Effects of Alloxan-Induced Diabetes on Ischemia-Reperfusion Injury in Rabbit Hearts

W. Mark Vogel and Carl S. Apstein

Hearts from rabbits with 8–16 weeks of alloxan-diabetes were compared with hearts from normal rabbits to determine whether diabetic myocardium is more sensitive to ischemic injury. In isolated buffer-perfused hearts, left ventricular developed pressure, diastolic pressure, time to peak pressure (TTPP), time to half-maximal relaxation (RT½), and positive and negative dP/dt were measured during generation of left ventricular filling curves before and after 90 minutes of low-flow ischemia. Hearts from diabetic rabbits (blood glucose, 384 ± 28 mg/dl, mean ± 95% confidence limits) had left ventricular developed and diastolic pressures similar to normal hearts but exhibited significant increases in TTPP and RT½, with decreased positive and negative dP/dt. Left ventricular chamber volume relative to heart mass was greater in diabetic than in normal hearts. Recovery of developed pressure after ischemia was similar in normal (41 ± 16%) and diabetic hearts (47 ± 13%). In diabetic hearts during recovery from ischemia, TTPP and RT½ remained increased compared with normal hearts, with positive and negative dP/dt decreased compared with normal hearts, in proportion to the preischemic differences. After ischemia, high-energy phosphates were depleted to the same extent in normal and diabetic rabbits. In coronary ligation experiments, histochemically determined infarct size in diabetic rabbits after 30 minutes occlusion and 24 hours reperfusion was similar to that in normal rabbits when adjusted for a significantly smaller heart weight and a correspondingly smaller anatomic risk region in the diabetic animals. Thus, despite characteristic abnormalities of mechanical function in diabetic hearts, the severity of injury after ischemia with reperfusion was normal for diabetic hearts. (Circulation Research 1988;62:975–982)

The incidence of heart failure after myocardial infarction is significantly greater in patients with diabetes mellitus than in nondiabetic patients with myocardial infarction.1,2 Because the incidence and severity of heart failure after infarction are related to infarct size,3,4 larger infarct size in diabetics might explain this increased risk. Increased myocardial infarct size in diabetics could result from increased severity of coronary atherosclerosis5,6 and, thus, an increased anatomic region at risk. Alternatively, since survival of ischemic myocardium can depend on the ability to metabolize glucose in preference to other substrates,7 diabetic myocardium may be more sensitive to ischemic injury, with an increased extent of injury for a given area at risk. Evidence regarding the latter hypothesis is quite contradictory. Different investigators have reported that infarct size in diabetic patients was larger,4 smaller,2 or unchanged8 compared with that in nondiabetic patients.

Several isolated heart studies suggest that diabetic hearts are more sensitive than normal hearts to anoxic or ischemic injury,10–11 but this disparity in sensitivity was alleviated by increased glucose12 and was eliminated by provision of substrates other than glucose.13 Hypoglycemia can increase infarct size,14 whereas augmented glucose and insulin may decrease infarct size.15 In mildly diabetic dogs, myocardial injury after coronary occlusion was comparable to that in nondiabetic dogs,16 but infarct size was larger than normal in severely diabetic dogs.17 Severely diabetic rats had higher mortality after coronary occlusion than did nondiabetic rats, but surviving diabetic rats had lower plasma levels of cardiac enzymes than did nondiabetic rats.18 Myocardial enzyme release after coronary ligation was increased in hearts from diabetic rats with severe ketosis but not in diabetic rats with moderate ketosis,19 suggesting that the extent of myocardial injury was related to ketosis rather than to diabetes per se.

To test the hypothesis that diabetic myocardium is more sensitive than normal myocardium to ischemia, we compared hearts from rabbits with alloxan-induced diabetes with hearts from normal rabbits by measuring the acute recovery of mechanical function after global ischemia in isolated hearts and by measuring the extent of myocardial necrosis 24 hours after coronary occlusion and reperfusion in intact animals. We observed characteristic abnormalities of cardiac function in diabetic animals20 but found no evidence of increased sensitivity to ischemic injury.

Materials and Methods Induction and Characterization of Experimental Diabetics

Diabetes was induced in male New Zealand rabbits (0.8–1.2 kg) by slow intravenous bolus injection of 125
mg/kg alloxan. Rabbits were housed with ad libitum access to Purina Rabbit Chow and water. Animals were weighed weekly, and 100 µl blood was obtained from an ear vein to determine nonfasting blood glucose. Animals were used for isolated heart experiments at 10–16 weeks after administration of alloxan or were used for coronary ligation experiments at 7–9 weeks. Animals were included in the diabetic group if their mean weekly blood glucose was greater than 250 mg/dl. Data from animals given alloxan whose blood glucose averaged between 125 and 250 mg/dl are not included in the report. Isolated heart function and metabolic measurements other than blood glucose for these animals did not differ in any respect from that of normal animals. On the day of an experiment, a blood sample was obtained, deproteinized by addition of an equal volume of 30% perchloric acid, and centrifuged, and the supernatant was stored at −20°C for measurement of acetoacetate and β-hydroxybutyrate. The inguinal fat pad was removed and weighed as an indicator of body fat. The normal comparison group consisted of age-matched animals not given alloxan and an equal number that were given alloxan but who were normoglycemic (blood glucose <125 mg/dl). Metabolic measurements and cardiac function of normoglycemic rabbits that had received alloxan did not differ from those of rabbits that did not receive alloxan.

Isolated Heart Preparation

Hearts were isolated and perfused by the cannulated aortic root. Coronary flow was kept constant at a rate initially adjusted to provide a perfusion pressure of 80 mm Hg. Perfusion rates were 4.6 ± 0.5 ml/min/g for normal hearts and 4.7 ± 0.5 ml/min/g for diabetic hearts. A water-filled balloon in the left ventricle controlled chamber volume. The cannulated balloon was attached to a transducer to measure ventricular pressure and electronically derived dP/dt. Hearts were paced at 3 Hz, and temperature was kept at 37°C. The nonrecirculated perfuse contained (mmol/l): NaCl 118, KCl 4.7, CaCl2 2.0, KH2PO4 1.2, MgSO4 1.2, NaHCO3 25, Na2-EDTA 0.4, glucose 5.5, sodium lactate 1.0, and octanoic acid 0.25. The venae cavae were ligated, and the pulmonary artery was cannulated with nitroblue tetrazolium (NBT). A solution of 35 mg NBT in 70 ml perfusion buffer was filtered through a Whatman GF/A glass fiber filter. Hearts were perfused for 12 minutes with this recirculated solution and then were fixed by perfusion with 15 ml buffered glutaraldehyde-formaldehyde fixative. After fixation, the coronary artery was ligated at the previous site of occlusion, and the heart was perfused with 25 ml fluorescent dye (6 mg/dl Rhodamine B in the fixative buffer) to outline the anatomic region at risk of infarction. Six slices about 2 mm thick were cut, perpendicular to the base-apex axis, below the point of coronary occlusion. Heart slices were photographed under white and ultraviolet light. Photographic slides were projected and traced on paper; areas of infarction and region at risk were determined by weighing the cut tracings. Infarct size and risk region were calculated by

Baseline Cardiac Function and Response to Isoproterenol

Hearts from 12 normal and 10 diabetic rabbits underwent the following protocol. After 30 minutes of equilibration, left ventricular–function curves were inscribed by increasing chamber volume in 0.2-ml increments so that ventricular diastolic pressure varied between 0 and 30 mm Hg. Between volume increments, traces were recorded for measurements of left ventricular diastolic pressure, developed pressure (systolic minus diastolic pressures), maximum positive and negative dP/dt, time to peak pressure (TTTP), and time from peak pressure to half-maximal relaxation (RTW). Adrenergic responses were then tested. Balloon volume was adjusted to obtain a left ventricular developed pressure of 80 mm Hg, pacing was ceased (intrinsic rate exceeded the pacing rate of 3 Hz during adrenergic stimulation), and isoproterenol was infused by syringe pump up to perfusate concentrations of 10−6 M. The infusion rate was increased until a maximal increase in +dP/dt was obtained. In a separate group, six normal and six diabetic hearts were isolated and perfused and then freeze-clamped and stored under liquid nitrogen until analyzed for ATP and creatine phosphate.

Effects of Global Ischemia

After generation of the isoproterenol dose-response curve, chamber volume was adjusted to a left ventricular diastolic pressure of 10 mm Hg. Arterial and venous perfusate samples were taken for measurement of oxygen tension and lactate concentration. Coronary flow was reduced to 11% of its baseline value for 90 minutes and was then returned to the initial rate for 30 minutes of reperfusion. Left ventricular pressures, dP/dt, venous-arterial lactate gradient, and oxygen consumption were measured at 15-minute intervals during ischemia and reperfusion. After 30 minutes of reperfusion, ventricular-filling curves were repeated, and hearts were freeze-clamped for measurement of ATP and creatine phosphate. This ischemia protocol was completed in seven of the 12 normal hearts; three hearts were subjected to more severe ischemia (5% of basal flow for 90 minutes), but that protocol was abandoned because recovery of developed pressure was less than 10%; two hearts did not complete the protocol because of an accidental air embolus and ventricular fibrillation during ischemia. The entire protocol was completed in all 10 diabetic hearts.

Infarct Size After Coronary Artery Ligation

A branch of the left circumflex coronary artery supplies the anterior-lateral and apical regions of the rabbit left ventricle. In pentobarbital anesthetized rabbits, this artery was reversibly occluded for 30 minutes midway between base and apex with a silk suture snare. After 24 hours of reperfusion, hearts were isolated and perfused as described above. To identify infarcted myocardium, hearts were stained with nitroblue tetrazolium (NBT). A solution of 35 mg NBT in 70 ml perfusion buffer was filtered through a Whatman GF/A glass fiber filter. Hearts were perfused for 12 minutes with this recirculated solution and then were fixed by perfusion with 15 ml buffered glutaraldehyde-formaldehyde fixative. After fixation, the coronary artery was ligated at the previous site of occlusion, and the heart was perfused with 25 ml fluorescent dye (6 mg/dl Rhodamine B in the fixative buffer) to outline the anatomic region at risk of infarction. Six slices about 2 mm thick were cut, perpendicular to the base-apex axis, below the point of coronary occlusion. Heart slices were photographed under white and ultraviolet light. Photographic slides were projected and traced on paper; areas of infarction and region at risk were determined by weighing the cut tracings.
multiplying the weight of each heart slice by the averaged basal and apical fractions of at-risk or infarcted tissue.

Statistical Comparisons

Data are presented as mean ± 95% confidence limits. For interpretation of graphs, differences between means are significantly different when the means fall outside each other's confidence limits with the group sizes used in these experiments. Differences between normal and diabetic groups were analyzed by Student's t test, Wilcoxon's rank test, analysis of covariance, or analysis of variance for repeated measures as indicated in the text. A probability less than 0.05 was used as the criterion for statistical significance in all tests.

Results

Characterization of Diabetic State

Characteristics of normal and diabetic animals used in the isolated heart experiments are shown in Table 1. In addition to increased blood glucose, diabetic rabbits exhibited increases of plasma acetoacetate and β-hydroxybutyrate concentrations, with decreases of body, inguinal fat pad, and heart weights. As indicated in Table 1, some differences were tested by the nonparametric Wilcoxon's rank test because the variability of these values was significantly greater in the diabetic group.

Adrenergic Responses

Isoproterenol dose-response curves in isolated hearts are illustrated in Figure 1. Dose-response curves were analyzed by an analysis of variance for repeated measures. This analysis demonstrated significant differences between normal and diabetic hearts for heart rate, TTPP, +dP/dt, and −dP/dt. The dose-response curves were shifted upward or downward in proportion to baseline differences between these values in the absence of adrenergic stimulation. There were no differences between normal and diabetic hearts in the extent of change caused by isoproterenol or in the concentration required to elicit half-maximal responses, suggesting that there was no alteration of adrenergic function per se in these diabetic hearts.

Isolated Heart Function and Response to Ischemia

Left ventricular function curves (developed pressure

Table 1. Characterization of Diabetic State in Rabbits

<table>
<thead>
<tr>
<th></th>
<th>Normal (n = 18)</th>
<th>Diabetic (n = 16)</th>
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<tbody>
<tr>
<td>Blood glucose (mg/dl)</td>
<td>104 ± 5</td>
<td>384 ± 28*</td>
</tr>
<tr>
<td>Plasma acetoacetate (μM)</td>
<td>63 ± 21</td>
<td>103 ± 41*</td>
</tr>
<tr>
<td>Plasma β-hydroxybutyrate (μM)</td>
<td>119 ± 38</td>
<td>288 ± 132*</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>3.4 ± 0.1</td>
<td>2.8 ± 0.3†</td>
</tr>
<tr>
<td>Inguinal fat pad weight (g)</td>
<td>12 ± 4</td>
<td>4 ± 2†</td>
</tr>
<tr>
<td>Heart weight (g)</td>
<td>11.2 ± 2.0</td>
<td>10.1 ± 1.0†</td>
</tr>
</tbody>
</table>

Values are mean ± 95% confidence limits.
*Variation is unequal in normal and diabetic groups, p<0.05 by the Wilcoxon's rank test; †p<0.05 by Student's t test.

Figure 1. Isoproterenol dose-response curves. Responses are on the y-axes, isoproterenolol concentration in log molar units is on the x-axis. Baseline values measured in the absence of isoproterenol are shown at the left of each panel, labeled “B.” Values are mean ± 95% confidence limits for 12 normal and 10 diabetic hearts. Left ventricular chamber volume was constant at the volume that produced a ventricular developed pressure near 80 mm Hg in the absence of isoproterenol.

Figure 2. Ventricular function curves before and after global ischemia in isolated rabbit hearts. Left ventricular developed pressure (systolic minus diastolic) is plotted as a function of left ventricular end-diastolic pressure (LVEDP). Values are mean ± 95% confidence limits for 12 normal hearts before ischemia, 7 normal hearts after ischemia, and 10 diabetic hearts before and after ischemia. Postischemic values were measured after 30 minutes of reperfusion at the initial coronary flow rate.
versus diastolic pressure) obtained before and after ischemia are shown in Figure 2. Developed pressure did not differ between normal and diabetic hearts before ischemia, and it was depressed to a similar extent after ischemia. After 30 minutes of reperfusion, maximal developed pressure recovered to 41 ± 16% of its preischemic value in normal hearts and to 47 ± 13% in diabetic hearts.

Left ventricular compliance curves (diastolic pressure versus chamber volume) obtained before and after ischemia are shown in Figure 3. Ventricular compliance was decreased after ischemia in normal and diabetic hearts as indicated by significant leftward shifts and increased slopes of the compliance curves. At a left ventricular diastolic pressure of 10 mm Hg, slopes of the compliance curves before ischemia were similar, 26 ± 3 mm Hg/ml for normal and 31 ± 8 mm Hg/ml for diabetic hearts. Corresponding values after ischemia were 50 ± 16 and 44 ± 13 mm Hg/ml (p < 0.05 for preischemia versus postischemia by paired t test; p > 0.05 for normal versus diabetic hearts by unpaired t test). There were no significant differences between normal and diabetic hearts in their diastolic pressure-volume curves. Relative to heart mass, however, chamber volume was significantly greater in diabetic hearts (Figure 4). There was a linear relation between heart mass and left ventricular chamber volume measured at a diastolic pressure of 10 mm Hg. Analysis of
Ischemia and Reperfusion in Diabetic Rabbit Hearts

Vogel and Apstein

**TABLE 2. Oxygen and Lactate Metabolism in Isolated Rabbit Hearts**

<table>
<thead>
<tr>
<th></th>
<th>Before ischemia</th>
<th>During ischemia (90 min)</th>
<th>After reperfusion (30 min)</th>
</tr>
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<tbody>
<tr>
<td>O₂ Consumption (ml/min/100 g)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>6.0 ± 0.4</td>
<td>0.9 ± 0.1</td>
<td>4.4 ± 0.9</td>
</tr>
<tr>
<td>Diabetic</td>
<td>5.7 ± 0.8</td>
<td>0.9 ± 0.1</td>
<td>4.2 ± 0.9</td>
</tr>
<tr>
<td>V-A lactate difference (mM)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>-0.05 ± 0.08</td>
<td>0.91 ± 0.27</td>
<td>-0.01 ± 0.07</td>
</tr>
<tr>
<td>Diabetic</td>
<td>-0.03 ± 0.04</td>
<td>0.88 ± 0.29</td>
<td>0.00 ± 0.03</td>
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</table>

Values are mean ± 95% confidence limits for seven normal and 10 diabetic hearts.
V-A, venous minus arterial lactate levels; positive values indicate net lactate production.

Covariance demonstrated that the slope of this relation was similar in normal and diabetic hearts but the relation was shifted toward greater volumes in the diabetic hearts. At a heart mass of 10 g, there was a difference of 0.44 ± 0.34 ml (p < 0.02), a 33% increase in diabetic relative to normal animals.

Positive and negative dP/dt, TTPP, and RT₁/₂ measured before and after ischemia are shown in Figures 5 and 6. These values varied in proportion to developed pressure during inscription of the filling curves and are presented as functions of developed pressure. Differences between normal and diabetic hearts in preischemic values were tested by analysis of variance for repeated measures. This test could not be used for the postischemic values because all hearts did not exhibit the same range of developed pressures (postischemic peak developed pressure ranged from 10 to 60 mm Hg in normal and from 11 to 65 mm Hg in diabetic hearts). The postischemic values and confidence limits illustrated in Figures 5 and 6 were derived from linear regression versus developed pressure, and differences between normal and diabetic hearts were tested by analysis of covariance. Before ischemia, TTPP and RT₁/₂ were significantly increased in diabetic hearts relative to values in normal hearts; both positive and negative dP/dt in diabetic hearts were significantly decreased relative to values in normal hearts. There was a significant interaction between developed pressure and groups with respect to RT₁/₂, indicating an increased slope of the relation in the diabetic hearts with greater differences in RT₁/₂ between normal and diabetic hearts at higher developed pressures. The abnormalities of diabetic hearts persisted after ischemia but were not exacerbated. Analysis of covariance showed that positive and negative dP/dt were decreased in diabetic compared with normal hearts, TTPP was increased in diabetic compared with normal hearts, and the slope of the relation between RT₁/₂ and developed pressure was greater in diabetic than in normal hearts.

Coronary resistance increased during the experiment. With coronary flow held constant, coronary perfusion pressure increased significantly from the initial value of 80 to 122 ± 26 mm Hg in normal hearts and 108 ± 13 mm Hg in diabetic hearts after 30 minutes of reperfusion.

**Isolated Heart Metabolism and Response to Ischemia**

Myocardial oxygen consumption and lactate metabolism (Table 2) were measured before and after ischemia at the left ventricular chamber volume that initially produced a diastolic pressure of 10 mm Hg. Before and after ischemia, normal and diabetic hearts exhibited net lactate balance with venous-arterial lactate differences not significantly different from zero. During ischemia, normal and diabetic hearts exhibited similar net lactate production. Oxygen consumption in normal and diabetic hearts was similar before ischemia, decreased to the same extent during ischemia, and recovered to the same extent after ischemia. Water and high-energy phosphate contents (Table 3) in normal and diabetic nonischemic hearts did not differ significantly. In postischemic hearts, water content increased and ATP content decreased compared with nonischemic hearts with no significant differences between normal and diabetic hearts.

**Risk Region and Infarct Size After Coronary Occlusion**

Coronary occlusion was performed on 16 normal and 17 diabetic rabbits. Mortality rate was 50% in normal and 53% in diabetic animals, yielding eight hearts in each group for measurement of infarct size. Blood glucose in the diabetic animals that did not survive (350 ± 29 mg/dl) was similar to that of diabetic survivors (360 ± 38 mg/dl). Risk region and infarct size are presented in Table 4. Body weight and heart weight were smaller in diabetic than in normal animals. Risk region and infarct size were correspondingly smaller in diabetic hearts, but the relation between infarct and risk region was similar to that of normal rabbits (Figure 7). Values for normal and diabetic hearts fell along the linear relation for infarct (MI) versus risk region (RR) established from 34 normal hearts that we studied previously: MI = (0.81 RR) - 0.17; r = 0.90. Infarct size adjusted for the difference in absolute risk region

**TABLE 3. High-Energy Phosphate and Water Content in Isolated Rabbit Hearts**

<table>
<thead>
<tr>
<th></th>
<th>Nonischemic</th>
<th>Postischemic</th>
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<tbody>
<tr>
<td></td>
<td>Normal (n = 6)</td>
<td>Diabetic (n = 6)</td>
</tr>
<tr>
<td>Water content (ml/g dry wt)</td>
<td>6.5 ± 0.9</td>
<td>6.6 ± 0.7</td>
</tr>
<tr>
<td>Adenosine triphosphate (μmol/g dry wt)</td>
<td>16 ± 4</td>
<td>18 ± 2</td>
</tr>
<tr>
<td>Creatine phosphate (μmol/g dry wt)</td>
<td>33 ± 5</td>
<td>29 ± 9</td>
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Values are mean ± 95% confidence limits.
*Significant difference compared with nonischemic value by Student's t test. There are no significant differences between normal and diabetic groups.
by analysis of covariance was similar in normal and diabetic hearts.

**Discussion**

This study was designed to test whether diabetes renders the myocardium more sensitive to ischemic injury independently of the severity of coronary disease. The rabbit is an appropriate animal for this study because diabetes does not accelerate atherogenesis in rabbits fed a normal diet.26,27 The same perfusate composition was used for both normal and diabetic hearts because we wanted to test whether diabetic myocardium per se was more sensitive to ischemia, independently of altered metabolic substrates (e.g., increased glucose and free fatty acids). In this study, diabetes produced abnormalities of systolic function (decreased rate of pressure development and prolonged TTPP) and abnormalities of diastolic function (decreased relaxation rate and prolonged RT 1/2). These abnormalities are characteristic of those described for diabetic rats23,28-30 and rabbits.31-33 The increase of left ventricular chamber volume relative to heart mass in diabetic rabbit hearts confirms a similar observation in diabetic rat hearts.34 Abnormal mechanical responses to adrenergic stimulation in diabetic rat hearts have been reported in some studies,35-38 but not in others.12,28,39,40 In papillary muscles from diabetic rabbits, responses to norepinephrine were essentially normal with slight blunting of the effect on relaxation rate.33 Thus, experimental diabetes can depress cardiac adrenergic responses, but this was not a characteristic of our model. Because occupation of a small fraction of cardiac β-adrenergic receptors19 is sufficient to elicit maximal responses (i.e., there are "spare receptors"), a substantial reduction in receptor number may be needed to alter mechanical responses. We did not find increased susceptibility to ischemic injury in isolated diabetic hearts. Coronary flow reduction to 11% of initial autoregulated flow was chosen to simulate the reduction observed after coronary occlusion in dogs16,42 or rabbits.43 Diabetes did not alter the ability to sustain anaerobic glycolysis during ischemia, judging from lactate production, nor did it affect residual oxidative metabolism during ischemia, judging from oxygen consumption. The degree of ischemic contracture, observed as a shift of the diastolic compliance curve, was similar in diabetic and normal hearts. Recovery of developed pressure after reperfusion was also similar in the two groups. Baseline diabetic abnormalities of positive and negative dP/dt, TTPP, and RT, were not exacerbated by ischemia. Water content increased and ATP content decreased to the same extent after ischemia in normal and diabetic hearts. Characteristically for this buffer-perfused preparation, nonischemic hearts were edematous because of the lack of colloid osmotic pressure and the arteriolar vasodilation that attends the low oxygen content of the perfusate. In our previous experience, reversal of edema by addition of dextran to the perfusate did not alter systolic function before or after ischemia,44 but myocardial edema probably increased baseline and postischemic diastolic stiffness.44,45

After coronary ligation in the rabbit, myocardial infarction progresses transmurally from endocardium to epicardium.35 Infarct size measured after 60 minutes of coronary occlusion is significantly greater than that measured after 30 minutes and is essentially transmural by 24 hours.35 Thus, the progression of infarction is incomplete at 30 minutes, and a significant increase in the rate of cell death should result in a larger infarct. In dogs46 and in rabbits,47 we found that infarct size measured by the NBT technique after coronary occlusion and reperfusion correlates very well with tetracycline deposition, another marker of myocardial cell

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**TABLE 4. Coronary Occlusion in Intact Rabbits**

<table>
<thead>
<tr>
<th></th>
<th>Normal (n = 8)</th>
<th>Diabetic (n = 8)</th>
</tr>
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<tbody>
<tr>
<td>Body weight (kg)</td>
<td>3.5 ± 0.5</td>
<td>2.6 ± 0.5 †</td>
</tr>
<tr>
<td>Left ventricular weight (g)</td>
<td>5.3 ± 0.5</td>
<td>4.0 ± 0.9 †</td>
</tr>
<tr>
<td>Risk region (g)</td>
<td>1.44 ± 0.21</td>
<td>0.98 ± 0.45 †</td>
</tr>
<tr>
<td>Infarct (g)</td>
<td>1.11 ± 0.14</td>
<td>0.66 ± 0.45 †</td>
</tr>
<tr>
<td>Adjusted infarct* (g)</td>
<td>0.94 ± 0.14</td>
<td>0.84 ± 0.14</td>
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Values are mean ± 95% confidence limits.
*Adjusted to equal the risk region by analysis of covariance.
†Significant difference compared with normal heart by Student's t test.

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**Figure 7. Infarct size as a function of region at risk measured 24 hours after temporary (30 minutes) coronary occlusion in eight normal and eight diabetic rabbits. Dashed line and stippled area represent the linear relation ± 95% confidence limits for infarct vs. risk region calculated from 34 normal rabbits from previous studies in this laboratory.**

Because we thought that baseline abnormalities in mechanical function of diabetic hearts might be exaggerated during adrenergic stimulation, we studied responses to isoproterenol. Diabetes altered basal and isoproterenol-stimulated values of several mechanical properties, but adrenergic responses were not altered with respect to the maximal changes caused by isoproterenol or the concentrations required to produce half-maximal effects. Several investigators have observed decreased β-adrenergic receptor number in diabetic rat hearts.34 Abnormal mechanical responses to adrenergic stimulation in diabetic rat hearts have been reported in some studies,35-38 but not in others.12,28,39,40 In papillary muscles from diabetic rabbits, responses to norepinephrine were essentially normal with slight blunting of the effect on relaxation rate.33 Thus, experimental diabetes can depress cardiac adrenergic responses, but this was not a characteristic of our model. Because occupation of a small fraction of cardiac β-adrenergic receptors is sufficient to elicit maximal responses (i.e., there are "spare receptors"), a substantial reduction in receptor number may be needed to alter mechanical responses. We did not find increased susceptibility to ischemic injury in isolated diabetic hearts. Coronary flow reduction to 11% of initial autoregulated flow was chosen to simulate the reduction observed after coronary occlusion in dogs16,42 or rabbits.43 Diabetes did not alter the ability to sustain anaerobic glycolysis during ischemia, judging from lactate production, nor did it affect residual oxidative metabolism during ischemia, judging from oxygen consumption. The degree of ischemic contracture, observed as a shift of the diastolic compliance curve, was similar in diabetic and normal hearts. Recovery of developed pressure after reperfusion was also similar in the two groups. Baseline diabetic abnormalities of positive and negative dP/dt, TTPP, and RT, were not exacerbated by ischemia. Water content increased and ATP content decreased to the same extent after ischemia in normal and diabetic hearts. Characteristically for this buffer-perfused preparation, nonischemic hearts were edematous because of the lack of colloid osmotic pressure and the arteriolar vasodilation that attends the low oxygen content of the perfusate. In our previous experience, reversal of edema by addition of dextran to the perfusate did not alter systolic function before or after ischemia,44 but myocardial edema probably increased baseline and postischemic diastolic stiffness.44,45

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death. The extent of myocardial injury determined by NBT staining also correlates well with the extent of myocardial enzyme depletion. In unpublished experiments, significantly reduced infarct size was detected by our technique for assessing risk region and infarction in rabbits pretreated with the ß-adrenergic antagonist propranolol.

Diabetes did not alter the relation between risk region and infarct size in this study. The percentage of ventricular mass at risk of infarction was similar in normal and diabetic hearts, but in absolute terms, the smaller diabetic hearts had smaller risk regions and infarcts. Like others, we observed a linear relation between risk region and infarct size that does not pass through zero (i.e., no infarct develops in the smallest risk regions). Thus, the ratio of infarct to risk region is not constant, and analysis of covariance was used to adjust for the disparity in risk region rather than expressing infarct size as a percentage of the region at risk. If expressed as a percentage of region at risk, infarct size in the diabetic group (63 ± 13%) appears to be less than that in the normal group (78 ± 9%). A significant difference in infarct size between normal and diabetic hearts was not observed after adjusting for the regression of infarct on risk region. The coronary ligation studies were performed after a shorter duration of diabetes (7–9 weeks) than in the isolated heart series (10–16 weeks). A longer duration of diabetes might conceivably have resulted in altered infarct size, but heart weight was decreased by diabetes in the coronary ligation series, indicating a significant effect of diabetes on the heart at that time. Cardiac abnormalities observed at 1 month in a diabetic rabbit model similar to ours were not more severe at 3 or 6 months.

Some previous studies of the isolated heart suggest that diabetic hearts are more sensitive than normal hearts to ischemic or anoxic injury. Several factors complicate interpretation of those studies. First, coronary flow, which is determined by systolic pressure in the ejecting hearts used in those studies, was in some cases lower during ischemia in diabetics because of a more rapid decline in contractile function. Secondly, the previous studies used glucose as the only substrate, unlike our preparation in which lactate and octanoate were available as substrates. Provision of substrates other than glucose during recovery from anoxia has completely normalized the function of diabetic hearts. Finally, severely diabetic rats used in previous studies can be markedly ketotic, with serum ketone concentrations as high as 17 mM. In Sinclair et al, the effects of ketosis on ischemic injury were examined with enzyme release as the measure of injury. Greater-than-normal injury was observed in hearts from diabetic rats with plasma ß-hydroxybutyrate concentrations greater than 2 mM but not in hearts from severely diabetic rats with lower ketone concentrations. Plasma ß-hydroxybutyrate of our diabetic rabbits (0.3 ± 0.1 mM) in the present study was well below the level of 2 mM defined as ketotic by Sinclair et al. Thus, previous studies of the isolated heart have reported normal sensitivity of diabetic hearts to anoxic or ischemic injury in the absence of severe ketosis and with substrates other than glucose available during recovery. But, diabetes was of short duration (2–9 days) in those studies, and baseline abnormalities of cardiac function were not observed. Our study extends those observations in a model of chronic diabetes with clearly demonstrable abnormalities of baseline cardiac function. Infarct size was greater than normal after coronary occlusion in severely diabetic dogs. As discussed above, this may have been due to an effect of ketoacidosis independent of diabetes; in mildly diabetic dogs, the extent of injury after coronary occlusion was normal. Clinical studies have produced contradictory results regarding the effect of diabetes on infarct size, but the methods available for human studies could not measure infarct size as a function of region at risk.

In summary, we studied global and regional myocardial ischemia and reperfusion in a model of chronic diabetes without severe ketoacidosis. Despite derangements of cardiac function, ischemic injury was not increased in diabetic hearts. The poor prognosis for myocardial infarction in diabetic patients may not be related to a myocardial defect that increases sensitivity to ischemia but rather to other factors, such as preexisting cardiomyopathy, impaired infarct healing, or increased severity of coronary artery disease.

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