Onset of diabetes in Zucker diabetic fatty (ZDF) rats leads to improved recovery of function after ischemia in the isolated perfused heart

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Wang, Peipei, and John C. Chatham. Onset of diabetes in Zucker diabetic fatty (ZDF) rats leads to improved recovery of function after ischemia in the isolated perfused heart. Am J Physiol Endocrinol Metab 286: E725–E736, 2004.—The aim of this study was to determine whether the transition from insulin resistance to hyperglycemia in a model of type 2 diabetes leads to intrinsic changes in the myocardium that increase the sensitivity to ischemic injury. Hearts from 6-, 12-, and 24-wk-old lean (Control) and obese Zucker diabetic fatty (ZDF) rats were isolated, perfused, and subjected to 30 min of low-flow ischemia (LFI) and 60 min of reperfusion. At 6 wk, ZDF animals were insulin resistant but not hyperglycemic. By 12 wk, the ZDF group was hyperglycemic and became progressively worse by 24 wk. In spontaneously beating hearts rate-pressure product (RPP) was depressed in the ZDF groups compared with age-matched Controls, primarily due to lower heart rate. Pacing significantly increased RPP in all ZDF groups; however, this was accompanied by a significant decrease in left ventricular developed pressure. There was also greater contracture during LFI in the ZDF groups compared with the Control group; surprisingly, however, functional recovery upon reperfusion was significantly higher in the diabetic 12- and 24-wk ZDF groups compared with age-matched Control groups and the 6-wk ZDF group. This improvement in recovery in the ZDF diabetic groups was independent of substrate availability, severity of ischemia, and duration of diabetes. These data demonstrate that, although the development of type 2 diabetes leads to progressive contractile and metabolic abnormalities during normoxia and LFI, it was not associated with increased susceptibility to ischemic injury.

cardiomyopathy; hyperglycemia; contractile function; type 2 diabetes

DIABETES LEADS to an increased incidence of myocardial ischemia as well as increased mortality following ischemia (18, 19). The increased incidence of ischemia can be attributed in large part to increased vascular disease; however, there is accumulating clinical and experimental evidence that a specific diabetic cardiomyopathy may contribute to the worse outcome following ischemia (7). For example, Lehto et al. (28) found that the increased mortality after ischemia was primarily a result of pump failure and was not related to infarct size. They concluded that changes at the level of the myocardium caused by diabetes were the primary cause of increased mortality after ischemia (28). However, experimental studies into the impact of diabetes on the response of the heart to ischemia have shown that diabetes increases, decreases, or has no effect on the tolerance of the heart to ischemia (16, 34). This inconsistency is particularly striking in light of the clear clinical evidence that, after myocardial infarction, the outcome for diabetic patients is substantially worse than for nondiabetic patients (18, 19).

Several factors may contribute to the variability seen in the results from studies into the effects of diabetes on the heart during ischemia. For example, the sensitivity to ischemic injury after diabetes appears to be substrate dependent, with hearts from diabetic animals showing decreased tolerance compared with nondiabetic groups when fatty acids were present (16). The severity and type of ischemia (i.e., low-flow vs. no-flow ischemia) are also reported to affect the outcome when hearts are compared from diabetic and nondiabetic animals (16). Furthermore, the duration and severity of the diabetic state also appear to be related to the sensitivity of the diabetic heart to ischemia (16). Another factor to be considered is that the majority of experimental investigations into the impact of diabetes on the heart have been performed using the streptozotocin-induced model of diabetes. This is typically a model of uncontrolled type 1 diabetes, associated with rapid-onset, severe insulin deficiency and the resulting severe metabolic disturbance. In contrast, >90% of diabetic patients have type 2 diabetes, which is a chronic, progressive disease involving obesity and insulin resistance. Thus, to understand the impact of type 2 diabetes on the heart and its response to ischemia, it would seem appropriate to consider different stages of the disease from the prediabetic, insulin-resistant stage, to the newly diabetic, and finally to the late diabetic stage.

Therefore, the purpose of this study was to test the hypothesis that the transition from insulin resistance to type 2 diabetes leads to changes at the level of the myocardium that result in increased sensitivity to ischemia and reperfusion. To account for substrate-dependent effects, studies were carried out in which hearts were perfused either with a physiological substrate mixture containing glucose, lactate, pyruvate, and palmitate or with glucose alone. We also used a low-flow ischemic protocol and modulated the severity of the ischemic insult either by allowing hearts to beat spontaneously or by pacing during the ischemic period. To evaluate the impact of the duration of diabetes, animals were studied in the prediabetic state, shortly after the onset of diabetes, and after a prolonged period of diabetes. We used the Zucker diabetic fatty rat (ZDF)
as a model of type 2 diabetes and studied animals at 6, 12, and 24 wk of age, times that span the range from insulin resistance alone to overt diabetes and severe, uncontrolled diabetes (11, 36). To ensure that any differences between the groups could be attributed to changes at the level of the myocardium and not a consequence of systemic factors, all experiments were carried out on isolated perfused hearts from ZDF and age-matched lean littermates (Control).

METHODS

Animals

Animal experiments were approved by the Institutional Animal Care and Use Committee and followed the Guide for the Care and Use of Laboratory Animals (National Academy of Sciences, 1996). Male Zucker diabetic fatty (ZDF/Gmi-fa/fa) rats and age-matched, lean control (7/fi) littermates were obtained from Charles River Laboratories Genetic Models (Indianapolis, IN). Animals were studied at 6, 12, and 24 wk of age. All animals were maintained on Purina 5008 diet as recommended by the supplier. The composition of this diet by weight is 23% protein, 58.5% carbohydrate, and 6.5% fat. The standard rodent diet used at our institution (Harlan Teklad 22.5 Rodent Diet W) consists of ~22% protein, 51% carbohydrate, and 5% fat by weight. It should be noted that the Purina 5008 diet is still a relatively low-fat diet, with fat contributing only 16.7% of the calories. In contrast, in rodent studies of diet-induced obesity, the diets consist of relatively low-fat diet, with fat contributing only 16.7% of total calories (14, 47); however, it is lower than the mean arterial pressure of 75 mmHg during the periods of equilibration and reperfusion as previously described (8, 9). Flow was controlled by a perfusion pump, and coronary flow was adjusted to maintain a constant perfusion pressure of 75 mmHg during the periods of equilibration and reperfusion. This pressure was consistent with our previously published studies (8, 9), as well as studies by others using similar preparations (12, 26). Flow was controlled by a perfusion pump, and coronary flow was adjusted to maintain a constant perfusion pressure of ~100 mmHg typically seen in vivo in rats. The perfusate consisted of Krebs-Henseleit buffer containing 1% BSA (essentially fatty acid free, Intergen, Mansfield, MA), 0.5 mM glucose, and 50 μU/ml insulin. In the majority of experiments, the perfusate contained 5 mM glucose, 0.32 mM sodium palmitate, 0.5 mM sodium lactate, and 0.05 mM sodium pyruvate as substrates. The perfusate was not recirculated. The substrate concentrations were chosen to try to mimic the normal fed state as previously described (30); however, although 0.32 mM palmitate was in close agreement with that found in the Control animals (see Table 1), 5 mM glucose was below the levels found in the Control groups. In another series of experiments, 5 mM glucose was the only substrate added into the perfusate (Glucose only); however, 1% BSA was also present in these experiments. Heart function was monitored via a fluid-filled balloon placed into the left ventricle and connected to a pressure transducer (HPA-7 Heart Performance Analyzer and BPA Blood Pressure Analyzer; Digi-Med, Louisville, KY). End-diastolic pressure (EDP) was set to 5 mmHg by adjusting balloon volume.

Analysis of Serum Metabolites

Blood samples were collected immediately after decapitation from all animals. Enzymatic colorimetric methods were used to analyze serum levels of glucose, triglycerides, cholesterol (Ektachem DK60 Analyzer), and free fatty acids (FFAs) (Wako NEFA C kit; Wako Chemicals, Neuss, Germany). Insulin and leptin levels were determined by radioimmunoassay (RI-13K and RL-84K, Linco Research, St. Charles, MO). IGF-I levels were determined by radioimmunoassay (DSL-2900, Diagnostic Systems Laboratories, Webster, TX).

Measurement of Troponin I Release in Effluent

As a marker of tissue injury, cardiac troponin I (cTn-I) concentration in coronary effluent was determined at 4, 30, and 60 min after reperfusion by use of ELISA (Troponin I ELISA kit; Life Diagnostics, cat. no. 2010). Total cTn-I release is expressed as nanograms per minute per gram wet weight. Before ischemia, there was no measurable release of cTn-I from the hearts. cTn-I has been shown to be a highly sensitive and specific marker of myocardial tissue injury in the isolated perfused heart, showing similar kinetics of release to both creatine kinase and lactate dehydrogenase (3).

Measurements of Lactate Efflux and Uptake

In a subset of experiments, [3-13C]lactate (99% enriched) was added to the perfusate to replace unlabeled lactate of the same concentration. Because the perfusate was not recirculated, 1H-NMR spectroscopic analysis of the perfusate and effluent samples combined with measurements of total lactate concentrations enabled us to differentiate between the 13C-labeled lactate added to the perfusate and the unlabeled lactate formed from metabolism of exogenous glucose or glycogen, as previously described (6). The ratios of 13C-labeled to unlabeled lactate were calculated from the 1H-NMR spectra; these data were multiplied by the total lactate concentration in the effluent to determine the concentrations of 13C-labeled and unlabeled lactate in effluent and perfusate. The rate of exogenous lactate uptake was then determined by the difference in the [3-13C]lactate concentrations in the perfusate and effluent multiplied by the coronary flow rate. Glycolytic lactate efflux was determined by unlabeled lactate concentration in the effluent multiplied by the coronary flow rate. All values have been normalized by wet heart weights and are expressed as micromoles per minute per gram.

Lactate concentration in the perfusate and coronary effluent was determined by use of enzymatic colorimetric methods [Ektachem DT Slide (LAC); Johnson & Johnson Clinical Diagnostic, Rochester, NY].

Experimental Protocol

All hearts were initially allowed to beat spontaneously and equilibrate for 30 min. In one group, hearts continued to beat spontaneously throughout the experiment (Unpaced). In the second group, hearts were paced at ~300 beats/min (SD9 Stimulator; Grass, Quincy, MA) from 10 min before ischemia throughout the experiment (Paced). In both Unpaced and Paced groups, low-flow ischemia (LFI) was induced by reducing the flow to 0.3 ml/min for 30 min. Constant-flow perfusion was used during LFI, because under constant pressure conditions, flow gradually decreases during the ischemic period as contracture develops. The use of constant flow during LFI results in greater control over the severity of the ischemic injury and consequently increases reproducibility of the experiments. Reperfusion was initiated by restoring the flow rate to achieve a perfusion pressure of 75 mmHg, which was maintained for a further 60 min. Coronary effluent samples were collected before ischemia, every 5 min during ischemia, every 1 min during the first 4 min of reperfusion, and after 30 and 60 min of reperfusion. The numbers of experiments in each group were as follows: Unpaced experiments: n = 6, 9, and 4 for 6-, 12-, and 24-wk Control groups and n = 6, 7, and 5 for 6-, 12-, and 24-wk ZDF groups; Paced experiments: n = 6, 7, and 5 for 6-, 12-, and 24-wk Control groups and n = 6, 6, and 5 for 6-, 12-, and 24-wk ZDF groups.

In the majority of experiments, hearts from Control and ZDF rats at 6, 12, and 24 wk of age were used. However, to assess whether the response of the hearts to ischemia and diabetes was substrate dependent, hearts from 12-wk old Control (n = 8) and ZDF (n = 5) rats...
were subjected to the Unpaced protocol with glucose as the only metabolic substrate throughout the entire protocol.

Statistics

All data are presented as means ± SE. Paired t-tests and one-way and repeated-measures ANOVA were used where appropriate and combined with Scheffe’s post hoc test. A P value of <0.05 was considered significant (Statview; Abacus Concepts, Berkeley, CA).

RESULTS

Animal Characteristics

Body weight, heart weight, and serum metabolite data for age-matched Control and ZDF rats are summarized in Table 1. As expected, at 6 and 12 wk the ZDF rats were 33 and 32% heavier than Controls; however, at 24 wk the ZDF group weighed significantly less than the Control group. The lower weight gain in the ZDF group between 12 and 24 wk reflects the severity of the diabetic state and is consistent with the natural history of severe, uncontrolled type 2 diabetes in humans. There were no consistent differences in frozen heart weights between ZDF and Control groups. The heart weight-to-body weight ratio was significantly lower in both the 6- and 12-wk ZDF groups compared with age-matched Controls; however, this reflects the higher body weight in the ZDF group rather than any differences in heart weight.

At 6 wk of age, serum glucose was modestly elevated in the ZDF group; since the animals were in the fed state, this presumably reflects the glucose intolerance present at this age. At this age, triglycerides were about sixfold higher in ZDF compared with Control groups, but FFA levels were not elevated. By 12 wk of age, glucose levels in the ZDF group were threefold higher than for age-matched Controls; triglycerides were almost 10 times higher, and fatty acids had increased by ~50%. At 24 wk, glucose, fatty acid, and triglyceride levels had all increased further in the ZDF group. High-density lipoprotein cholesterol (HDLC) levels were also higher in the ZDF group compared with age-matched Controls at all ages. Total cholesterol was significantly higher in the 6- and 24-wk ZDF groups. The higher HDLC level in the ZDF group is consistent with other reports of serum lipoproteins in ZDF rats (43, 44) and may reflect altered lipoprotein metabolism in these animals (4). In the 6-wk groups, both insulin and leptin levels were ~10 times higher in ZDF animals compared with Controls. At 12 wk of age, insulin and leptin levels in ZDF rats were still higher than in Controls; however, they had both declined significantly compared with 6-wk ZDF rats. By 24 wk, insulin levels in the ZDF group had declined markedly and were significantly lower than in the Control group. At this age, leptin levels were still significantly higher in ZDF rats; however, they were lower than in the 6- and 12-wk ZDF groups. Differences in IGF-I levels followed those for insulin, being significantly elevated in 6- and 12-wk ZDF rats and depressed in the 24-wk ZDF rats compared with age-matched Controls.

These data are entirely consistent with previously published data on ZDF rats (36, 48) and clearly demonstrate that the ZDF animals exhibit a type 2 diabetic phenotype.

Preischemic Cardiac Function

Unpaced group. Baseline, preischemic cardiac function of spontaneously beating hearts from 6-, 12-, and 24-wk-old Control and ZDF groups is summarized in Table 2. The intrinsic heart rate was significantly lower in all ZDF groups compared with age-matched Control animals. There was also a significant age effect for heart rate in both Control and ZDF groups, which was especially pronounced in the ZDF groups. However, there were no differences in left ventricular developed pressure (LVPD = peak systolic pressure − EDP) between the Control and ZDF groups at any age. Consequently, because of the lower heart rates, the rate-pressure product (RPP = heart rate × LVPD) was significantly depressed in the 12- and 24-wk ZDF groups compared with age-matched Controls. The RPP in the 24-wk ZDF group was also significantly depressed compared with both 6- and 12-wk ZDF groups. RPP normalized to heart weight (RPP/HW) showed a similar pattern to RPP alone, demonstrating both a significant difference between Control and ZDF groups and a significant age effect in both groups (ANOVA). In the 24-wk ZDF group, positive and negative changes in pressure over time (+dP/dt and −dP/dt) were also significantly depressed compared with age-matched Control groups. In the ZDF group, there was also a significant age effect for both +dP/dt and −dP/dt; there was no age effect in the Control group. Coronary flow was lower in the 12-wk ZDF group compared with age-matched Controls, but there were no differences between ZDF and Control groups at either

Table 1. Characteristics and serum metabolic data from fed Control and ZDF rats at 6, 12, and 24 wk of age

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>ZDF</th>
<th></th>
<th>Control</th>
<th>ZDF</th>
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<th>Control</th>
<th>ZDF</th>
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<tbody>
<tr>
<td>6 Wk</td>
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<td>12 Wk</td>
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<td>24 Wk</td>
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<tr>
<td>Body wt, g</td>
<td>182±3</td>
<td>242±6*</td>
<td>289±5</td>
<td>381±7*</td>
<td>466±8</td>
<td>429±6*</td>
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<tr>
<td>Heart wt, g</td>
<td>1.20±0.04</td>
<td>1.28±0.05</td>
<td>1.58±0.04</td>
<td>1.89±0.07*</td>
<td>2.22±0.05</td>
<td>1.93±0.04*</td>
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<tr>
<td>HW/BW, × 104</td>
<td>6.6±0.2</td>
<td>5.3±0.2*</td>
<td>5.6±0.1</td>
<td>5.0±0.2*</td>
<td>4.8±0.2</td>
<td>4.5±0.1</td>
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<td>Glucose, mM</td>
<td>8.3±0.2</td>
<td>10.8±0.6*</td>
<td>9.2±0.2</td>
<td>28.1±1.0*</td>
<td>11.3±0.3</td>
<td>39.2±2.4*</td>
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<td>FFA, mM</td>
<td>0.27±0.02</td>
<td>0.29±0.02</td>
<td>0.26±0.02</td>
<td>0.37±0.03*</td>
<td>0.39±0.03</td>
<td>0.73±0.06*</td>
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<tr>
<td>TG, μM</td>
<td>0.58±0.06</td>
<td>3.38±0.24*</td>
<td>0.70±0.08</td>
<td>6.62±0.18*</td>
<td>1.77±0.29</td>
<td>6.32±0.68*</td>
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<tr>
<td>HDLC, mM</td>
<td>108.6±4.4</td>
<td>164.0±16.3*</td>
<td>108.9±11.6</td>
<td>157.2±2.8*</td>
<td>135.0±12.4</td>
<td>365.7±30.8*</td>
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<tr>
<td>CHO, mM</td>
<td>0.17±1.01</td>
<td>0.26±0.01*</td>
<td>0.19±0.02</td>
<td>0.23±0.02</td>
<td>0.20±0.01</td>
<td>0.51±0.02*</td>
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<tr>
<td>Insulin, nM</td>
<td>0.53±0.04</td>
<td>3.90±0.49*</td>
<td>0.74±0.07</td>
<td>2.17±0.34*</td>
<td>0.57±0.04</td>
<td>0.29±0.01*</td>
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<tr>
<td>Leptin, nM</td>
<td>0.14±0.03</td>
<td>2.10±0.06*</td>
<td>0.19±0.03</td>
<td>1.55±0.10*</td>
<td>0.81±0.05</td>
<td>1.12±0.06*</td>
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<tr>
<td>IGF-I, μM</td>
<td>0.28±0.01</td>
<td>0.40±0.02*</td>
<td>0.30±0.01</td>
<td>0.41±0.01*</td>
<td>0.30±0.01</td>
<td>0.24±0.01*</td>
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</table>

Values are means ± SE; n, no. of rats/group. HW, heart wt; BW, body wt; FFA, free fatty acids; TG, triglycerides; CHO, total cholesterol; HDLC, high-density lipoprotein cholesterol; IGF-I, insulin-like growth factor I. *P < 0.01, ZDF vs. age-matched Control.
6 or 24 wk. There was a significant age effect for coronary flow in both Control and ZDF groups.

**Paced group.** Because the predominant difference in cardiac function between Control and ZDF groups was a lower heart rate, we decided to normalize heart rate by pacing hearts at ~300 beats/min, and the results are summarized in Table 2. After pacing, heart rates ranged from 301 to 305 beats/min; the mean heart rate across all experiments was 304 ± 0.3 beats/min. There were no between- or within-group differences in LVDP after pacing; however, in the 12- and 24-wk ZDF groups and the 24-wk Control group, LVDP decreased significantly after pacing. ANOVA indicated a significant difference in the decrease in LVDP after pacing in the ZDF compared with the Control groups. After pacing, there were no significant differences between or within groups in RPP; ANOVA of the complete data set indicated that the increase in RPP after pacing was significantly greater in the ZDF groups compared with the Control groups. After pacing, there was no significant difference in RPP/HW between Control and ZDF groups at 6 and 24 wk; however, at 12 wk, RPP/HW was still significantly lower in the ZDF group. There was also a significant age effect in RPP/HW after pacing for both Control and ZDF groups.

There were no between-group differences in either +dP/dt or −dP/dt after pacing; however, there were still significant age effects for both parameters in the ZDF group. No such age effect was seen in the Control group. The age effect was primarily a result of a significantly lower +dP/dt in the 24-wk ZDF groups compared with the 6-wk ZDF group and a lower −dP/dt in the 24-wk ZDF group compared with the 12-wk ZDF group. There was a slight increase in coronary flow in all groups after pacing, and the percent increase in coronary flow was greater for the ZDF than for the Control groups. After pacing, there were no significant differences in coronary flow between Control and ZDF groups at any age.

### Effect of LFI

The onset of LFI led to a cessation of contractile function within ~2–3 min in all Unpaced and Paced experiments. During 30-min LFI, there was a gradual increase in EDP characteristic of the development of contracture that was particularly pronounced in the Paced experiments, as shown in Fig. 1A. In the Unpaced experiments, although repeated-measures ANOVA demonstrated a significant time effect, there was no significant difference between preischemic EDP and either maximum EDP or end-ischemic EDP in any of the groups. Repeated-measures ANOVA also indicated that there was no significant group or age effect in the development of contracture in the Unpaced experiments. However, in all the Paced groups there was marked contracture development, starting after ~10 min of ischemia. There was no difference in the time of onset of contracture or the time to peak contracture between any two of the groups (data not shown). When all groups were combined, repeated-measures ANOVA indicated a significantly greater contracture in the ZDF compared with Control groups and a greater contracture with increasing age. Maximum and end-ischemic EDP values were significantly increased compared with preischemic values in all groups and were higher overall in the ZDF compared with Control groups (ANOVA).

The coronary flow normalized to HW during LFI is summarized in Fig. 1B. Because of the increase in HW with age, there was a significant age effect for coronary flow in both Control and ZDF groups, resulting in significantly lower flow rates in both Control and ZDF groups at 12 and 24 wk compared with 6-wk groups. This lower flow could contribute to the greater contracture seen at 12 and 24 wk in Paced groups. In the 12-wk groups, flow was ~15% lower in the ZDF group, and at 24 wk it was ~15% higher in the ZDF group.
whereas there were no differences in flow between Control and ZDF groups at 6 wk. Thus differences in contracture in the Paced experiments between Control and ZDF groups do not appear to be a result of different flow rates during the ischemic period.

**Functional Recovery**

In all groups, restoration of flow led to rapid recovery of contractile function. Function in both Unpaced and Paced groups at the end of reperfusion is summarized in Fig. 2. In the Unpaced groups, in contrast to preischemic function, heart rate was not significantly different between Control and ZDF groups at 6 and 12 wk of age; in fact, in 12- and 24-wk ZDF groups, heart rate was significantly increased compared with preischemic values. LVDP was significantly depressed compared with preischemic levels in 6- and 12-wk Control and ZDF groups, but not in either of the 24-wk groups; however, there were no differences in LVDP between Control and ZDF groups at any age. Surprisingly, in the 24-wk ZDF group, RPP was significantly higher than preischemic levels, although it was still lower compared with the 24-wk Control group. At the end of reperfusion, EDP was not significantly different from preischemic or end-ischemic levels in any of the Unpaced groups. RPP was significantly lower than preischemic levels only in the 6-wk Control group. There were no differences in +dP/dt at the end of reperfusion between Control and ZDF groups at 12 and 24 wk of age. Due to technical problems, +dP/dt was not recorded during reperfusion in the 6-wk group.

In the Paced experiments, LVDP and RPP at the end of reperfusion were significantly depressed compared with preischemic values in all Control groups and in the 6-wk ZDF group. RPP was significantly lower than preischemic levels only in the 6-wk Control group. There were no differences in +dP/dt at the end of reperfusion between Control and ZDF groups at 12 and 24 wk of age. Due to technical problems, +dP/dt was not recorded during reperfusion in the 6-wk group.

In the Paced experiments, LVDP and RPP at the end of reperfusion were significantly depressed compared with preischemic values in all Control groups and in the 6-wk ZDF group. In both 6-wk Control and ZDF groups, the recovery of RPP was ~40% of preischemic values. This compares with ~80% recovery of RPP in comparable groups in the Unpaced experiments, which is consistent with the observation that
Pacing during ischemia leads to greater ischemic injury (20). Surprisingly, at both 12 and 24 wk, the ZDF groups had significantly better recovery of LVDP and RPP compared with age-matched Control groups and 6-wk ZDF groups. The improved recovery of LVDP and RPP in the 12- and 24-wk ZDF groups was associated with significantly lower EDP. Although +dP/dt at the end of reperfusion was significantly depressed compared with preischemic value; n.m., not measured because of technical problems. Unpaced experiments: n = 6, 9, and 4 for 6-, 12-, and 24-wk Control groups, respectively, and n = 6, 7, and 5 for 6-, 12-, and 24-wk ZDF groups, respectively. Paced experiments: n = 6, 7, and 5 for 6-, 12-, and 24-wk Control groups, respectively, and n = 6, 6, and 5 for 6-, 12-, and 24-wk ZDF groups, respectively.

As an index of the degree of tissue injury, we measured cTn-I levels in the coronary effluent at 4, 30, and 60 min after reperfusion in 12-wk Control and ZDF groups, and the results are summarized in Fig. 3. Before ischemia and at earlier times during reperfusion, cTn-I levels were below the limit of detection in both groups. It can be seen that, despite the differences in functional recovery between 12-wk Control and ZDF groups, there were no significant differences in cTn-I release between the groups at any of the time points examined.

Glucose-Only Experiments

In the experiments we have described, hearts were perfused with glucose, lactate, pyruvate, and palmitate as substrates. To
assess the effects of altered substrate availability, hearts from 12-wk-old Control and ZDF animals were perfused with glucose as the sole substrate throughout the experiment, allowed to beat spontaneously (i.e., Unpaced), and subjected to the LFI protocol. The results from these experiments are summarized in Fig. 4. Consistent with the previous Unpaced experiments, before ischemia RPP was significantly reduced in the ZDF compared with the Control group, primarily due to lower heart rate. Surprisingly, the development of contracture during LFI was substantially greater in both Control and ZDF groups compared with all of the other Unpaced groups. Interestingly, consistent with the observations in the Paced experiments, the development of contracture was greater in the ZDF groups compared with Controls with glucose only. However, despite the greater contracture in the ZDF group, the recovery of LVDP and RPP was similar in the Control and ZDF groups. Overall, when glucose was the sole available metabolic substrate, the differences in functional recovery between Control and ZDF groups were similar to the previous experiments when a physiological substrate mixture was provided. However, it is noteworthy that, in both Control and ZDF groups, RPP before ischemia and at the end of reperfusion was significantly higher in hearts perfused with the physiological substrate mixture than in those perfused with glucose alone.

Lactate Metabolism

In the Paced experiments, we calculated total lactate release determined by arteriovenous differences in lactate concentra-

![Graph](image-url)
tion combined with measurements of coronary flow. Before ischemia, in all groups there was no measurable difference in arteriovenous lactate concentration; thus net lactate release was zero, and net lactate release returned to zero within 4 min of reperfusion. During ischemia and early reperfusion, there was marked increase in arterial lactate concentration in Control and ZDF groups at all ages, reflecting net lactate release from the heart (Fig. 5). There were no significant differences in lactate release between Control and ZDF groups at any age during either LFI or reperfusion.

As noted above, during normoxic perfusion the arteriovenous lactate concentration difference was zero. However, we have previously reported that, in hearts perfused with both lactate and glucose, there was simultaneous uptake of exogenous lactate and efflux of lactate from glucose, resulting in a negligible arteriovenous lactate difference (6). Therefore, to determine the absolute rates of lactate uptake and efflux, in a number of experiments hearts in the Paced study were perfused with $^{13}$C-labeled lactate rather than unlabeled lactate, and $^1$H-NMR spectroscopy was used to determine the fractional enrichment of lactate in the coronary effluent (6). In Fig. 6, the rates of lactate uptake and efflux at baseline, at end LFI, and at end reperfusion are shown for Control and ZDF groups at 6, 12, and 24 wk of age. Interestingly, at baseline the rate of lactate efflux was depressed in 12- and 24-wk ZDF groups but was not significantly altered in the prediabetic 6-wk ZDF group. This is consistent with previously reported impaired glycolysis in ZDF rats at 12 wk of age (10). Lactate uptake at baseline was significantly lower only in the 24-wk ZDF group; this is consistent with impaired lactate uptake seen in hearts from more severe uncontrolled type 1 diabetic rats (8). The fact that lactate uptake was unaltered in the 12-wk ZDF animals is also consistent with a recent study showing that, in the newly diabetic ZDF rats, glucose but not lactate oxidation was decreased (10).

During LFI, the rate of lactate efflux was significantly lower than at baseline, possibly due to flow-related limitation in glucose uptake or lactate efflux; however, there was no difference between Control and ZDF groups at any age. As shown in Fig. 5, total lactate release included release during LFI and washout during early reperfusion; thus the rate of lactate efflux during LFI could be underestimated if lactate washout during early reperfusion were not included. When the data in Fig. 5 were used and adjusted for lactate washout on reperfusion, the rate of lactate efflux in Control vs. ZDF groups was 1.7 ± 0.1 vs. 1.4 ± 0.0, 1.7 ± 0.1 vs. 1.4 ± 0.1, and 2.0 ± 0.3 vs. 1.7 ± 0.3 μmol·min$^{-1}$·g$^{-1}$ in 6-, 12-, and 24-wk rats, respectively. Although this adjustment increased the rates of lactate efflux during LFI, there were still no differences between Control and ZDF groups at any age. During LFI there was no measurable uptake of lactate. At the end of reperfusion, both lactate uptake and efflux rates were significantly lower than baseline rates. Interestingly, the differences in lactate uptake and efflux observed before ischemia were no longer present during reperfusion.

DISCUSSION

Experimental studies into the effects of diabetes on the response of the heart to ischemia have resulted in contradictory conclusions, i.e., that diabetes increases, decreases, or has no effect on the sensitivity of the heart to ischemic injury (16, 34). It has been proposed that factors contributing to these disparate conclusions include differences in the severity of the diabetic state, the severity of the ischemic insult, and substrate presentation (34). In this study, hearts were isolated from rats that were insulin resistant but not diabetic, newly diabetic, and diabetic for >3 mo. We also perfused hearts with physiological substrate mixtures as well as with glucose as the sole substrate. The severity of the ischemic insult was also modulated by either allowing hearts to beat spontaneously or pacing throughout. Thus, for the first time, many of the potentially confounding factors noted above have been examined in a single study. We have shown that, regardless of the conditions, after LFI the recovery of cardiac function was greater in the 12- and 24-wk ZDF diabetic groups compared with the nondiabetic, age-matched Control groups as well as with the 6-wk nondiabetic ZDF group. These results suggest that the development of type 2 diabetes does not result in intrinsic changes to the myocardium that increase its sensitivity to ischemic injury. Although the development of diabetes did not increase the sensitivity to ischemia, there was evidence of impaired contractile function in the 12- and 24-wk ZDF groups. This was characterized in part by lower heart rate, as well as significant decreases in $+dP/dt$ and $-dP/dt$ with age that were not apparent in the Control groups. Zhou et al. (48) demonstrated a significant decrease in fractional shortening in 20-wk ZDF rats compared with age-matched controls, but they found no functional differences at 7 wk of age. Depressed cardiac function was also reported in severely diabetic $db/db$ mice (2). The impaired function in these older type 2 diabetic animals is consistent with our finding of depressed RPP in the 24-wk ZDF group. As noted above, reduced heart rate was a major contributing factor to the functional differences between Control and ZDF groups (Table 2). However, pacing normalized RPP across all groups; RPP correlation with total energy demand in
the isovolumic perfused heart suggests that the ZDF groups were capable of sustaining workloads similar to those of the Control groups. Whether this is also true at the higher workloads seen in vivo remains to be determined. However, whereas pacing increased RPP in the ZDF groups, there was a significant decrease in LVDP in response to pacing (Table 2). This may reflect subtle alterations in calcium handling similar to those reported in isolated myocytes from diabetic and insulin-resistant rats (15, 37, 38).

To our knowledge, the only study that has addressed issues similar to those raised here was by Aasum et al. (1), who investigated age-dependent changes in contractile function and sensitivity to ischemic injury in hearts from type 2 diabetic db/db mice. In female db/db mice they found a decrease in cardiac function in hyperglycemic 10- to 12-wk-old animals compared with age-matched lean, nondiabetic db/+ mice and 6-wk old normoglycemic db/db mice. This is consistent with the decrease in function between 6 and 12 wk in our ZDF groups. However, they saw no further decline in function in 16- to 18-wk-old animals, whereas we found that function had deteriorated further in our 24-wk-old group. Consistent with our finding of reduced heart rate in 12- and 24-wk-old ZDF animals, the decrease in function in the older db/db mice was also at least in part due to lower heart rate. However, the effects of pacing were not examined in that study; thus it is unknown whether cardiac function in the female db/db mice could be normalized by increasing heart rate. Aasum et al. (1) found that, after no-flow ischemia, functional recovery in 12-wk-old diabetic male db/db mice was depressed compared with nondiabetic age-matched controls. This is in contrast with our results, which surprisingly showed improved recovery in both 12- and 24-wk ZDF groups. The discrepancy could be due to a number of factors. For example, the glucose and fatty acid concentrations were about twofold higher in the db/db experiments, and there was no insulin present. In addition, they used a no-flow ischemia protocol rather than the low-flow ischemia protocol used here. It is also possible that the ejecting heart preparation used in the db/db study may be more sensi-
tive to changes in contractility than the isovolumic preparation used here.

Although we found improved functional recovery after reperfusion, during LFI there was significantly greater contracture development in the ZDF groups (Fig. 1). Ischemic contracture is frequently associated with increased severity of ischemic injury (23, 45), and increasing glycolysis has been shown to prevent contracture during ischemia (13, 46). Because diabetes is usually associated with impaired glucose utilization (7), it is possible that this contributed to the increased contracture seen in the 12-wk obese groups. Consistent with our earlier studies of carbohydrate metabolism in ZDF rats (10), we found a decreased rate of lactate efflux before ischemia in the 12- and 24-wk ZDF groups. However, during LFI, total lactate release and the rate of lactate efflux were similar in all groups (Figs. 5 and 6). These data demonstrate that defects in glycolysis associated with diabetes during normoxia are not apparent during LFI. This suggests that, during LFI, substrate delivery is the primary determinant of glycolytic flux in both Control and ZDF groups and that this dominates any differences in glycolysis between the two groups that were present before ischemia. These results also suggest that the greater contracture development in the ZDF groups is not a consequence of differences in glycolysis.

As noted above, greater contracture development is normally associated with greater ischemic injury and poorer functional recovery on reperfusion (45); however, this is not a universal finding (24, 25). Despite the greater contracture development in the 12- and 24-wk ZDF groups, functional recovery after reperfusion was significantly higher in both of these groups compared with age-matched Control and 6-wk ZDF groups. There was also no difference in tissue injury, as indicated by cTn-I release. Kolocassides and colleagues (24, 25) also reported a dissociation between ischemic contracture and functional recovery on reperfusion in the setting of ischemic preconditioning. They concluded that ischemic contracture was not a reliable index of tissue injury (24, 25), and the results presented here confirm that conclusion. They also found that the onset of contracture was associated with depletion of ATP but that improved recovery of function appeared to be related to less severe acidosis (25). The impact of type 2 diabetes on the relationship between ATP levels and intracellular pH during LFI remains to be determined.

Increased glycogen levels have been reported to be protective during LFI (13), and diabetes has been shown to increase cardiac glycogen levels (27). Therefore, it is possible that the improved functional recovery in the 12- and 24-wk ZDF groups could be attributed to higher glycogen levels. However, the protection associated with increased glycogen levels was also associated with increased lactate production (13), and we found no difference in either total lactate production or lactate efflux rates during LFI (Figs. 5 and 6). Thus, even if glycogen levels were increased in the diabetic ZDF groups, glycolytic flux does not appear to be enhanced; consequently, it seems unlikely that differences in glycogen content contributed to the improved functional recovery seen here. However, it should be noted that measurement of lactate production accounts only for anaerobic glycolysis and does not take into account any oxidation of pyruvate that may occur during LFI due to the presence of residual oxygen. Furthermore, since diabetes increases triglyceride levels in the heart (9, 35), it is also possible that increased oxidation of endogenous lipid stores could contribute to improved functional recovery in the diabetic groups. Clearly, more studies are required to better understand the contributions of both endogenous and exogenous substrates to energy production during LFI under both normal and diabetic conditions.

It is popularly believed that the presence of fatty acids leads to poorer outcome in response to ischemia (31). It has also been suggested that the recovery of diabetic hearts from ischemia is worse when fatty acids are present (16). However, we found that, in 12-wk Control and ZDF groups, functional recovery after LFI and reperfusion in hearts perfused with a physiological substrate mixture was similar to that in hearts perfused with glucose as the sole substrate (Fig. 4). Interestingly, the development of contracture was greater in hearts perfused with glucose alone, suggesting, as discussed above, more rapid depletion of ATP compared with hearts perfused with palmitate, lactate, and pyruvate. King et al. (22) proposed that, during LFI, the presence of residual oxygen combined with additional substrates during LFI might increase ATP synthesis compared with perfusions with glucose alone. Our results are certainly consistent with that conclusion, although the relative contributions of oxidative and nonoxidative metabolic pathways to ATP synthesis during LFI remain to be determined. Nevertheless, these results clearly indicate that the presence of fatty acids during ischemia is not inherently detrimental and suggest that more studies are required to investigate the influence of substrate availability on the response to ischemia and reperfusion.

Improved functional recovery of diabetic hearts after ischemia has been previously reported in acute, severely insulin-deficient models of diabetes (16, 29). However, this is the first such report in a model of type 2 diabetes. It is clear that the improved recovery of function is associated with the onset of hyperglycemia, since it is not seen in the insulin-resistant but nondiabetic 6-wk ZDF group. It is also evident that the phenomenon is unaffected by prolonged diabetes, since it is also present in the 24-wk ZDF group. Thus the onset of hyperglycemia appears to afford some protection of the heart to ischemic injury. Interestingly, high glucose incubation of isolated myocytes causes upregulation of the antiapoptotic factor Bcl-2 and inactivation of the proapoptotic factor Bad, which leads to increased tolerance to hypoxia (39). Hyperglycemia also activates protein kinase C (5), which has been shown to have a protective effect against ischemia (41). It remains to be determined whether these factors contribute to the improved functional recovery seen in the diabetic ZDF groups.

It is important to note that, in these experiments, the hearts have been removed from their metabolic and neurohumoral environment and perfused under identical conditions. Thus it is possible that the impaired preischemic function in the diabetic ZDF groups characterized primarily by lower spontaneous heart rate may be a consequence of this in vitro preparation. Furthermore, whereas the data clearly demonstrate that the onset of type 2 diabetes increases the sensitivity of the myocardium to injury resulting from ischemia and reperfusion, it is possible, if not likely, that in vivo diabetes may result in systemic changes that could result in increased ischemic injury. For example, both obesity and diabetes are associated with increased sympathetic activity, higher serum lipid levels, and increased levels of inflammatory cytokines (17), all of which
may lead to worse outcome in response to ischemic injury (33, 42). Interestingly, McDonagh et al. (32) reported that, when hearts from diabetic rats were perfused with red blood cells from diabetic animals, functional recovery was markedly impaired compared with those hearts perfused with normal red blood cells (32). This is supported by the observation that infarct size was higher in diabetic ZDF rats after ischemia and reperfusion in vivo (21). Recently, Scheuermann-Freestone et al. (40) showed in an elegant study that the cardiac bioenergetic status was significantly impaired in type 2 diabetic patients compared with nondiabetic controls; a reduction in myocardial energy reserve could clearly have a negative impact on the response to ischemia. It remains to be determined whether similar alterations in bioenergetic status are present in vivo in rodent models of type 2 diabetes.

This study has demonstrated for the first time, in a model of type 2 diabetes, abnormalities in contractile function in the isolated perfused heart even before the onset of overt hyperglycemia. These alterations in function become progressively worse with the duration of the disease. The onset of diabetes was also associated with decreased lactate efflux and lactate uptake, consistent with impaired glycolysis and carbohydrate metabolism. Paradoxically, despite these defects, the development of overt hyperglycemia also resulted in improved recovery of function after ischemia and reperfusion, although the degree of tissue injury was similar between groups. These results show that the onset of type 2 diabetes does not increase the sensitivity of the myocardium to ischemic injury. Thus the increased infarct size observed in vivo in ZDF rats (21) and possibly the higher mortality seen in diabetic patients after myocardial infarction are most likely a consequence of systemic factors rather than diabetes-induced changes in the myocardium.

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