Response of Isolated Working Hearts to Fatty Acids and Carnitine Palmitoyltransferase I Inhibition During Reduction of Coronary Flow in Acutely and Chronically Diabetic Rats

Gary D. Lopaschuk and Marguerite Spafford

The effects of palmitate on mechanical failure of ischemic hearts were studied in acutely (48-hour) and chronically (6-week) streptozotocin diabetic rats. Coronary flow was reduced by 50% in isolated working hearts perfused at a 15 cm H2O preload and 100 mm Hg afterload by the one-way ball valve model of ischemia. Peak systolic pressure (PSP) and cardiac output (CO) decreased 40% by 4 minutes in control hearts perfused with 11 mM glucose and paced at 280 beats/min, compared with 50% in hearts from acutely diabetic rats. Addition of 1.2 mM palmitate to the perfusate accelerated failure rates, with PSP and CO decreasing 65% and 80% by 4 minutes in control and acutely diabetic rat hearts, respectively. In chronically diabetic rats, mechanical function could not be maintained in palmitate-perfused hearts paced at 280 beats/min, even in the absence of ischemia. If these hearts were paced at 250 beats/min and subjected to ischemia, PSP and CO decreased 90% by 4 minutes, regardless of whether palmitate was added to the perfusate. Under these conditions, PSP decreased less than 10% by 4 minutes in both palmitate- or glucose-perfused control hearts. Etomoxir (10^-9 M), a carnitine palmitoyltransferase I inhibitor, markedly decreased the rate of mechanical failure in both acutely and chronically diabetic rat hearts, in the presence and absence of palmitate. The beneficial effect of Etomoxir on mechanical function did not occur as a result of a decrease in either myocardial long chain acyl-coenzyme A or long chain acylcarnitine levels. These data demonstrate that a reversible metabolic disorder involving fatty acids contributes to the increased susceptibility of the diabetic to myocardial ischemic injury. (Circulation Research 1989;65:378-387)

Metabolic alterations that occur as a result of diabetes can contribute to the severity of myocardial ischemic injury.1-3 A prominent metabolic alteration that occurs in diabetics is an elevation in circulating free fatty acids and an increased reliance of the heart on fatty acids as an energy substrate.4-8 This results in an increase in myocardial levels of fatty acid intermediates, such as long chain acyl-coenzyme A (CoA) and long chain acylcarnitine.2,9 Accumulation of these intermediates has been implicated in the accelerated failure rates of ischemic hearts from diabetic rats.2,3

In nondiabetics, a correlation between the severity of a myocardial infarction and serum levels of free fatty acids has been shown.10-13 In addition, a number of experimental studies demonstrate that fatty acids can potentiate ischemic injury.14-21 The mechanism by which this occurs has not been determined, but it has been suggested that accumulation of potentially harmful intermediates of fatty acid metabolism may be involved.14,17,19 The specific effects of fatty acids on mechanical failure of diabetic hearts, however, has not been assessed.

Carnitine palmitoyltransferase I (CPT 1) inhibitors can protect the heart from the adverse effects of ischemia.19-22 Contrary to other groups, however, we demonstrated that the protective effect of CPT 1 inhibitors can occur independent of a lowering of long chain acylcarnitine or long chain acyl-CoA levels.20,21 Instead, we observed that the CPT 1 inhibitor Etomoxir (ethyl 2-[6-(4-chlorophenoxy)hexyl]oxirane-2-carboxylate; Byk Gulden Pharmaceutika, Konstanz, FRG) stimulates glucose oxidation in fatty acid-perfused hearts. This occurs at concentrations of Etomoxir that protect the ischemic
Heart perfusions

Diabetes was produced as previously described in ether-anesthetized male Wistar rats (200–250 g) with a single tail vein injection of 60 mg/kg streptozotocin (Sigma Chemical, St. Louis, Missouri) dissolved in 50 mM citrate buffer, pH 4.5 (an approximate volume of 0.2 ml was injected). Control animals were injected with citrate buffer alone. Animals were allowed food and water ad libitum and were used either 48 hours or 42 days after the onset of diabetes. Streptozotocin-treated animals with a serum glucose above 400 mg/dl before perfusion procedures were used in this study.

Heart Perfusions

Hearts from sodium pentobarbital-anesthetized rats were excised and placed in ice cold buffer; the aorta was subsequently cannulated. A 10-minute Langendorff perfusion was initiated with Krebs-Henseliet buffer (pH 7.4) gassed with 95% O2-5% CO2, containing 2.5 mM free calcium and 11 mM glucose. During this time, the left atrium and pulmonary artery were cannulated. Hearts were then switched to the working mode and perfused with buffer containing 11 mM glucose or 11 mM glucose and 1.2 mM palmitate. All working heart perfusate contained 3% bovine serum albumin (Fraction V, Sigma Chemical) that contained approximately 0.05 mM fatty acids. Palmitate, when used, was bound to the albumin. All perfusate containing albumin was dialyzed against 10 volumes of Krebs-Henseliet buffer for 16 hours and filtered before use. Hearts were perfused at a left atrial filling pressure of 15 cm H2O and a hydrostatic aortic afterload of 80 mm Hg. Hearts were paced at a rate of 280 beats/min (acute diabetes study) or 250 beats/min (chronic diabetes study).

Myocardial low-flow ischemia was produced with a one-way ball valve technique developed by Neery’s laboratory. After 15-minute aerobic perfusion, aortic outflow was diverted through a one-way ball valve resulting in a 50% reduction in diastolic reperfusion of the coronary arteries. Mechanical and metabolic demand were maintained by pacing hearts at either 280 or 250 beats/min. If hearts failed during the ischemic period, coronary flow decreased to 20% of original values. This is because coronary flow is a function of aortic pressure and changes in direct relation to left ventricular pressure development. If coronary flow is corrected for pressure differences, no change in coronary flow occurs throughout the perfusion period.

Etomoxir, when used, was added directly to the perfusate before the onset of ischemia. A stock solution of Etomoxir was freshly prepared for each experiment by dissolving the Na+ salt of Etomoxir in distilled H2O. After 15 minutes of low-flow ischemia, hearts were frozen with Wollenberger clamps cooled to the temperature of liquid N2 and weighed. A series of hearts were also frozen before the onset of ischemia for metabolite determinations. Atrial tissue remaining on the cannula was removed, dried, and eventually used in the calculation of whole heart weight.

Frozen ventricular tissue was weighed and powdered with a mortar and pestle cooled to the temperature of liquid N2. A portion of the powder was used to determine the dry-to-wet ratio. Total dry weight was calculated using the wet ventricular weight and the dry atrium weight. Long chain acyl-CoA, long chain acylcarnitine, ATP, and creatine phosphate were extracted from the frozen tissue with perchloric acid. Extracted long chain acyl-CoA and acylcarnitine were subsequently hydrolyzed to free CoA and carnitine. Free CoA was measured fluorometrically, and free carnitine was measured radiometrically, as described previously. Measurement of ATP and creatine phosphate levels from perchloric extracts was determined spectrophotometrically with a standard coupled enzyme assay involving glucose 6-phosphate dehydrogenase, hexokinase, and creatine phosphokinase.

Data were analyzed by a two-way analysis of variance, followed by comparison of means with the Newman-Keuls test. A value of \( p < 0.05 \) was set as a level of significance.

Results

Effect of One-way Ball Valve Ischemia in Diabetic Rat Hearts

To produce ischemia in isolated working rat hearts, aortic outflow was redirected through a one-way ball valve. To prevent a decrease in heart rate during reduced coronary flow, hearts were paced at 280 beats/min. This heart rate was chosen because it resulted in a gradual decrease in peak systolic pressure (PSP) development over a 15-minute period in glucose-perfused control hearts. Figure 1 shows the decrease in PSP and cardiac output (CO) in control and acutely diabetic rat hearts subjected to a 50% reduction in coronary flow. In control hearts, PSP and CO decreased 40% within 4 minutes after diversion of aortic outflow through the one-way ball valve. If palmitate was present in the perfusate of control hearts, PSP and
CO decreased 65% within 4 minutes of diverting flow through the ball valve. In diabetic rat hearts, the decrease in PSP and CO was significantly accelerated, in both the absence and presence of palmitate (a 65% and 80% decrease, in the absence and presence of palmitate, respectively). These experiments demonstrate that both fatty acids and diabetes accelerate heart failure if coronary flow is reduced 50% and metabolic demand is maintained.

The effects of ischemia were also studied in chronically diabetic rat hearts to determine if the duration of diabetes altered the response of the heart to fatty acids and ischemia. In the presence of palmitate, however, chronically diabetic rat hearts could not be paced at 280 beats/min. These hearts would fail even in the absence of ischemia. Therefore, all hearts were paced at 250 beats/min, a rate at which function was maintained. As would be expected, hearts from control animals, when made ischemic at this paced rate, failed more slowly than those paced at 280 beats/minute (Figures 1 and 2). PSP and CO decreased less than 50% throughout the entire 15-minute period of ischemia in glucose-perfused control hearts paced at 250 beats/min. At this lower heart rate, addition of palmitate to the perfusate did not accelerate failure in control hearts. This result suggests that the detrimental effect of palmitate on mechanical function during ischemia is dependent on the work performed by the heart.

**Figure 1.** Graphs of heart function in glucose- and palmitate-perfused ischemic working hearts from acutely diabetic rats. Peak systolic pressure development and cardiac output were measured in hearts paced at 280 beats/min and perfused at a 15-cm H2O preload, 80-mm Hg afterload. Ischemia was produced by diverting aortic outflow through a one-way ball valve as described in “Materials and Methods.” Hearts from control or acutely diabetic rats were perfused with either 11 mM glucose or 1.2 mM palmitate and 11 mM glucose. Values are the mean±SEM of at least six hearts in each group. *Significantly different from glucose-perfused control hearts. †Significantly different from palmitate-perfused control hearts.

**Figure 2.** Graphs of heart function in glucose- and palmitate-perfused ischemic working hearts from chronically diabetic rats. Peak systolic pressure and cardiac output were measured in hearts paced at 250 beats/min and perfused at 15-cm H2O preload, 80-mm Hg afterload. Ischemia was produced by diverting aortic outflow through a one-way ball valve as described in “Materials and Methods.” Hearts from control or 6-week streptozotocin diabetic rats were perfused with either 11 mM glucose or 1.2 mM palmitate and 11 mM glucose. Values are the mean±SEM of at least six hearts in each group. *Significantly different from glucose-perfused control hearts. †Significantly different from palmitate-perfused control hearts.
When ischemia was produced in chronically diabetic rat hearts paced at the lower rate of 250 beats/min, a rapid failure occurred (PSP and CO decreased by 90% within 4 minutes of initiating ischemia). This occurred whether or not palmitate was present in the perfusion medium. These data demonstrate that the sensitivity of diabetic rat hearts to ischemic failure markedly increases as the duration of diabetes increases.

**Effects of the Carnitine Palmitoyltransferase I Inhibitor Etomoxir on Ischemic Hearts**

To get a better understanding of the involvement of fatty acids in ischemic injury to the diabetic rat heart, hearts were perfused with the carnitine palmitoyltransferase I inhibitor Etomoxir (10^{-9} M). This concentration of Etomoxir was chosen because it was found to be effective in protecting nondiabetic rat hearts from ischemic injury (S. Wall and G.D. Lopaschuk, submitted manuscript). The effects of Etomoxir on PSP and CO in acutely diabetic rat hearts paced at 280 beats/min are shown in Figure 3.

In glucose-perfused hearts from diabetic rats, Etomoxir prevented any decrease in PSP and CO from occurring throughout the 15-minute period of ischemia. In palmitate-perfused hearts, Etomoxir also decreased the rate of heart failure with PSP and CO decreasing by less than 40% after 15 minutes of ischemia. In chronically diabetic rat hearts, Etomoxir also markedly decreased the rate of mechanical failure (Figure 4). In hearts paced at 250 beats/min, Etomoxir prevented any significant decrease in PSP and CO from occurring throughout the 15-minute period of ischemia. In control hearts paced at 250 beats/min, Etomoxir also completely prevented any decrease in PSP and CO from occurring (data not shown).

**Long Chain Acyl-CoA and Long Chain Acylcarnitine Levels in Ischemic Hearts**

To determine if accumulation of fatty acid intermediates are involved in ischemic failure in these hearts, long chain acyl-CoA and long chain acylcarnitine levels were measured in control and acutely diabetic rat hearts frozen before and after the 15-minute period of ischemia (Table 1). Long chain acyl-CoA levels in the absence of ischemia were significantly elevated in glucose-perfused acutely diabetic rat hearts compared with glucose-perfused control hearts. This finding confirms previously reported results.\(^2,9\) Similarly, addition of palmitate to the perfusate of control hearts also significantly increased long chain acyl-CoA.\(^9\) In addition to these results, we found that palmitate perfusion of acutely diabetic rat hearts results in a further increase in long chain acyl-CoA levels.

Long chain acyl-CoA levels were also measured at the end of 15 minutes of ischemia. The only significant difference in long chain acyl-CoA occurred in the palmitate-perfused acutely diabetic rat hearts, in which a decrease was seen. The absence of change in glucose-perfused hearts parallels earlier studies that used this mild ischemia perfusion protocol.\(^2\)

Long chain acylcarnitine levels measured in non-ischemic hearts followed a similar pattern to the long chain acyl-CoA values (Table 1). Again the highest levels in nonischemic hearts were seen in palmitate-perfused hearts from acutely diabetic rats. No significant difference, however, was seen between the glucose-perfused hearts from control or diabetic rats. At the end of 15 minutes of ischemia, a small increase in long chain acylcarnitine
levels was seen in glucose-perfused control and diabetic rat hearts, again similar to earlier studies that used this experimental model of mild ischemia. Long chain acylcarnitine levels actually decreased in palmitate-perfused diabetic rat hearts.

Long chain acyl-CoA and long chain acylcarnitine were also measured in control and chronically diabetic rat hearts (Table 2). In nonischemic control hearts, long chain acyl-CoA levels were similar to control hearts paced at 280 beats/min for both glucose- and palmitate-perfused hearts. In chronically diabetic rat hearts perfused with palmitate, however, long chain acyl-CoA was significantly lower than in acutely diabetic rats. After a 15-minute period of ischemia, long chain acyl-CoA levels again remained the same or decreased.

Differences in long chain acylcarnitine levels were also observed in nonischemic control hearts paced at 250 beats/min compared with hearts perfused at 280 beats/min. Acylcarnitine levels were significantly elevated in glucose-perfused hearts paced at 250 beats/min compared with glucose-perfused hearts paced at 280 beats/min. In addition, acylcarnitine levels were elevated in palmitate-perfused hearts paced at 250 beats/min compared with palmitate-perfused hearts paced at 280 beats/min. These elevated levels of long chain acylcarnitine were present at the end of the 15 minutes of ischemia. Elevated levels of carnitine esters were also found in control hearts that still maintained 60% of their preischemic function.

In chronically diabetic rat hearts perfused with palmitate, a significant decrease in long chain acylcarnitine levels was observed compared with acutely diabetic rat hearts. Opposite to what was seen in hearts from acutely diabetic rat hearts, a significant increase in long chain acylcarnitine after ischemia was seen in chronically diabetic rat hearts.

Long chain esters of carnitine and CoA were also measured in diabetic rat hearts in which Etomoxir was added to the perfusate (Table 3). In acutely diabetic rat hearts perfused with glucose, Etomoxir (10⁻⁹ M) did result in a small, but significant, decrease in long chain acylcarnitine levels after 15-minute ischemia (from 600 to 451 nmol/g dry wt). In palmitate-perfused hearts, however, addition of Etomoxir resulted in a large increase in long chain acylcarnitine (from 752 to 1,540 nmol/g dry wt). This large increase in long chain acylcarnitine levels occurred even though a marked protective effect of Etomoxir was seen on mechanical function. A dissociation between the beneficial effect of Etomoxir on mechanical function and a lowering of long chain acylcarnitine levels was also seen in control hearts (data not shown). In chronically diabetic rat hearts, Etomoxir did not significantly alter long chain acylcarnitine levels, either in the presence or absence of palmitate (Tables 2 and 3). In both acutely and chronically diabetic rat hearts, Etomoxir was without effect on long chain acyl-CoA levels (Tables 1, 2, and 3). However, in acutely diabetic rat hearts perfused with palmitate, a significant decrease was noted.

Combined, these metabolite levels suggest that no association between accumulation of long chain esters of CoA or carnitine and the degree of mechanical failure can be made.

ATP and Creatine Phosphate Levels in Ischemic Hearts

As shown in Table 4, ATP and creatine phosphate levels were significantly depressed in control
Diabetes in each group. (preischemia) or after 15 minutes of work and 15 minutes of ischemia (postischemia). Values are the mean±SEM of at least five hearts in each group.

**TABLE 1. Long Chain Acyl-CoA and Long Chain Acylcarnitine Levels in Acutely Diabetic Rat Hearts Before and After 15 Minutes of Low-Flow Ischemia**

<table>
<thead>
<tr>
<th>Perfusion condition</th>
<th>Long chain acyl-CoA (nmol/g dry wt)</th>
<th>Long chain acylcarnitine (nmol/g dry wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Preischemic</td>
<td>Postischemic</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>60±13</td>
<td>85±13</td>
</tr>
<tr>
<td>Palmitate plus glucose</td>
<td>92±13*†</td>
<td>86±9</td>
</tr>
<tr>
<td>Diabetic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>102±9*</td>
<td>89±10</td>
</tr>
<tr>
<td>Palmitate plus glucose</td>
<td>195±44*†</td>
<td>93±13</td>
</tr>
</tbody>
</table>

Hearts from control or acutely diabetic rats were perfused with either 11 mM glucose or 1.2 mM palmitate and 11 mM glucose. Hearts perfused at a 15-cm H₂O preload and 80-mm Hg afterload and paced at 280 beats/min were frozen after 15 minutes of work (preischemia) or after 15 minutes of work and 15 minutes of ischemia (postischemia). Values are the mean±SEM of at least five hearts in each group. *Significantly different from preischemic glucose control hearts. †Significantly different from same group in absence of palmitate.

This study demonstrates the importance of fatty acids in mediating myocardial ischemic injury in the diabetic rat. Even in the absence of diabetes, free fatty acids accelerated mechanical failure of ischemic hearts. This may have important consequences in the diabetic patient because the plasma can contain high levels of free fatty acids and the heart can contain an increased intracellular source of fatty acids in the form of triglycerides. Our study also demonstrates that the susceptibility of diabetic rat hearts to ischemia increases as the duration of diabetes increases. It is not certain whether this increased susceptibility of chronically diabetic rats to ischemia is mediated by fatty acids because hearts failed rapidly, even in the absence of added palmitate. It could be that fatty acids originating from elevated levels of endogenous triglycerides may be contributing to ischemic injury. Some evidence for this is provided by data demonstrating that the CPT 1 inhibitor Etomoxir had a marked beneficial effect in ischemic hearts from diabetic rats even in the absence of palmitate.

**TABLE 2. Long Chain Acyl-CoA and Long Chain Acylcarnitine in Chronically Diabetic Rat Hearts Before and After 15 Minutes of Low-Flow Ischemia**

<table>
<thead>
<tr>
<th>Perfusion condition</th>
<th>Long chain acyl-CoA (nmol/g dry wt)</th>
<th>Long chain acylcarnitine (nmol/g dry wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Preischemic</td>
<td>Postischemic</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>54±1</td>
<td>56±10</td>
</tr>
<tr>
<td>Palmitate plus glucose</td>
<td>131±13*†</td>
<td>105±6*†</td>
</tr>
<tr>
<td>Diabetic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>145±8*</td>
<td>75±5*</td>
</tr>
<tr>
<td>Palmitate plus glucose</td>
<td>87±5*†</td>
<td>69±5*</td>
</tr>
</tbody>
</table>

Hearts from control or chronically diabetic rats were perfused with either 11 mM glucose or 1.2 mM palmitate and 11 mM glucose. Hearts perfused at a 15-cm H₂O preload and 80-mm Hg afterload and paced at 250 beats/min were frozen after 15 minutes of work (preischemia) or after 15 minutes of work and 15 minutes of ischemia (postischemia). Values are the mean±SEM of at least five hearts in each group. *Significantly different from preischemic glucose control hearts. †Significantly different from same group in absence of palmitate.
TABLE 3. Effect of Etomoxir on Long Chain Acyl-CoA and Long Chain Acylcarnitine Levels in Acutely and Chronically Diabetic Rat Hearts After 15 Minutes of Low-Flow Ischemia

<table>
<thead>
<tr>
<th>Perfusion condition</th>
<th>Long chain acyl-CoA (nmol/g dry wt)</th>
<th>Long chain acylcarnitine (nmol/g dry wt)</th>
<th>ATP (µmol/g dry wt)</th>
<th>Creatine-phosphate (µmol/g dry wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Acute diabetes plus Etomoxir</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>59±10</td>
<td>451±68</td>
<td>16.7±1.1</td>
<td>9.1±1.1</td>
</tr>
<tr>
<td>Palmitate plus glucose</td>
<td>97±12*</td>
<td>1660±391*</td>
<td>17.8±1.5</td>
<td>8.7±0.9</td>
</tr>
<tr>
<td><strong>Chronic Diabetes plus Etomoxir</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>77±10</td>
<td>764±246*</td>
<td>21.2±1.6</td>
<td>8.1±1.6</td>
</tr>
<tr>
<td>Palmitate plus glucose</td>
<td>48±3</td>
<td>1075±285*</td>
<td>18.8±1.9</td>
<td>8.1±1.4</td>
</tr>
</tbody>
</table>

Hearts are those shown in Figures 3 and 4. Etomoxir (10⁻⁵ M) was added 6 minutes before the onset of ischemia. *Significantly different from same group in the absence of palmitate.

activity of the heart. In addition, long chain acyl-CoA has been shown to exert specific effects on both mitochondrial and sarcolemmal membrane proteins. Whether accumulation of long chain acylcarnitine or long chain acyl-CoA mediates ischemic injury is controversial. Liedtke et al demonstrated that increased circulating fatty acids in the pig impair heart function and suggested that this was due to increased levels of fatty acid intermediates. Neely's group, however, fails to see a correlation between accumulation of these intermediates and the degree of ischemic injury. The involvement of long chain acyl-CoA and long chain acylcarnitine to ischemic injury may depend on the type of hypoxic or ischemic insult. The greatest accumulation of these intermediates occurs in hearts subjected to hypoxia or restrictions of coronary flow to less than 30% of normal values in hearts subjected to high workloads. If hearts are subjected to both hypoxia and a reduction of coronary flow to less than 10% of normal, this accumulation is most dramatic, with acylcarnitines accounting for up to 70% of the total cellular carnitine. Regardless of these levels, however, a dramatic recovery of heart function occurs upon reperfusion. During severe ischemia, such as in the complete absence of coronary flow, however, the accumulation of these intermediates is not dramatic. Accumulation of long chain acyl-CoA and long chain acylcarnitine in hearts subjected to reductions in coronary flow (such as our experimental model) depends on the degree by which coronary flow is reduced. Neely's group found that severe restrictions of flow to 10–30% of normal result in large increases in the levels of these intermediates. A more moderate reduction of coronary flow to 50% of control values, however, results in only modest increases. Our data confirm these observations and suggest that in the presence of moderate reductions of coronary flow and maintained metabolic demand, accumu-

TABLE 4. ATP and Creatine Phosphate Levels in Diabetic Rat Hearts Before and After 15 Minutes of Low-Flow Ischemia

<table>
<thead>
<tr>
<th>Perfusion condition</th>
<th>ATP (µmol/g dry wt)</th>
<th>Creatine phosphate (µmol/g dry wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Preischemic</td>
<td>Postischemic</td>
</tr>
<tr>
<td><strong>Acute Diabetes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>18.6±3.0</td>
<td>13.6±0.8*</td>
</tr>
<tr>
<td>Palmitate plus glucose</td>
<td>16.3±1.5</td>
<td>12.2±1.2*</td>
</tr>
<tr>
<td>Diabetic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>18.6±1.2</td>
<td>12.5±1.7*</td>
</tr>
<tr>
<td>Palmitate plus glucose</td>
<td>17.4±0.8</td>
<td>10.7±0.9*</td>
</tr>
<tr>
<td><strong>Chronic Diabetes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>18.3±2.1</td>
<td>16.9±1.1</td>
</tr>
<tr>
<td>Palmitate plus glucose</td>
<td>18.5±1.9</td>
<td>19.5±4.4</td>
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<tr>
<td>Diabetic</td>
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<td></td>
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<tr>
<td>Glucose</td>
<td>16.2±1.5</td>
<td>8.5±1.0*</td>
</tr>
<tr>
<td>Palmitate plus glucose</td>
<td>17.7±1.7</td>
<td>8.2±0.5*</td>
</tr>
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</table>

Hearts from control or acutely diabetic rats were perfused with either 11 mM glucose or 1.2 mM palmitate and 11 mM glucose. Hearts were perfused at a 15-cm H₂O preload and 80-mm Hg afterload and paced at 280 beats/min (acute diabetes) or 250 beats/min (chronic diabetes). Hearts were frozen after 15 minutes of work (preischemia) or after 15 minutes of work and 15 minutes of ischemia (postischemia). Values are the mean±SEM of at least five hearts in each group.

*Significantly different from preischemic glucose control hearts.
†Significantly different from postischemic glucose control hearts.
‡Significantly different from postischemic palmitate control hearts.
tion of tissue long chain acyl-CoA or long chain acylcarnitine is not the primary reason for differences in rates of mechanical failure. This conclusion is based on the observation that no correlation was found between the accumulation of these intermediates during ischemia and the rate of mechanical failure. In palmitate-perfused control hearts paced at 250 beats/min, long chain acylcarnitine levels were markedly elevated after 15 minutes of ischemia, while mechanical function was only slightly decreased. In addition, Etomoxir had a beneficial effect even though long chain acyl-CoA and long chain acylcarnitine were not decreased. It should be noted, however, that intramitochondrial long chain acyl-CoA was not measured in this study. Therefore, it cannot be concluded that intramitochondrial long chain acyl-CoA does not play a role in ischemic injury.

Previous studies have shown that CPT 1 inhibitors can have a beneficial effect in ischemic heart.\textsuperscript{17,19-22} Using the "in situ" ischemic pig heart, Molaparast-Sales et al\textsuperscript{19} demonstrated that the CPT 1 inhibitor, oxefencine, both improves function and decreases levels of CoA and carnitine esters. This same group, however, found that the CPT 1 inhibitor tritraglycidic acid, which also lowered the levels of these intermediates, does not improve heart function after ischemia.\textsuperscript{18} In reperfused ischemic rat hearts, Paulson et al\textsuperscript{22} demonstrated that the CPT 1 inhibitor POCA (sodium 2[5-(4-chlorophenyl)-pentyl]oxirane-2-carboxylate) both lowers long chain acylcarnitines and improves recovery of mechanical function. Using a similar protocol, we recently found that Etomoxir also improves reperfusion recovery of ischemic hearts.\textsuperscript{20,21} The beneficial effect, however, was not correlated to a decrease in the levels of long chain acylcarnitine. For example, a low dose of Etomoxir (10^{-9} M) markedly decreases long chain acylcarnitine levels during reperfusion but does not improve mechanical function. At a higher dose (10^{-8} or 10^{-6} M), Etomoxir improves function but does not always lower acylcarnitine levels because of a probable inhibition of CPT 2. The mechanism by which we believe Etomoxir exerts its beneficial effect is by a stimulation of glucose use in the ischemic heart.\textsuperscript{20} We have demonstrated that Etomoxir stimulates glucose oxidation in both nonischemic hearts and in hearts reperfused after a period of ischemia.\textsuperscript{20,21} A similar increase in glucose oxidation in hearts from this study may contribute to the preservation of ATP during ischemia.

Glucose oxidation in the diabetic heart is markedly impaired, not only as a result of impaired glucose transport but also from a decrease in pyruvate dehydrogenase complex activity. A major part of this decrease in pyruvate dehydrogenase activity results from the increased use of fatty acids for oxidative metabolism.\textsuperscript{27} A number of possibilities exist as to why some degree of glucose oxidation may be important in the heart of a diabetic rat. A controversial explanation is that glycolytically produced ATP in the cytosol is preferentially used for membrane proteins, such as the sarcolemmal ATPases.\textsuperscript{15} Another explanation is that increasing flux through the pyruvate dehydrogenase complex will prevent the accumulation of glycolytic products such as lactate, which may accumulate under ischemic conditions.\textsuperscript{38} A third explanation is a decrease in O_{2} consumed per ATP produced. Complete oxidation of glucose produces 3.17 mol ATP/mol O_{2} compared with 2.80 mol ATP/mol O_{2} consumed when palmitate is oxidized.

In ischemic hearts, glucose oxidation decreases to a lesser extent than palmitate oxidation.\textsuperscript{39} The relative increase in glucose use in ischemic hearts may lessen the detrimental effects of ischemia by decreasing myocardial O_{2} requirements. Addition of fatty acid to the perfusate or the presence of diabetes, however, markedly decrease the hearts' use of glucose.\textsuperscript{37} (S.R. Wall and G.D. Lopaschuk, submitted manuscript). Therefore, it would appear advantageous to decrease the hearts' reliance on fatty acids. Recently, we demonstrated that the adverse effects of fatty acids on reperfusion recovery of ischemic hearts can be overcome by the addition of Etomoxir. A parallel series of experiments in non-ischemic hearts demonstrated that Etomoxir also increases myocardial glucose oxidation rates.\textsuperscript{20,21} Therefore, the protective effect of Etomoxir in our diabetic rat hearts may have been a result of a relative increase in glucose use. Unfortunately, this cannot be tested directly under the experimental conditions used in this study because hearts are not under steady-state conditions. Direct measurements of glucose oxidation during ischemic failure is complicated by the fact that work performed by the heart is a major determinant of oxidative metabolism.\textsuperscript{7} We would predict that in these hearts, 10^{-9} M Etomoxir is stimulating glucose oxidation. In nonischemic hearts and during reperfusion of globally ischemic hearts, 10^{-9} M Etomoxir does not stimulate glucose oxidation. A dose of 10^{-8} M Etomoxir, however, will stimulate glucose oxidation.\textsuperscript{4} It is possible that under the present perfusion conditions, where glucose use is stimulated, a lower dose of Etomoxir is now effective in stimulating glucose oxidation.

Sarcolemmal and sarcoplasmic reticulum membrane alterations that occur during ischemia and reperfusion may contribute to cell injury.\textsuperscript{40-43} Defects in cardiac membrane function are also evident 4 to 6 weeks after the onset of diabetes in rats, even in the absence of ischemia.\textsuperscript{23,24,43} It is conceivable, therefore, that alterations in membrane function in diabetic rats may contribute to the increased susceptibility of hearts to ischemic injury. In this study, we do demonstrate that failure of mechanical function during ischemia is accelerated in chronically diabetic rats compared with acutely diabetic rats. However, we were able to protect the heart during ischemia by adding Etomoxir to the perfus-
ate. This suggests that metabolic abnormalities in the chronically diabetic rat heart are a major determinant in its increased susceptibility to ischemic injury. Defects in membrane protein function may be more important in decreasing the maximal work that the heart can perform in the absence of ischemia.

In summary, we demonstrate that both exposure of hearts to high concentrations of fatty acids and increasing the duration of diabetes increase the rate of mechanical failure of hearts during decreased coronary flow. The CPT 1 inhibitor Etomoxir could significantly decrease the rate of mechanical failure in these hearts. Accumulation of long chain acyl-CoA or long chain acylcarnitine in the myocardium could account for neither the detrimental effects of fatty acids and diabetes in ischemic hearts nor the beneficial effect of Etomoxir. We suggest that impaired glucose use in diabetic rat hearts and in fatty acid perfused hearts contributes to the increased susceptibility to ischemic injury.

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Response of isolated working hearts to fatty acids and carnitine palmitoyltransferase I inhibition during reduction of coronary flow in acutely and chronically diabetic rats.

G D Lopaschuk and M Spafford

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