Myocardial infarction and heart failure in the \( \text{db/db} \) diabetic mouse

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**Greer, James J. M., Derek P. Ware, and David J. Lefer.** Myocardial infarction and heart failure in the \( \text{db/db} \) diabetic mouse. *Am J Physiol Heart Circ Physiol* 290: H146–H153, 2006. First published August 19, 2005; doi:10.1152/ajpheart.00583.2005.—Clinical studies have reported that the incidence and severity of myocardial infarction is significantly greater in diabetics compared with nondiabetics after correction for all other risk factors. The majority of studies investigating the pathophysiology of myocardial ischemia-reperfusion injury have focused on otherwise healthy animals. At present, there is a paucity of experimental investigations on the pathophysiology of heart failure in diabetic animals. We hypothesized that the severity of myocardial reperfusion injury and the development of congestive heart failure would be markedly enhanced in the \( \text{db/db} \) diabetic mouse. Accordingly, we studied the effects of varying durations of in vivo myocardial ischemia and reperfusion on the incidence of heart failure in \( \text{db/db} \) diabetic mice. Nondiabetic and \( \text{db/db} \) diabetic mice (10 wk of age) were subjected to 30, 45, or 60 min of left coronary artery occlusion and 28 days of reperfusion. Survival at 24 h of reperfusion was 100% in nondiabetic mice subjected to 30 min of myocardial ischemia and 88% in nondiabetic mice subjected to 45 min of myocardial ischemia. In contrast, survival was 53% in \( \text{db/db} \) diabetic mice subjected to 30 min of myocardial ischemia and 44% in \( \text{db/db} \) mice after 45 min of myocardial ischemia. Prolonged survival in nondiabetic mice was not significantly attenuated when compared during the 28-day follow-up period with all groups experiencing >90% survival. Prolonged survival was significantly decreased in \( \text{db/db} \) mice after both 30 and 45 min of myocardial ischemia compared with sham controls. Furthermore, we observed a significant degree or left ventricular dilatation, cardiac hypertrophy, and cardiac contractile dysfunction in \( \text{db/db} \) mice subjected to 45 min of myocardial ischemia and 28 days reperfusion. In nondiabetic mice subjected to 45 min of myocardial ischemia, we failed to observe any changes in left ventricular dimensions or fractional shortening. These studies provide a feasible experimental model system for the investigation of heart failure secondary to acute myocardial infarction in the \( \text{db/db} \) diabetic mouse.

**DIABETES MELLITUS (TYPE 2)** is a metabolic disease that develops as a result of insulin resistance leading to impaired glucose management. Characteristics of this disease include central obesity, elevated circulating glucose levels, increased serum insulin, and random glycosylation of proteins resulting in a loss of function. Obesity and a sedentary lifestyle play a major role in the development of Type 2 diabetes and are becoming more prevalent throughout the world (8). As a result, there has been a dramatic increase in the number of Type 2 diabetic patients and a concomitant rise in the incidence of cardiovascular disease. Diabetics tend to suffer unduly from cardiovascular disease complications because the morbidity and mortality from cardiovascular disease states are significantly increased in diabetics when compared with nondiabetics (12, 21). More specifically, diabetes mellitus increases the frequency and severity of myocardial infarction (MI) (13, 17, 32) as compared with age-matched nondiabetics. Diabetic patients have a two- to threefold greater risk of developing congestive heart failure (CHF) after MI and suffer from increased mortality after acute MI (10, 30). Although clinical studies have clearly established a correlation between diabetes mellitus and increased severity of MI, very little is currently known regarding the pathophysiological mechanisms involved in the response of the diabetic myocardium to ischemia. The development of an in vivo model of heart failure in diabetic animals would provide the necessary model system to further our understanding of the pathophysiology of CHF in diabetes mellitus.

Previous studies of heart failure in diabetes mellitus have been focused in large animal models (23, 39, 42). The development of a murine model of heart failure in the setting of diabetes mellitus allows for the investigation of gene-targeted mice and facilitates the use of gene therapy. Multiple murine models of diabetes are now currently used in the study of Type 2 diabetes. In particular, the \( \text{db/db} \) diabetic mouse suffers from a leptin receptor mutation resulting in the inability to regulate metabolism (7). Leptin receptor dysfunction results in obesity, elevated plasma glucose and insulin levels, and increased plasma free fatty acids (1, 36). Previous studies have demonstrated that the \( \text{db/db} \) mouse has increased susceptibility to myocardial ischemia-reperfusion injury (17, 27). The tendency for the increased severity of MI in the \( \text{db/db} \) mouse offers an excellent model system for the investigation of heart failure in the setting of diabetes mellitus.

In the present study, we subjected both nondiabetic and \( \text{db/db} \) diabetic mice to escalating durations of myocardial ischemia followed by reperfusion for 28 days. After reperfusion, we assessed survival, left ventricular (LV) remodeling, LV contractile function, and pulmonary edema.

**MATERIALS AND METHODS**

**Animals.** All animals received humane care in compliance with the “Principles of Laboratory Animal Care” formulated by the National Society of Medical Research and the **Guide for the Care and Use of Laboratory Animals** published by the National Institutes of Health (NIH Publication No. 85-23, Revised 1996). The experimental protocol was reviewed and approved by the Institutional Animal Care and Use Committee of LSU Health Sciences Center Shreveport. Ten-week-old male C57BL/KsJ-lepr\(^{+/-}\)/lepr\(^{db}\) diabetic (\( \text{db/db} \)) and age- and matched C57BL/6/J nondiabetic control mice were obtained from Jackson Laboratories (Bar Harbor, ME).

**Blood glucose levels.** Blood was obtained from diabetic and nondiabetic mice by tail snip, and blood glucose levels were measured in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
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with the use of One Touch SureStep test strips and meter. Glucose levels are expressed as milligrams per deciliter.

**Serum insulin levels.** Whole blood was obtained from the carotid artery of 10- and 12-week-old nondiabetic and diabetic mice. Serum was separated by centrifugation. Serum samples were then analyzed with the use of a commercially available mouse insulin ELISA kit (Crystalchem, Downers Grove, IL). This kit is highly specific for mouse insulin and has a sensitivity of 39 pg/ml.

**Hemoglobin A1c.** Whole blood was collected from the carotid artery of 10- and 12-week-old db/db diabetic and C57BL1/6 nondiabetic control mice. Plasma was isolated and hemoglobin A1c levels were measured in the clinical laboratories at the Louisiana State University Health Sciences Center.

**Myocardial ischemia-reperfusion protocol.** Surgical procedures used in the myocardial ischemia-reperfusion protocol were similar to methods previously described (20). Briefly, nondiabetic and db/db diabetic mice were weighed and anesthetized via intraperitoneal injections of pentobarbital sodium (50 mg/kg) and ketamine (60 mg/kg). Mice were secured to a surgical board ventral side up, and two-dimensional echocardiography was performed before the initiation of ischemia. Mice were then orally intubated and connected to a Harvard Apparatus Rodent Ventilator (model 835). Supplemental oxygen was supplied, and body temperature was monitored with the use of a rectal probe thermometer and controlled with an infrared heat lamp. A median sternotomy was performed, and the left coronary artery (LCA) was visualized and ligated with the use of a 7-0 silk suture mounted on a BV-1 tapered needle. A piece of PE-10 tubing was placed between the LCA and the 7-0 suture to minimize coronary injury induced by occlusion and to facilitate reperfusion. Mice were randomized for subjection to 30, 45, or 60 min of myocardial ischemia followed by reperfusion. After reperfusion, the chest wound was reapproximated and mice were extubated and allowed to recover with supplemental oxygen until mobile. All mice received butorphanol tartrate (0.2 mg/kg im) and cefazolin (50 mg/kg im) to minimize pain and infection. Mice surviving 24 h were enrolled into the survival study and were monitored for 28 days. Mice surviving the 28-day reperfusion protocol were anesthetized with pentobarbital sodium (50 mg/kg ip) and ketamine (60 mg/kg ip), and follow-up two-dimensional echocardiography was performed. The hearts from these mice were excised and weighed to calculate heart-to-body weight ratios. In addition, lungs were extracted to obtain accumulated pulmonary fluid measurements (Fig. 1).

**Two-dimensional echocardiography.** In vivo transthoracic echocardiography of the LV with the use of a 15-MHz linear array transducer (15L8) interfaced with a Sequoia C256 (Acuson, Mountain View, CA) was performed as previously described (20, 19). LV end-diastolic dimension (LVEDD), LV end-systolic dimensions (LVESDs), aortic diameter (AoD), aortic velocity time interval (AoVTI), and heart rate were measured at baseline and at 28 days after myocardial ischemia and reperfusion. LV percent fractional shortening (LV%FS) was calculated according to the following equation: $\text{LV}\%\text{FS} = [(\text{LVEDD} - \text{LVESD})/\text{LVEDD}] \times 100$. Stroke volume was calculated from the product of the aortic cross-sectional area, i.e., $\pi \times (\text{AoD}/2)^2$, and the AoVTI. Cardiac output was calculated from the product of the stroke volume and heart rate. All data were collected and calculated from 10 cardiac cycles per experiment.

**Cardiac hypertrophy.** Cardiac hypertrophy was assessed by heart-to-body weight ratios. Each excised heart weight at 28 days post-MI was divided by the weight of the mouse resulting in a ratio representative of cardiac hypertrophy.

**Pulmonary edema.** Extracted lungs were weighed (wet weight) and then placed in a drying oven at 40°C for 7 days. At day 7, the lungs were again weighed and the dry weight was subtracted from the wet weight. The resulting sum represents the accumulated pulmonary fluid.

**Statistical analysis.** Data were analyzed by Student’s unpaired t-test or ANOVA with Bonferroni’s post hoc analysis with the use of StatView (SAS Institute, Cary, NC) software. Kaplan-Meier survival curves and statistics were performed with the use of StatView software. Data are reported as means ± SE with differences accepted as significant when $P < 0.05$.

**RESULTS**

**Body weight.** Body weight measurements (Fig. 2A) demonstrated an obese phenotype because the db/db diabetic mouse ($n = 41$) body weight was significantly increased ($P < 0.01$) compared with nondiabetic control mice ($n = 38$). Body weight was 49 ± 0.4 g in the db/db diabetic mice compared with 24 ± 0.4 g in nondiabetic control animals.

**Blood glucose.** Fasted blood glucose levels were measured in db/db diabetic and nondiabetic control animals. Blood glucose levels (Fig. 2B) were significantly ($P < 0.01$) elevated in the db/db diabetic mice ($301 ± 15$ mg/dl) compared with nondiabetic control animals ($95 ± 5$ mg/dl).

**Insulin levels.** Serum insulin levels (Fig. 2C) were significantly increased from 1.7 ± 0.1 ng/ml in nondiabetic mouse to 12.5 ± 0.8 ng/ml in db/db diabetic mice ($P < 0.01$ between groups).

**Hemoglobin A1c.** Measurements of plasma hemoglobin A1c levels for nondiabetic and db/db diabetic mice are displayed in Fig. 2D. Diabetic mice exhibited a significant ($P < 0.01$) increase in the percentage of glycosylated hemoglobin (9.2 ± 0.2%) when compared with age-matched nondiabetic mice (4.5 ± 0.3%).

**Acute survival.** Survival data for both nondiabetic and diabetic mice at 24 h after myocardial ischemia and reperfusion are presented in Fig. 3, A and B. Nondiabetic mice subjected to sham ischemia-reperfusion or 30 min of myocardial ischemia experienced 100% survival at 24 h. In contrast, nondiabetic mice subjected to 45 min of ischemia and reperfusion showed limited mortality with 88% of mice surviving (Fig. 3A). Diabetic mice subjected to sham surgery had 100% survival at 24 h (Fig. 3B). Diabetic mice were also subjected to 30, 45, and 60 min of LCA ischemia-reperfusion and exhibited survival rates of 71%, 53%, and 18%, respectively, at 24 h after reperfusion (Fig. 3B). As a result of the high mortality rate at 24 h in diabetic mice subjected to 60 min of myocardial ischemia, this group was not enrolled in the 28-day heart failure study.
Prolonged survival. Survival data for both nondiabetic and db/db diabetic mice during the 28 days after reperfusion are presented in Fig. 4, A and B. Nondiabetic mice subjected to sham myocardial ischemia surgery (n = 10) or 30 min of myocardial ischemia (n = 12) experienced 100% survival over the course of the 28-day experimental protocol. Nondiabetic mice subjected to 45 min of ischemia (n = 14) had very limited mortality with a survival rate of 93%; \( P < 0.05 \) vs. sham (Fig. 4A).

Survival data for the db/db diabetic mice subjected to myocardial ischemia and reperfusion are presented in Fig. 4B. Sham-operated diabetic mice (n = 17) enrolled in the survival study demonstrated 100% survival at 28 days. Diabetic mice subjected to 30 min of myocardial ischemia experienced a survival rate of 58% (\( P < 0.05 \) vs. sham). Diabetic mice subjected to 45 min of LCA occlusion experienced a survival rate of 44% at 28 days after reperfusion (\( P < 0.01 \) vs. sham).

LVEDD. LVEDD data at baseline and after 28 days post-MI are presented in Fig. 5, A and B. Data are presented for animals subjected to either 30 or 45 min of myocardial ischemia for both nondiabetic (Fig. 5A) and db/db diabetic mice (Fig. 5B). No significant changes (\( P = \text{NS} \) vs. baseline) in LVEDD were observed in nondiabetic mice subjected to either 30 or 45 min of myocardial ischemia and 28 days of reperfusion. Baseline LVEDD was 3.2 ± 0.1 mm in the nondiabetic mice subjected to 30 min of myocardial ischemia and 3.3 ± 0.1 mm at 28 days post-MI. Similarly, LVEDD was 3.1 ± 0.1 mm at baseline in mice subjected to 45 min of myocardial ischemia and 3.2 ± 0.1 mm at 28 days after MI. Sham-operated nondiabetic mice displayed no significant differences in LVEDD between baseline and at 28 days post-MI (Table 1).

LVEDD data for db/db diabetic mice are presented in Fig. 5B. Diabetic mice subjected to 30 min of myocardial ischemia had similar (\( P = \text{NS} \)) measurements of LVEDD at baseline and 28 days post-MI (3.1 ± 0.1 vs. 3.5 ± 0.1 mm). In contrast, however, LVEDD in diabetic mice subjected to 45 min of ischemia displayed a significant increase in diastolic dimension at 28 days post-MI (3.8 ± 0.1 vs. 4.3 ± 0.1 mm). 

Fig. 2. Diabetic state in db/db diabetic mice. Body weight (g, A); blood glucose (mg/dl, B); serum insulin (ng/ml, C); and hemoglobin A1c (%, D) are measured in nondiabetic control and db/db diabetic mice at 10 wk of age. The db/db diabetic mice were obese, hyperglycemic, and hyperinsulinemic and displayed significant increases in hemoglobin A1c levels compared with nondiabetic controls. Numbers within circles represent number of animals included in each group. **\( P < 0.01 \) vs. nondiabetic mice.

Fig. 3. Acute survival after myocardial ischemia and reperfusion in nondiabetic and db/db diabetic mice. A: survival of nondiabetic mice at 24 h after sham surgery, 30 min of myocardial ischemia and reperfusion, or 45 min of myocardial ischemia and reperfusion. B: survival of diabetic mice subjected to sham surgery and 30, 45, or 60 min of myocardial ischemia and reperfusion. Numbers within circles indicate number of animals that survived divided by total number of animals enrolled in each study group.
sions from a baseline value of 3.2 ± 0.2 mm to 28-day post-MI measurements of 4.1 ± 0.2 mm at 28 days postreperfusion (P < 0.01 between baseline and 28 days). LVEDD of sham-operated mice were similar at baseline and 28 days after sham surgery (Table 1).

LVESD. LVESD data for both nondiabetic and diabetic mice are presented in Fig. 5, C and D. LVESD in the nondiabetic 30 min myocardial ischemia mice (Fig. 5C) were also similar at baseline and 28 days post-MI (2.1 ± 0.1 and 2.4 ± 0.1 mm, respectively). In addition, nondiabetic mice in the 45-min myocardial ischemia group (Fig. 5C) showed no changes in LV dimensions in systole between baseline and 28 days post-MI (2.2 ± 0.1 vs. 2.4 ± 0.2 mm). Similarly, sham-operated mice displayed no differences in baseline and 28-day LVESD measurements (Table 1).

LVESD measurements in diabetic mice subjected to 30 min of myocardial ischemia (Fig. 5D) revealed no change in baseline conditions compared with 28 days post-MI (1.9 ± 0.2 vs. 2.3 ± 0.2 mm, respectively). Interestingly, diabetic mice experiencing 45 min of myocardial ischemia and 28 days of reperfusion (Fig. 5D) exhibited significant (P < 0.01 vs. baseline) dilation from 2.1 ± 0.01 mm at baseline to 3.3 ± 0.3 mm at 28 days post-MI. LVESD were unchanged (P = NS) in sham-operated db/db diabetic mice at 28 days when compared with baseline (Table 1).

LV fractional shortening in nondiabetic mice. Data for LV fractional shortening in nondiabetic mice subjected to myocardial ischemia and reperfusion are presented in Fig. 6A. Nondiabetic mice subjected to 30 min of myocardial ischemia demonstrated no change in the LV fractional shortening when comparing baseline measurements (33.6 ± 2%) with measurements obtained 28 days post-MI (29.2 ± 2%). Similarly, 45 min of myocardial ischemia had no effect on fractional shortening in nondiabetic mice, (30 ± 2% vs. 25 ± 1%, respectively). Furthermore, no changes in fractional shortening were

![Fig. 4. Kaplan-Meier survival curves representing percentage of surviving mice subjected to sham surgery, 30 min of ischemia, or 45 min of ischemia during the 28-day experimental protocol in nondiabetic mice (A) and db/db diabetic mice (B). The 28-day survival was not significantly different in nondiabetic mice subjected to sham myocardial ischemia (n = 10), 30 min of myocardial ischemia (n = 12), and 45 min of ischemia (n = 14). In contrast, survival in db/db mice after 30 min of myocardial ischemia was significantly (P < 0.05) less than that observed in sham-operated controls. The 28-day survival was also significantly attenuated in db/db mice subjected to 45 min of myocardial ischemia compared with sham (P < 0.01). MI, myocardial infarction.](http://ajpheart.physiology.org/)

![Fig. 5. LV end-diastolic dimensions at baseline and 28 days after 30 or 45 min of myocardial ischemia in nondiabetic (A) and in db/db diabetic mice (B). LV end-systolic dimensions at baseline and 28 days post 30 or 45 min of myocardial ischemia in nondiabetic (C) and db/db diabetic mice (D). Numbers within circles represent number of mice investigated in each group. **P < 0.01 vs. baseline.](http://ajpheart.physiology.org/)
observed in sham-operated nondiabetic controls at 28 days after surgery when compared with baseline values (Table 1).

LV fractional shortening in diabetic mice. LV fractional shortening data for \textit{db/db} diabetic mice are shown in Fig. 6B. Diabetic mice subjected to 30 min of myocardial ischemia demonstrated no significant \((P = \text{NS})\) decrement in LV fractional shortening when comparing baseline measurements to 28-day post-MI measurements (37 \(\pm\) 3\% vs. 35 \(\pm\) 3, respectively). Diabetic mice subjected to 45 min myocardial ischemia, however, displayed a 30\% reduction in fractional shortening from 34 \(\pm\) 2\% at baseline to 21 \(\pm\) 2\% post-MI \((P < 0.01)\). No differences in LV fractional shortening were observed in diabetic, sham-operated controls at 28 days after sham surgery (Table 1).

Cardiac hypertrophy in nondiabetic mice. Heart-to-body weight ratio data for nondiabetic mice subjected to sham surgery, 30 and 45 min of myocardial ischemia, and 28 days of reperfusion are shown in Fig. 7A. No evidence of cardiac hypertrophy was observed in mice subjected to 30 min of myocardial ischemia compared with sham-operated control mice \((P = \text{NS})\). There was, however, a significant \((P < 0.05)\) level of cardiac hypertrophy in the nondiabetic 45-min myocardial ischemia group, 5.4 \(\pm\) 0.2 mg/g, compared with sham-operated animals, 4.6 \(\pm\) 0.1 mg/g.

Cardiac hypertrophy in diabetic mice. Heart-to-body weight ratio data for the \textit{db/db} diabetic mice subjected to sham surgery, 30 and 45 min of myocardial ischemia, and 28 days of reperfusion are presented in Fig. 7B. Assessment of heart-to-body weight ratios in 30-min myocardial ischemia diabetic mice revealed no significant cardiac hypertrophy compared with sham-operated diabetics \((P = \text{NS} \text{ vs. sham})\). Diabetic mice subjected to 45 min of LCA occlusion experienced approximately a 25\% increase in heart-to-body weight ratio compared with sham-operated diabetic mice \((P < 0.01 \text{ vs. sham})\).

Pulmonary edema. Pulmonary lung weight data obtained at 28 days after sham surgery, 30 min of myocardial ischemia, and 45 min of myocardial ischemia are depicted in Fig. 8. Data

![Fig. 6. LV percent fractional shortening measurements obtained by two-dimensional echocardiography at baseline and at 28 days of reperfusion in nondiabetic (A) and db/db diabetic mice (B) subjected to 30 or 45 min of myocardial ischemia. No significant changes were observed in LV fractional shortening in nondiabetic mice after either 30 or 45 min of myocardial ischemia. In contrast, LV percent fractional shortening was significantly reduced in diabetic mice subjected to 45 min of myocardial ischemia. Numbers within circles are number of mice per group. **\(P < 0.01\) vs. baseline.](image)

![Fig. 7. Cardiac hypertrophy in nondiabetic (A) and diabetic hearts (B) at 28 days after sham surgery, 30 min of myocardial ischemia, and 45 min of myocardial ischemia. Cardiac hypertrophy was determined by the heart-to-body weight ratio. Significant increases in cardiac hypertrophy were observed in both nondiabetic and db/db diabetic mice after 45 min of myocardial ischemia and 28 days of reperfusion. Numbers within circles are number of mice per group. *\(P < 0.05\) vs. sham nondiabetic; **\(P < 0.01\) vs. sham diabetic.](image)
For nondiabetic mice are shown in Fig. 8A and for db/db diabetic mice in Fig. 8B. No evidence of pulmonary edema was observed in nondiabetic or diabetic mice subjected to myocardial ischemia and 28 days of reperfusion when compared with respective sham-operated controls.

**DISCUSSION**

In the present study, we present data regarding the effects of coronary artery ligation and reperfusion on the development of heart failure in the db/db diabetic mouse. Both nondiabetic and diabetic mice were subjected to 30, 45, or 60 min of LCA ligation followed by 28 days of reperfusion. We investigated survival, LV function (i.e., fractional shortening), cardiac hypertrophy, and pulmonary edema during a 28-day follow-up period. The results clearly indicate that the db/db mouse develops LV dilatation, cardiac hypertrophy, and significant reductions in LV contractile function after 45 min of LCA occlusion and reperfusion. The db/db diabetic mice also experienced significantly greater mortality after both 30 and 45 min of myocardial ischemia when compared with either sham-operated diabetic mice or nondiabetic mice subjected to LCA ligation and reperfusion. This pathological response is generally consistent with the development of heart failure. In contrast, the nondiabetic control mice subjected to 45 min of coronary ischemia displayed very modest changes in survival and no changes in LV morphology, remodeling, or contractile function. The present study reveals a novel model of heart failure in the diabetic myocardium that allows for the investigation of the pathophysiology of heart failure in Type 2 diabetes mellitus.

Data obtained from this study further substantiate the severe diabetic state of the C57BL/KsJ-lepr<sup>db</sup>/lepr<sup>db</sup> (i.e., db/db diabetic) mouse and provides additional support for this mouse model as a relevant model of Type 2 diabetes mellitus for cardiovascular research. In the present study, we observed central obesity, elevated blood glucose and serum insulin levels, as well as increased glycosylated hemoglobin in the db/db mice compared with nondiabetic controls. Our data regarding the diabetic phenotype of the db/db mouse coincide well with other studies (1, 2, 36). We also confirmed that the db/db diabetic mouse exhibits a decreased tolerance to MI as myocardial ischemic durations of 30 and 45 min resulted in excessive mortality when compared with the nondiabetic controls. These data are in agreement with the increased severity of MI that has been reported in humans (22).

One of the most salient findings of the present study is the significant increase in mortality observed with the db/db mice after myocardial ischemia and reperfusion. We observed that survival at 24 h was only 53% in the db/db diabetic mice after only 30 min of myocardial ischemia and only 44% after 45 min of myocardial ischemia. Furthermore, survival in the db/db mice was reduced to 18% after 60 min of myocardial ischemia. In contrast, survival at 24 h in nondiabetic mice was 100% after 30 min of myocardial ischemia and 88% after 45 min of myocardial ischemia. This finding correlates well with the increased mortality of diabetic patients suffering from acute MI (22, 32). This increased mortality observed in the db/db diabetic mice at 24 h after myocardial ischemia and reperfusion may be a result of a number of factors. One factor that may play a role is enhanced inflammatory response in ischemia-reperfusion injury accompanied by diabetes as described previously (17, 31, 34). Another possible explanation for the increased mortality of the db/db mouse may likely be a result of increased reactive oxygen species (ROS) during myocardial ischemia and reperfusion (11, 22). Previous studies have clearly demonstrated that baseline ROS production is markedly enhanced in diabetic animals (41, 40, 14) and diabetic patients (3, 4, 37). It is likely that following reperfusion of the ischemic myocardium in the db/db mice results in an overwhelming increase in ROS production that leads to lethal cardiac arrhythmias and sudden death. In addition, the resulting mortality due to ischemic insult in the present study could be affected by the underlying cardiomyopathies associated with the diabetic state (21, 22, 35) that in concert with ischemia can result in sudden death. Given the complex pathology of Type 2 diabetes mellitus, there are numerous explanations for the increased injury and mortality due to ischemic insult in the db/db diabetic mouse. The present study presents a model that allows for the investigation of various pathological factors involved in the development of heart failure as well as the increased morbidity and mortality that is observed in the setting of diabetes mellitus.

Animal models of heart failure have offered great insight into the pathophysiology of CHF (18, 19, 26). The superimposition of a diabetic phenotype onto myocardial ischemia-reperfusion injury dramatically increases the clinical relevance of the experimental model. Diabetes in the absence of MI and heart failure results in a number of pathologies that are perpetuated in heart failure, which might be targeted for the study of interventional approaches.

One area of great interest in the pathology of diabetes mellitus is the potential role of endothelial dysfunction in Type 2 diabetes (5, 24, 28, 29). It has previously been reported that...
endothelial nitric oxide (NO) synthase (eNOS)-derived NO is diminished in the setting of Type 2 diabetes (6, 9). Pharmacological agents and gene therapy approaches that increase eNOS function or NO bioavailability have previously been shown to improve survival in humans in heart failure (25) as well as animal models of heart failure (16, 18, 26). Conversely, in a murine model of heart failure, hypertrophy and dysfunction are exacerbated in the absence of eNOS (15). Therapeutic approaches that increase eNOS-derived NO decrease collagen and fibrinogen deposition in the viable myocardium, increase vascularityization of the insulted myocardium (26), and decrease afterload (18). Given that diabetes mellitus and hyperglycemia are known to result in protein modifications and impair cell signaling (4), it would be interesting to determine whether eNOS/NO therapy would prove beneficial in the diabetic myocardium in the setting of heart failure.

In the current study utilizing the db/db diabetic mouse and myocardial ischemia-reperfusion protocols, we were able to reproduce the exacerbation of injury and incidence of heart failure that is observed in diabetic patients. In the present study, we observed a significant degree of mortality in diabetic mice as well as diminution of cardiac function caused by LV dilatation, which by itself promotes the progression of heart failure and mortality (38). This diabetic model of MI and heart failure also results in the development of cardiac hypertrophy that develops as a result of compensatory mechanisms but later becomes detrimental and perpetuates morbidity by increasing the demands of a failing heart (33). We did not observe any signs of pulmonary edema in the db/db mice at 28 days after myocardial ischemia as evidenced by pulmonary fluid weights. Thus it does not appear that the heart failure induced in this model resembles CHF. It is likely that longer periods of myocardial ischemia or longer durations of reperfusion after MI would result in CHF. This study provides a novel animal model that allows for the advancement of our current understanding of heart failure pathophysiology in the setting of diabetes mellitus. Future studies employing this model system will also allow for the formulation of pharmacological or genetic interventions to treat heart failure in diabetic patients.

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