (4)	2.4 ± 0.4 (7)*

Values are mean ± SE. Numbers in parentheses indicate the number of animals in each group. **P* < 0.05 compared with *db/+* mice at the same age. FFA, free fatty acids

the left atrium was cannulated and connected to the preload reservoir (12). The heart was thereafter perfused in the working (left ventricle ejecting) mode, using a modified KHB buffer supplied with 11 mmol/l glucose and 0.7 mmol/l fatty acids (0.4 mmol/l added palmitate plus 0.3 mmol/l endogenous fatty acids) bound to 3% BSA (fraction V, Sigma) (7). The preload pressure was 12.5 mmHg, and the afterload column was set to a height corresponding to a pressure of 50 mmHg (7). Perfused hearts were allowed to beat spontaneously.

Measurements of cardiac metabolism and function. Cardiac glucose and palmitate oxidation were measured simultaneously in isolated working hearts as described previously (6,7). Glucose oxidation was determined by measuring ¹⁴CO₂ released by the metabolism of [¹⁴C]glucose, whereas palmitate oxidation was determined by measuring ³H₂O released from [9,10-³H]palmitate (7,11). Heart metabolism was measured during a 40- or 60-min perfusion period; a sample from the recirculating perfusate (40 ml total volume) was taken every 10 min for determinations of metabolite content. At the end of the perfusion, hearts were frozen and total dry mass was determined.

A modest accumulation of lactate in the perfusate (to 0.25 mmol/l) occurred during the 40-min normoxic perfusion, which increased further to ~0.75 mmol/l after reperfusion. Although lactate oxidation was not measured directly, metabolism of radiolabeled lactate, generated from glycolysis of radiolabeled exogenous glucose and taken up from the perfusate, will also yield ¹⁴CO₂ upon subsequent oxidation. The measurements of ¹⁴CO₂ production will therefore reflect total carbohydrate oxidation from either glucose or lactate.

Blood samples and plasma analyses. Blood samples (fed conditions) taken from the body cavity after excision of the heart were centrifuged; plasma glucose and nonesterified free fatty acid concentrations were measured using kits from Boehringer Mannheim (catalog no. 1442449; Mannheim, Germany) and Wako Chemicals (catalog no. 994-75409; Neuss, Germany), respectively. Plasma insulin levels were determined using an enzyme-linked immunosorbent assay kit (Chrysal Chem, Chicago, IL).

Determination of myocardial triacylglycerol. Myocardial triacylglycerol (TG) was extracted from ~10 mg of freeze-dried heart tissue (perfused hearts) by the method of Folch. Dried lipids were mixed with 300 μl tert-butyl butanol and 150 μl Triton X 100/methanol (1:1, vol/vol), and TG content was thereafter measured with a Triglyceride 25 kit from ABX Diagnostics (Montpellier, France) (7).

Experimental protocols. Cardiac metabolism and contractile function were measured during 40 min of normoxic perfusion of hearts from diabetic (*db/db*) and nondiabetic (*db/+*) female mice at 6, 10–12, and 16–18 weeks of age, reflecting early, established, and advanced stages in their diabetic progression (8,9). Another series of experiments examined the impact of ischemic stress on *db/db* and *db/+* hearts from 6- and 12-week-old male mice. After 20 min of stabilization, the hearts were subjected to 13 or 15 min of no-flow global ischemia, followed by 70 min of reperfusion (10 min in Langendorff mode and 60 min in working mode) (13). Temperature measurements in the left atrium showed that cardiac temperature dropped <0.5°C during the ischemic period. Rates of substrate oxidation were measured in the working reperfusion phase only. Finally, a group of 9-week-old diabetic male mice were treated for 3 weeks with the PPAR-α ligand BM 17.0744 (Roche Pharmaceuticals) added to the drinking water (0.24 mg/ml) (14). We have previously reported that this treatment of *db/db* mice normalizes blood parameters (glucose, free fatty acids, TG, and insulin), as well as ex vivo cardiac metabolism (7). Thus, PPAR-α treatment allowed us to test whether normalization of cardiac metabolism can alter the sensitivity of *db/db* hearts to ischemia-reperfusion.

Statistical analysis. Data are expressed as mean ± SE. Differences in cardiac function and substrate metabolism were determined by ANOVA followed by an unpaired Student's *t* test or a Mann-Whitney rank sum test. Bonferroni's method was applied in the case of multiple comparisons.

Differences between means were regarded as statistically significant at *P* < 0.05.

RESULTS

Age-dependent changes of function and metabolism in hearts from female *db/db* mice. At 6 weeks of age, female *db/db* mice already demonstrated increased body weight, with hyperinsulinemia but normoglycemia (Table 1). By 10–12 weeks, diabetic mice exhibited marked obesity, more pronounced hyperinsulinemia, and also hyperglycemia. A more severe hyperglycemia was evident in 16- to 18-week-old diabetic mice. The relatively high levels of plasma free fatty acids most likely were due to the administration of heparin before the blood sampling. Nevertheless, plasma free fatty acids were elevated to approximately the same extent in *db/db* mice at all age groups. Heart weight increased with age for both *db/+* and *db/db* mice, with no significant differences between diabetic and control hearts. Thus *db/db* mice showed no signs of cardiac hypertrophy. At 6 weeks, myocardial TG content was slightly but nonsignificantly higher in *db/db* compared with *db/+* hearts (14.2 ± 1.0 vs. 10.3 ± 0.9 mol/g dry wt; *n* = 6 and 8, respectively). In the older mice (10–18 weeks), this difference was pronounced, being approximately twofold higher in *db/db* (16.6 ± 1.3 μmol/g dry wt; *n* = 8) compared with *db/+* hearts (8.8 ± 0.8 μmol/g dry wt; *n* = 17, *P* < 0.05).

Aortic and coronary flows and cardiac output (CO) were calculated as milliliters per minute per gram of dry weight (Fig. 1A–C), to correct for the increase in heart size with increasing age. Hearts from *db/+* mice showed no changes with age for any of the measured parameters of contractile function. CO was reduced slightly in 6-week-old *db/db* hearts, entirely as a result of decreased aortic flow because coronary flow was unchanged. Heart rate (HR; Fig. 1) and peak systolic pressure PSP (not shown) were not changed in 6-week-old *db/db* hearts. The product of PSP and HR, as well as of PSP and CO (Fig. 1), were not significantly reduced in 6-week-old *db/db* hearts. More pronounced signs of cardiac contractile dysfunction were evident in perfused hearts from 10- to 12-week-old *db/db* mice as indicated by a significant reduction in CO, aortic flow, HR, PSP·HR, and PSP·CO (Fig. 1). Perfused 16- to 18-week-old *db/db* hearts exhibited the same features of contractile dysfunction as the 10- to 12-week-old group (Fig. 1).

Steady-state rates of carbohydrate and palmitate oxidation are presented in Fig. 2A and B. There were no significant age-dependent changes in these parameters for

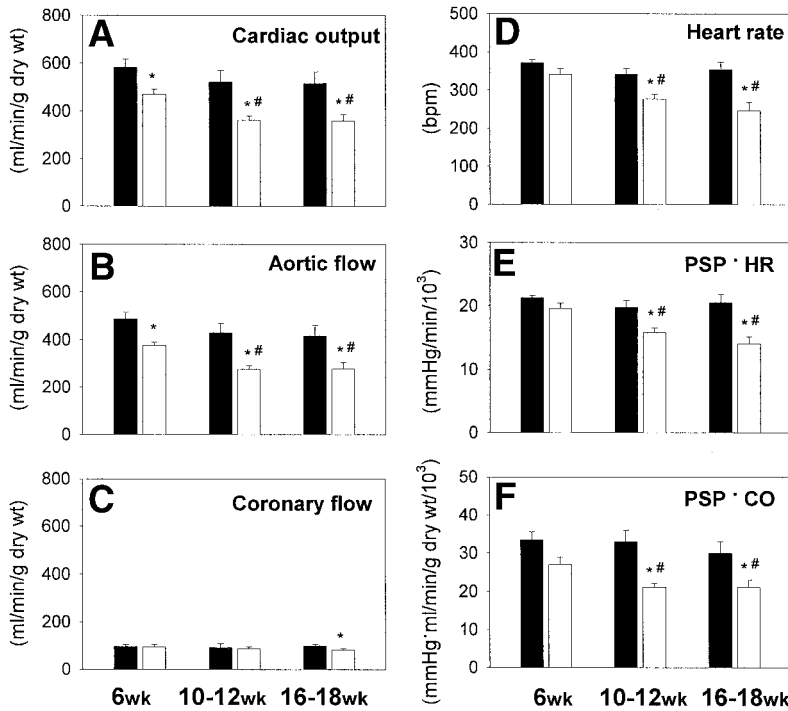


FIG. 1. Cardiac contractile function in perfused working hearts from nondiabetic *db/+* (■) and diabetic *db/db* female mice (□) at 6, 10–12, and 16–18 weeks of age. In *A* (CO), *B* (Aortic flow), and *C* (coronary flow), values are adjusted for small differences in heart weight ($\text{ml} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ dry wt). PSP and HR (*D*) were recorded in the aortic (afterload) line (7). Left ventricular performance was also calculated as the products of PSP and HR (*E*) as well as PSP and CO (*F*). Results are mean \pm SE for $n = 4$ –9 perfusions. *Significantly different ($P < 0.05$) from *db/+* hearts at the same age; #significantly different ($P < 0.025$) from 6-week-old *db/db* hearts.

perfused working hearts from *db/+* mice. At 6 weeks, carbohydrate oxidation was reduced slightly in *db/db* hearts, but palmitate oxidation was increased by 2.2-fold. Carbohydrate oxidation was significantly reduced (to 27% of control) in hearts from *db/db* mice at 10–12 weeks of age; rates of palmitate oxidation remained elevated. Re-

duced carbohydrate oxidation and increased palmitate oxidation were also evident in older (16- to 18-week-old) diabetic mice, but these changes were not more pronounced than at 10–12 weeks. Because the contractile performance of perfused hearts will influence metabolism, rates of carbohydrate and palmitate oxidation were nor-

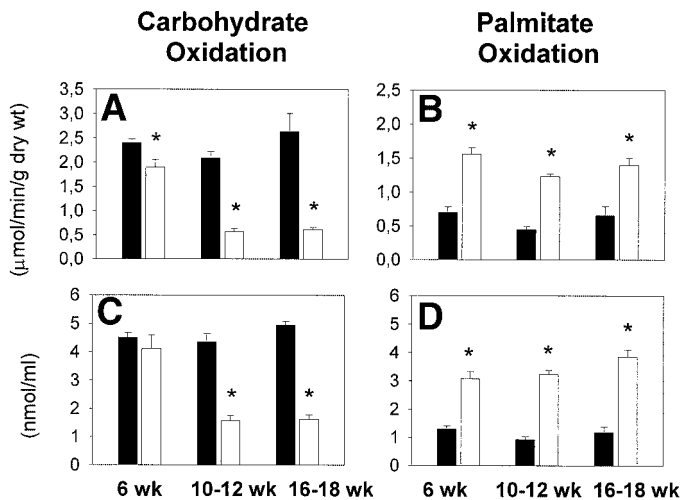


FIG. 2. Steady-state rates of carbohydrate (*A* and *C*) and palmitate oxidation (*B* and *D*) for perfused working hearts from nondiabetic *db/+* (■) and diabetic *db/db* (□) female mice at 6 ($n = 8$ and $n = 6$), 10–12 ($n = 5$ and $n = 9$), and 16–18 ($n = 4$ and $n = 8$) weeks of age. Results (mean \pm SE) are expressed as $\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ dry weight (*A* and *B*), or as nmol/ml after normalization for cardiac output (*C* and *D*). *Significantly different from age-matched *db/+* hearts. *Bottom*: Calculated acetyl-CoA production from glucose (■) and palmitate oxidation (□), for *db/+* and *db/db* mice from the age groups as indicated, based on rates of metabolism shown in the top panel. The theoretical yield of acetyl-CoA that could be expected from glucose and palmitate metabolism was calculated, using a stoichiometric ratio of 2 and 8 mol acetyl-CoA per mol of glucose and palmitate being metabolized, respectively (49).

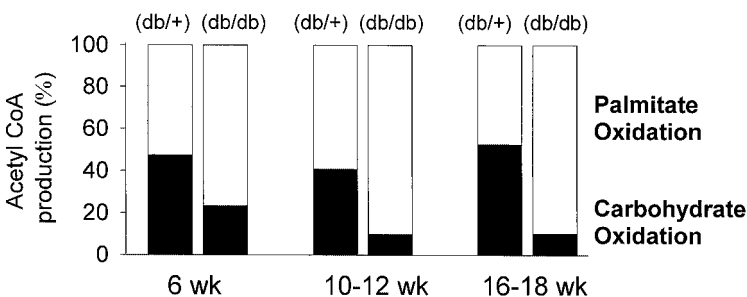


TABLE 2

Characteristics of nondiabetic (*db/+*) and diabetic (*db/db*) male mice, according to age and preischemic contractile function in perfused working hearts of these mice

Characteristics	6 weeks		12 weeks	
	<i>db/+</i>	<i>db/db</i>	<i>db/+</i>	<i>db/db</i>
Body weight (g)	24.3 ± 0.7 (8)	30.5 ± 0.9 (8)*	27.9 ± 0.5 (16)	43.6 ± 1.2 (11)*
Heart dry weight (mg)	24.5 ± 1.0 (8)	23.5 ± 1.9 (8)	29.0 ± 1.0 (16)	28.0 ± 1.0 (11)
Plasma glucose (mmol/l)	13.6 ± 0.6 (8)	23.8 ± 2.4 (8)*	12.7 ± 0.5 (16)	46.2 ± 2.3 (11)*
Plasma insulin (μU/ml)	24 ± 4 (6)	111 ± 19 (6)*	34 ± 9 (4)	165 ± 40 (7)*
Plasma FFA (mmol/l)	1.0 ± 0.1 (8)	1.8 ± 0.1 (8)*	1.4 ± 0.1 (10)	2.1 ± 0.2 (11)*
Cardiac function	(<i>n</i> = 5)	(<i>n</i> = 5)	(<i>n</i> = 14)	(<i>n</i> = 11)
HR (beat/min)	345 ± 22	307 ± 26	322 ± 16	272 ± 17*
Cardiac output (ml · min ⁻¹ · g ⁻¹ dry wt)	405 ± 40	418 ± 19	443 ± 22	371 ± 16*
Coronary flow (ml · min ⁻¹ · g ⁻¹ dry wt)	81 ± 11	85 ± 3	87 ± 4	86 ± 4
Aortic flow (ml · min ⁻¹ · g ⁻¹ dry wt)	324 ± 30	333 ± 19	355 ± 20	284 ± 17*
PSP·HR (mmHg·beats · min ⁻¹ · 10 ⁻³)	20.5 ± 1.3	18.1 ± 1.5	19.3 ± 0.9	16.3 ± 1.1*

Values are mean ± SE. Numbers in parentheses indicate the number of animals in each group. In the 12-week groups, preischemic function of hearts subjected to 13 and 15 min of ischemia is included. **P* < 0.05 compared with age-matched *db/+* mice.

malized for differences in CO (Fig. 2C and D). Thus, after correction, carbohydrate oxidation was not changed in 6-week-old *db/db* hearts but remained significantly reduced in hearts from 10- to 12-week-old and 16- to 18-week-old *db/db* mice. This indicates that the diabetes-induced decrease in carbohydrate metabolism was not secondary to reduced energy demand associated with decreased contractile performance. Corrected rates of palmitate oxidation were markedly increased for *db/db* hearts in all three age groups.

The relative contribution of glucose and palmitate oxidation to acetyl CoA generation was calculated (Fig. 2 bottom). For *db/+* hearts, palmitate oxidation contributed ~50% of the total calculated acetyl CoA production at all three ages. In contrast, the contribution of palmitate oxidation in diabetic *db/db* hearts was elevated to 77% at 6 weeks and to 90% for both the 10- to 12- and 16- to 18-week-old groups. Thus, fatty acids become the almost exclusive source of acetyl CoA in *db/db* hearts with increase in age. Acetyl CoA production normalized for cardiac output in 6-week-old *db/db* hearts was 38.2 ± 2.6 nmol/ml (*n* = 6) compared with 18.8 ± 0.9 nmol/ml in control *db/+* hearts (*n* = 8), suggesting that cardiac efficiency is reduced substantially. This difference persisted in *db/db* hearts from the older age groups.

Age-dependent changes in contraction and metabolism after ischemia-reperfusion in hearts from male *db/db* mice. Hearts from 6- and 12-week-old *db/db* and *db/+* male mice were subjected to 13 min of ischemia and reperfusion. The 6-week-old *db/db* male mice used for this protocol already showed both obesity and hyperglycemia (Table 2). Obesity and hyperglycemia, however, were more marked at 12 weeks of age. Compared with female *db/db* mice at the same ages, male *db/db* mice exhibited the diabetic phenotype at an earlier age and to a more severe degree. However, preischemic contractile function in working hearts from 6-week-old male *db/db* mice was not significantly different from age-matched *db/+* hearts (Table 2). Furthermore, the recovery of all functional parameters after 13 min of no-flow ischemia in 6-week-old *db/db* hearts was not different from the recovery of *db/+* hearts (Fig. 3). Preischemic contractile function in 12-week-old male *db/db* hearts was decreased relative to *db/+* hearts

(Table 2). In addition, we found that the recovery of mechanical function after ischemia (13 min), and reperfusion was significantly reduced in *db/db* hearts (Fig. 3). Extending the ischemic period to 15 min resulted in 22 ± 3, 6 ± 4, 18 ± 3, and 73 ± 6% recovery of CO, AF, PSP·CO, and PSP·HR, respectively, in 12-week-old *db/db* hearts (*n* = 5) compared with 46 ± 12, 39 ± 14, 47 ± 13, and 90 ± 5% recovery (all *P* < 0.05) of the same parameters in *db/+* hearts (*n* = 8).

Carbohydrate and palmitate oxidation rates were measured during the 60-min postischemic working heart reperfusion period (Fig. 4). There were no significant differences in postischemic carbohydrate or palmitate oxidation between hearts from 6- and 12-week-old *db/+* mice. In 6-week-old *db/db* hearts, postischemic carbohydrate oxidation was not different from age-matched *db/+* hearts, but palmitate oxidation was elevated. In hearts from 12-week-old *db/db* mice, postischemic carbohydrate oxidation was significantly reduced to 37% of age-matched *db/+* values, whereas the rates of palmitate oxidation remained elevated. Calculation of the relative contribution of glucose and palmitate oxidation to acetyl CoA generation showed that palmitate oxidation contributed ~45% of total calculated acetyl CoA production during reperfusion for *db/+* hearts. In diabetic *db/db* hearts, the contribution of palmitate oxidation was 70 ± 4% at 6 weeks and 86 ± 2% at 12 weeks. Thus, fatty acids were the predominant source of acetyl CoA in ischemic-reperfused diabetic hearts.

Treatment of male *db/db* mice with the PPAR-α ligand for 3 weeks (from 9 to 12 weeks of age) resulted in a significant reduction in plasma glucose and fatty acids (to 20.2 ± 3.3 and 1.3 ± 0.2 mmol/l, *n* = 5), with no changes in body weight or heart weight compared with untreated 12-week-old *db/db* mice. Administration of BM 17.0744 to *db/db* mice has previously been shown to increase glucose oxidation and reduce fatty acid oxidation in isolated working hearts subjected to normoxic perfusions (7). In the present study, carbohydrate and palmitate oxidation were measured in the reperfusion period after no-flow ischemia. BM 17.0744-treated *db/db* mice exhibited increased carbohydrate oxidation with a concomitant decrease in fatty acid oxidation during reperfusion (Fig. 5).

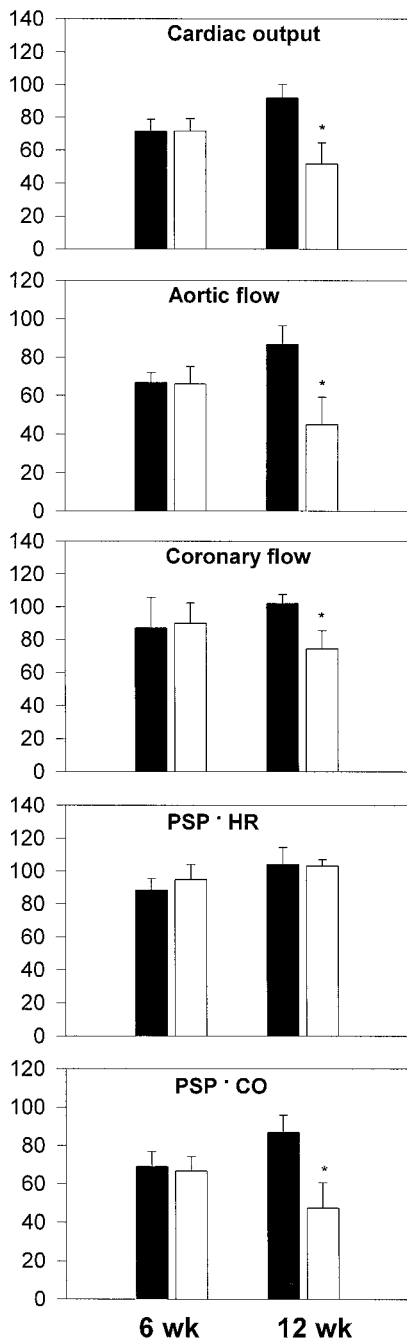


FIG. 3. Recovery of parameters of contractile function (as percentage of preischemic values) for perfused working hearts from nondiabetic *db/+* (■) and diabetic *db/db* (□) male mice at 6 and 12 weeks of age. Hearts were subjected to 13 min of no-flow ischemia, followed by 10 min of reperfusion in the Langendorff mode and 60 min of perfusion in the working heart mode. Results are mean \pm SE of five to six hearts. *Significantly different from *db/+* hearts at the same age.

In hearts from BM 17.0744-treated *db/db* mice, palmitate oxidation contributed $71 \pm 3\%$ of the total calculated acetyl CoA production, compared with $82 \pm 2\%$ from untreated *db/db* hearts.

Preischemic contractile function in hearts from BM 17.0744-treated *db/db* mice (heart rate 289 ± 8 beats/min; CO 348 ± 27 ml \cdot min $^{-1}$ \cdot g $^{-1}$ dry wt; coronary flow 74 ± 10 ml \cdot min $^{-1}$ \cdot g $^{-1}$ dry wt; aortic flow 274 ± 18 ml \cdot min $^{-1}$ \cdot g $^{-1}$ dry wt; PSP \cdot HR 17.4 ± 0.4 mmHg beat \cdot min $^{-1}$ \cdot 10 $^{-3}$; PSP \cdot CO 21 ± 2 mmHg \cdot ml \cdot min $^{-1}$ \cdot g $^{-1}$ dry wt \cdot 10 $^{-3}$) was

not different from untreated 12-week-old *db/db* mice (Table 2). Treatment of *db/db* mice with BM 17.044 had no effect on postischemic recovery (Fig. 6) compared with untreated *db/db* mice.

DISCUSSION

Previous evidence for the existence of a diabetic cardiomyopathy with altered cardiac metabolism and reduced contractile function in experimental animals has been obtained predominantly from experiments with type 1 (insulin-deficient) models of diabetes (15,16). This study demonstrates a progressive cardiomyopathy in a type 2 diabetic model, the *db/db* mouse; increased contractile dysfunction with age was associated with a progressive predominant reliance on fatty acid oxidation in hearts from female *db/db* mice. We also found increased susceptibility to ischemia-reperfusion injury in male *db/db* hearts with increasing age.

A small reduction in cardiac output was detectable in perfused working hearts from 6-week-old female *db/db* mice as compared with nondiabetic *db/+* mice. Conversely, cardiac output was not reduced in hearts from 6-week-old male *db/db* mice, even though they exhibited a modest degree of hyperglycemia compared with normoglycemia in female *db/db* mice. The functional differences for 6-week-old female *db/db* hearts, however, were marginal compared with much more pronounced signs of contractile dysfunction in hearts from older *db/db* mice (male and female). These results, showing progressive contractile dysfunction in ex vivo working *db/db* heart perfusions, are consistent with recent assessments of cardiac function in vivo by echocardiography (17), demonstrating both systolic and diastolic dysfunction in 12-week-old *db/db* mice, whereas cardiac function was unchanged in 6-week-old *db/db* male mice (17).

Age-dependent changes in cardiac metabolism were detected in hearts from female *db/db* mice. At 6 weeks, an increased rate of fatty acid oxidation was already evident in *db/db* hearts, whereas carbohydrate oxidation (adjusted for cardiac output) was not changed compared with *db/+* hearts. In accordance with previous studies, a marked decrease in carbohydrate oxidation was observed in 10- to 12-week-old *db/db* hearts. Thus, fatty acid oxidation becomes the predominant oxidative substrate in *db/db* hearts with increasing age. The biochemical mechanisms responsible for the reduction in carbohydrate oxidation in perfused hearts from *db/db* mice probably include both a translocation defect for the insulin-regulatable (GLUT4) glucose transporter (18) and inactivation of pyruvate dehydrogenase (19,20). The inactivation of pyruvate dehydrogenase in *db/db* hearts may be caused by the increased rate of fatty acid oxidation (21) that preceded the reduction in carbohydrate oxidation. The mechanism(s) for the increased rates of fatty acid oxidation in *db/db* hearts is (are) unknown but probably include increased sarcolemmal fatty acid uptake as demonstrated in giant vesicles from Zucker rat hearts (22) as well as increased mitochondrial fatty acid uptake (23,24). Investigation of potential mechanisms regulating fatty acid metabolism in *db/db* hearts is clearly an important future objective.

As demonstrated in the present and other studies (25), changes in cardiac metabolism appear early in the diabetic

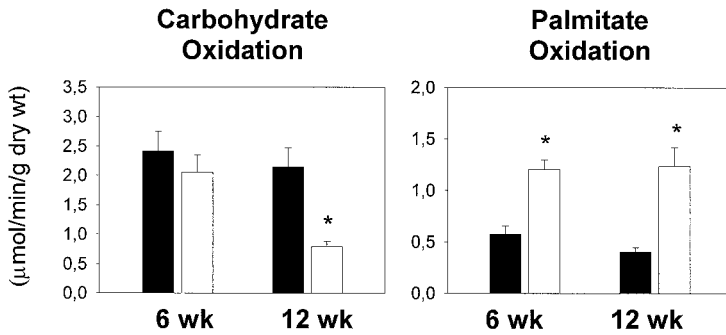


FIG. 4. Rates of postischemic carbohydrate and palmitate oxidation for perfused working hearts from nondiabetic $db/+$ (■) and diabetic db/db (□) male mice at 6 and 12 weeks of age (as described in Fig. 3). Hearts were subjected to 13 min of no-flow ischemia, followed by 10 min of reperfusion in Langendorff mode and 60 min of reperfusion in working heart mode. Postischemic metabolism was measured during the 60-min postischemic working heart perfusion. Results are mean \pm SE of five to six hearts and are expressed as $\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ dry wt. *Significantly different from age-matched $db/+$ hearts.

progression. Consequently, it has been proposed that these metabolic changes may contribute to the development of contractile dysfunction (25,26). Normalization of glucose and palmitate metabolism as a consequence of increased cardiac expression of the insulin-regulatable glucose (GLUT4) transporter also improved contractile function (6,17). This hypothesis is also supported by a recent study by Chatham and Seymour (25), demonstrating that cardiac metabolism was changed early in the diabetic progression in the ZDF rat, before signs of contractile dysfunction were detectable. Hearts from older ZDF rats showed ventricular dysfunction associated with myocardial TG accumulation, ceramide formation, and apoptosis (27). Therefore, Zhou et al. (27) proposed that lipotoxicity from overutilization of fatty acids may contribute to cardiac dysfunction in type 2 diabetes. Transgenic mice with increased fatty acid utilization as a result of cardiac-specific long-chain acyl-CoA synthetase overexpression (28) also demonstrated signs of reduced in vivo contractile function by echocardiography. The present study shows a progressive increase in TG content in db/db hearts. Furthermore, Giacomelli and Wiener (29) observed accumulation of lipid droplets and progressive degradation of mitochondria. It will therefore be interesting to examine db/db hearts for evidence of increased apoptosis in future investigations. In addition to lipotoxicity mechanisms, other nonmetabolic mechanisms, such as alterations in calcium handling, could contribute to contractile dysfunction.

Mouse hearts are much more susceptible to ischemia-reperfusion damage compared with other species; this is particularly evident in experiments with working (left ventricle ejecting) preparations as demonstrated by De Windt et al. (30). In this study, increasing the duration of global no-flow ischemia by 2 min (from 13 to 15 min)

decreased the recovery of cardiac output in control hearts from 92 ± 9 to $46 \pm 12\%$ of preischemic values.

The recovery of contractile function after ischemia-reperfusion for 6-week-old db/db hearts was unchanged relative to nondiabetic $db/+$ hearts. In contrast, the recovery of 12-week-old db/db hearts was reduced substantially compared with age-matched control hearts. Therefore, db/db hearts from mice at a relatively advanced stage in their diabetic progression exhibit enhanced susceptibility to ischemic damage, in addition to decreased normoxic function. Comparable experiments have not been performed with type 2 ZDF rat hearts. Perfused hearts from profoundly insulin-resistant but mildly hyperglycemic (31) corpulent JCR:LA-cp rats also showed enhanced sensitivity to an ischemia-reperfusion challenge, even though normoxic (preischemic) contractile function was not compromised (32). Results from experiments with insulin-deficient (type 1) diabetic models have been controversial with evidence for both increased and decreased tolerance to ischemia (33,34), probably as a result of differences in experimental conditions. It should be noted that db/db hearts were perfused with normal concentrations of 11 mmol/l glucose and 0.7 mmol/l fatty acids and no insulin. Future experiments will need to determine the influence of elevated glucose and fatty acid concentrations as well as insulin that mimic the in vivo diabetic condition in db/db mice at different ages. Elevated concentrations of fatty acids may also alter the degree of ischemic damage exhibited by diabetic db/db hearts, as has been demonstrated in type 1 diabetic models (35,36). Finally, because 12-week-old db/db mice demonstrated decreased postischemic recovery of coronary flow and because coronary flow is an important determinant of cardiac function, the decreased function can also reflect impaired flow as a result

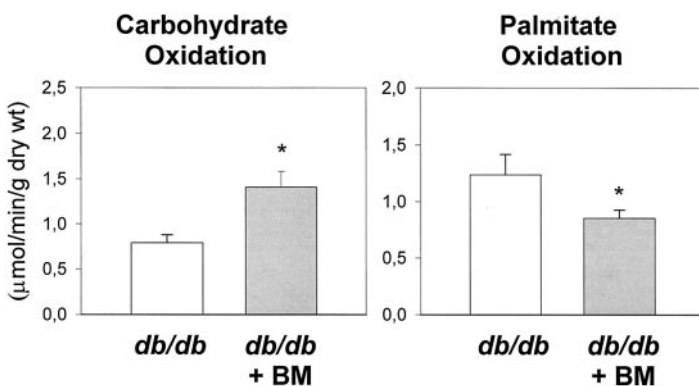


FIG. 5. Rates of postischemic carbohydrate and palmitate oxidation for perfused working hearts from 12-week-old diabetic db/db male mice (□; the same hearts as described in Fig. 4) and from 12-week-old db/db mice treated with BM 17.0744 for 3 weeks before ($db/db + BM$; ■; $n = 6$). Hearts were subjected to 13 min of no-flow ischemia, followed by 10 min of reperfusion in Langendorff mode and 60 min of perfusion in working heart mode. Postischemic metabolism was measured during the 60-min postischemic working heart reperfusion. Results are mean \pm SE and are expressed as $\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ dry wt. *Significantly different from db/db hearts.

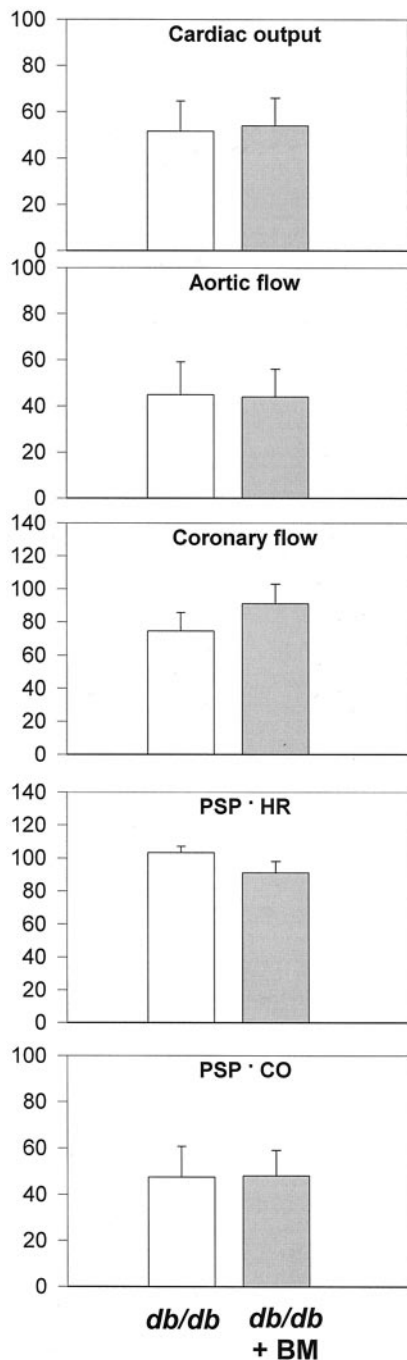


FIG. 6. Recovery of parameters of contractile function (as percentage of preischemic values) for perfused working hearts from diabetic *db/db* mice (□; same hearts as described in Fig. 3; 12-week-old *db/db* mice) and from BM 17.0744-treated *db/db* mice (*db/db* + BM; ■; $n = 6$). Hearts were subjected to 13 min of no-flow ischemia, followed by 10 min of reperfusion in Langendorff mode and 60 min of perfusion in working heart mode. Results are mean \pm SE.

of vascular or endothelial dysfunction in addition to myocyte dysfunction.

Treating *db/db* mice with the PPAR- α ligand BM 17.0744 resulted in a reduction in blood glucose consistent with previous investigations (7,37,38). BM 17.0744 also normalized plasma lipids (free fatty acids and TG) and reduced plasma insulin levels (7). The improvement of the diabetic status in *db/db* mice by BM 17.0744 also resulted in changes in cardiac metabolism; carbohydrate oxidation

was significantly increased with a concomitant reduction in fatty acid oxidation (7). The present study demonstrated that BM 17.0744-induced changes in cardiac metabolism were maintained during reperfusion. Stimulation of cardiac glucose metabolism in other experimental models has been demonstrated to be beneficial during several pathological conditions, including diabetes (39,40) and ischemia-reperfusion (41,42). Stimulation of glucose metabolism by acute dichloroacetate perfusion increased contractile function in hearts from insulin-deficient diabetic rats (39,40). In contrast, increased myocardial carbohydrate oxidation after 3 weeks of treatment with BM 17.0744 did not change normoxic cardiac function in diabetic *db/db* mice (present study, 7). Also, BM 17.0744 treatment did not improve mechanical recovery after ischemia-reperfusion in *db/db* mice (present study). This finding suggests that in the severe type 2 diabetic *db/db* mice, at least some of the adverse effects of diabetes on the recovery of contractile function after ischemia-reperfusion was not due to altered metabolism. This result, however, does not rule out the possibility that altered cardiac metabolism may still play a causal role in development of dysfunction, presumably related to development of irreversible changes in the myocardium.

Cardioprotective effects during ischemia-reperfusion have been reported after treatment with PPAR- γ ligands in isolated hearts from insulin-deficient diabetic rats (43,44), as well as in situ control rats (45) and pigs (46). PPAR- γ treatment also improved in vivo cardiac function in ZDF rats (27) and protected against ischemia-reperfusion in the Zucker rat (47). Therefore, improvement of normoxic cardiac function and enhanced ischemic recovery in diabetic hearts may be restricted to PPAR- γ activation.

It must be acknowledged that assessment of cardiac phenotype using mouse models has some major limitations. Most important, the very high heart rates and high basal level of systolic contraction of murine hearts limit applicability of experimental results to the human situation (48). Thus, although the use of murine models permits investigations of mechanisms underlying complex cardiac diseases such as diabetic cardiomyopathy (6), extrapolation to the human diabetic condition must take into consideration this limitation.

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