Beneficial Effect of Carnitine on Mechanical Recovery of Rat Hearts Reperfused After a Transient Period of Global Ischemia Is Accompanied by a Stimulation of Glucose Oxidation

Tom L. Broderick, MSc; H. Arthur Quinney, PhD; Collin C. Barker, BSc; and Gary D. Lopaschuk, PhD

Background. We have previously shown that increasing myocardial carnitine levels in fatty acid-perfused isolated working rat hearts dramatically increases glucose oxidation rates. Since high levels of fatty acids depress reperfusion recovery of ischemic hearts by inhibiting glucose oxidation, we determined what effect carnitine has on glucose oxidation during reperfusion of ischemic hearts.

Methods and Results. Isolated working rat hearts were perfused with 11 mM [5-3H/ul-14C]glucose, 1.2 mM palmitate, and 100 μU/ml insulin and subjected to a 35-minute period of global ischemia followed by aerobic reperfusion. Rates of glycolysis and glucose oxidation were determined by measuring tritiated water and 14CO2 production, respectively. Before ischemia, myocardial carnitine content was first increased by perfusing hearts during a 60-minute baseline aerobic perfusion with 10 mM L-carnitine. This resulted in a significant increase in total myocardial carnitine from 4,804±358 to 9,692±2,090 nmol/g dry wt (mean±SD). Glycolysis rates in carnitine-treated hearts were not significantly altered compared with control hearts during the aerobic perfusion (2,482±1,173 versus 1,640±1,365 nmol glucose · g dry wt−1· min−1, respectively). In contrast, glucose oxidation rates in carnitine-treated hearts were significantly increased before ischemia compared with control hearts (471±209 versus 158±75 nmol glucose · g dry wt−1· min−1, respectively). During reperfusion of previously ischemic hearts, glycolytic rates returned to presischemic values in both carnitine-treated and control hearts. Glucose oxidation rates also recovered to presischemic values in these hearts and remained significantly elevated in carnitine-treated hearts compared with control hearts (283±113 versus 130±27 nmol glucose · g dry wt−1· min−1, respectively).

Conclusions. These results suggest that the beneficial effects of carnitine in the ischemic heart can be explained by the actions of this compound on overcoming fatty acid inhibition of glucose oxidation. (Circulation 1993;87:972–981)

KEY WORDS • carnitine • fatty acids • ischemia • glycolysis • glucose oxidation

The contribution of free fatty acids to myocardial ischemic injury has received considerable attention. High levels of fatty acids are purported to contribute to infarct size and mortality in humans (see References 1 and 2 for reviews) and have been shown to potentiate ischemic injury in a variety of experimental models, including pig, dog, rabbit, and rat hearts.3–7 Blood free fatty acid concentrations can be dramatically elevated in patients suffering a myocardial infarction or undergoing cardiac bypass surgery.8–11 As a result, reperfusion of previously ischemic hearts exposes the myocardium to high levels of fatty acids. Increasing evidence suggests that the detrimental effects of fatty acids on ischemic myocardium are correlated with their effect of inhibiting overall myocardial glucose use.6,7,12,13 In both aerobic and reperfused ischemic myocardium, high concentrations of fatty acids markedly decrease glucose oxidation rates.6,12–15 Furthermore, pharmacological intervention aimed at increasing glucose oxidation rates during reperfusion enhances recovery of mechanical function of previously ischemic hearts.5,12,13 A large number of studies support the use of L-carnitine and its derivatives as agents for protecting the

From the Cardiovascular Disease Research Group, Lipid and Lipoprotein Research Group (G.D.L.), Departments of Exercise Physiology (T.L.B., H.A.Q.), Pediatrics (G.D.L.), and Pharmacology (C.C.B., G.D.L.), the University of Alberta, Edmonton, Canada.

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Address for reprints: Dr. Gary D. Lopaschuk, 423 Heritage Medical Research Building, The University of Alberta, Edmonton, Canada T6G2S2.

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ischemic myocardium. Studies in humans have shown that in patients with angina pectoris and coronary artery disease, intravenous administration of L-carnitine reduces ST segment elevation and improves both atrial pacing tolerance and myocardial lactate extraction.\textsuperscript{16-20} In open-chest anesthetized dogs, an intracoronary infusion of L-carnitine reduces ST segment elevation, partially restores high-energy phosphates, and prevents the incidence of ventricular fibrillation.\textsuperscript{21} In the fatty acid-supplemented porcine heart, L-carnitine improves overall hemodynamic and mechanical function,\textsuperscript{22} whereas L-propionylcarnitine partially restores depletion of tissue carnitines and reduces the uptake of free fatty acids into the myocardium.\textsuperscript{23-25} In ischemic hearts, L-carnitine prevents the loss of tissue carnitines, decreases the grade of ventricular arrhythmias,\textsuperscript{26} and raises the atrial fibrillatory threshold.\textsuperscript{27} In isolated rat hearts, L-carnitine, L-propionylcarnitine, and L-acetylcarnitine have also been shown to improve overall mechanical function in hearts reperfused after a period of transient ischemia.\textsuperscript{28}

The mechanism for the beneficial effects of L-carnitine in ischemic hearts has not been completely delineated, but it is generally believed to be related to the actions of carnitine in modulating the transport of long-chain fatty acids across the mitochondrial matrix to the site of $\beta$-oxidation. The commonly proposed theory to explain the actions of carnitine in the ischemic myocardium is to stimulate carnitine palmitoyltransferase I activity and thereby prevent the accumulation of potentially toxic esters of coenzyme A (CoA) and carnitine.\textsuperscript{28-31} Increased levels of long-chain acyl-CoA have been suggested to inhibit the mitochondrial ATP translocase, thereby limiting ATP supply.\textsuperscript{29,30} However, no consistent correlation between the reported effects of carnitine on the intermediates of fatty acids and myocardial ATP content has been found.\textsuperscript{27,31-34}

In addition to carnitine's well-documented role in the oxidation of fatty acids, it can also buffer the intramitochondrial acetyl-CoA/CoA ratio by stimulating acetyl carnitine synthesis from carnitine acetyltransferase.\textsuperscript{35} In heart mitochondria, carnitine increases CoA levels and reduces acetyl-CoA levels, resulting in a 10- to 20-fold reduction in the acetyl-CoA/CoA ratio, which correlates with an efflux of acetyl carnitine from the mitochondria.\textsuperscript{36,37} In the intact working rat heart, we have demonstrated that this stimulates the pyruvate dehydrogenase complex activity and thereby overcomes fatty acid inhibition of glucose oxidation.\textsuperscript{19} As a result, we believe that this role of carnitine is an alternative explanation for the beneficial effects of carnitine in the reperfused ischemic heart.

In this study, we used the isolated working rat heart to determine the effects of carnitine treatment on myocardial glucose use during reperfusion of ischemic hearts. To perform these experiments, hearts were initially perfused as described for a period of 60 minutes in the presence of 10 mM L-carnitine to increase intracellular carnitine content.\textsuperscript{15} We demonstrate that carnitine increases glucose oxidation rates during reperfusion of ischemic hearts and that this is accompanied by an enhanced mechanical recovery. Our results suggest that the beneficial effects of carnitine in the ischemic heart can be explained by overcoming fatty acid inhibition of glucose oxidation, which probably occurs secondarily to a decrease in the intramitochondrial acetyl-CoA/CoA ratio, thereby relieving inhibition of the pyruvate dehydrogenase complex.

**Methods**

L-Carnitine ($\beta$-hydroxy-\(\gamma\)-trimethylaminobutyric acid, inner salt) was purchased from Sigma Chemical Co., St. Louis, Mo. D-[\(\text{U}^{-14}\text{C}\)]glucose and D-[5-\(\text{H}(\text{N})\)]glucose were purchased from New England Nuclear, Boston. Bovine serum albumin (fraction V) was obtained from Boehringer Mannheim, Germany. Hyamine hydroxide (methylbenzethionium; 1 M in methanol solution) was obtained from New England Nuclear Research Products (Boston). Dowex 1-X4 anion exchange resin (200-400-mesh chloride form) was obtained from Bio-Rad Laboratories (Richmond, Calif.). ACS aqueous counting scintillant was purchased from Amersham Canada Ltd. (Oakville, Ontario). All other chemicals were reagent grade.

**Heart Perfusions**

Hearts from sodium pentobarbital–anesthetized male Sprague-Dawley rats were excised, the aorta was cannulated, and a retrograde perfusion with Krebs-Henseleit buffer (pH 7.4, gassed with 95% O\(2\)/5% CO\(2\), and containing 1.25 mM Ca\(^{2+}\)) was initiated as previously described.\textsuperscript{38} During this initial perfusion, the hearts were trimmed of excess tissue, the pulmonary artery was cut, and the opening to the left atrium was cannulated. Hearts were then switched to the working mode and perfused at an 11.5-mm Hg left atrial filling pressure and 80-mm Hg hydrostatic aortic afterload in a recirculating buffer system (100 ml) containing 11 mM glucose, 1.2 mM palmitate, and 100 $\mu$M insulin. Palmitate was prebound to 3% bovine serum albumin. A concentration of 1.2 mM palmitate was used because this concentration of fatty acid can be seen in the blood after a myocardial infarction\textsuperscript{1,8,9} or after cardiac bypass surgery.\textsuperscript{11} We chose to use 11 mM glucose in the perfusate because glucose uptake is saturated at this concentration and tissue glycogen levels do not decline under aerobic conditions, and because lower glycogen levels in the heart before ischemia (which occur at lower glucose levels and in the absence of fatty acids) may affect the ability of the heart to withstand the ischemic insult. In this study, spontaneously beating hearts were used so as not to impose a high metabolic demand on hearts during the first critical minutes of reperfusion. Heart rate, peak systolic pressure development, and developed pressure were monitored throughout the perfusion with a Spectramed P 23XL pressure transducer (Oxnard, Calif.) in the aortic afterload line. Signals were recorded with a Gould RS-3600 physiograph (Cleveland, Ohio).

1. Carnitine, when used, was added to the buffer at a concentration of 10 mM at the beginning of a 60-minute aerobic baseline perfusion. This condition was chosen because carnitine uptake across the sarcolemmal membrane occurs by both a slow Na\(^+\)-dependent diffusion and a carrier-mediated component.\textsuperscript{35,40} We have previously shown that this 60-minute aerobic perfusion with 10 mM carnitine is sufficient to significantly increase total myocardial carnitine content.\textsuperscript{15}
**Table 1. Effects of Carnitine Treatment on Total Myocardial Carnitine Content and Esters in Nonischemic and Reperfused Ischemic Hearts**

<table>
<thead>
<tr>
<th>Perfusion condition</th>
<th>Carnitine esters (nmol/g dry wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Free</td>
</tr>
<tr>
<td>After initial aerobic perfusion</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>3,242±418</td>
</tr>
<tr>
<td>Carnitine-treated</td>
<td>4,497±1,038*</td>
</tr>
<tr>
<td>After reperfusion after ischemia</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>3,707±456</td>
</tr>
<tr>
<td>Carnitine-treated</td>
<td>4,561±523*</td>
</tr>
</tbody>
</table>

Values are mean±SD for seven hearts in each group. Carnitine was added to the perfusate at a concentration of 10 mM for a 60-minute period.

*Significant compared with control hearts under the same perfusion conditions.

**Ischemic Heart Perfusions**

Global no-flow ischemia was produced in control and carnitine-treated hearts for a 35-minute period by clamping off both the left atrial and aortic flow. Throughout the ischemic period, hearts were maintained at 37°C. Left atrial and aortic flow was then restored, and recovery of mechanical function was monitored for a further 40-minute period.

At the end of the aerobic reperfusion period, a 5-minute Langendorff drip-out was initiated to remove any carnitine present in the extracellular space. Thereafter, hearts were rapidly frozen with Wollenberger clamps precooled to the temperature of liquid nitrogen.

A series of control and carnitine-treated hearts were also perfused for a 60-minute aerobic period. After a 5-minute drip-out period, these hearts were frozen for biochemical analysis.

**Measurement of Glycolysis and Glucose Oxidation**

Glycolysis and glucose oxidation were measured simultaneously by perfusing hearts with Krebs-Henseleit buffer containing 11 mM [5-3H/ul]C]glucose (specific activity of perfusate equaled 600,000 dpm/ml of 3H and 600,000 dpm/ml 14C), 1.2 mM palmitate, and 100 μU/ml insulin, as described previously.15

Steady-state glycolytic rates were determined by measuring triitated water production (released at the enolase step of glycolysis). Glycolytic rates were expressed as micromoles of glucose metabolized per minute per gram dry weight. Steady-state oxidative rates of glucose oxidation were determined by measurement of 14CO2 production in a closed system that allowed quantitative collection of both gaseous and perfusate 14CO2 (14CO2 is released at the level of the pyruvate dehydrogenase complex and in the tricarboxylic acid cycle). Glucose oxidation rates were expressed as nanomoles glucose oxidized per minute per gram dry weight. Both 14CO2 and tritiated water production rates were collected at 10-minute intervals before ischemia and at 10-minute intervals during the 40-minute reperfusion period after ischemia.

At the end of the perfusions, hearts were rapidly frozen with Wollenberger clamps cooled to the temperature of liquid nitrogen. The frozen ventricular tissue was then weighed and powdered in a mortar and pestle cooled to the temperature of liquid nitrogen. A portion of the powdered tissue was used to determine the dry-to-wet weight ratio of the ventricles. The atrial tissue remaining on the cannula was removed, dried in an oven for 12 hours at 100°C, and weighed. With the dried atrial tissue, total frozen ventricular weight, and the ventricular dry-to-wet weight ratio, the total dry weight of the heart was determined.

**Measurement of Tissue Metabolites**

ATP, creatine phosphate, lactate, long-chain acyl-CoA, and carnitine esters were extracted from frozen heart tissue by a perchloric acid extraction method described previously.38 ATP levels in neutralized extracts were determined spectrophotometrically by a coupled enzyme assay involving glucose-6-phosphate dehydrogenase and hexokinase, whereas creatine phosphate levels were determined by creatine kinase.41 Excerpted lactate levels were determined spectrophotometrically by an enzymatic assay involving glutamate pyruvate transaminase and creatine kinase.42 Long-chain acyl-CoA was measured fluorometrically by an enzymatic reaction involving α-ketoglutarate dehydrogenase.43 Long-chain acylcarnitine was hydrolyzed and free carnitine measured radioenzymatically by use of carnitine acetyltransferase.44

**Statistical Analysis**

The unpaired t test was used for the determination of statistical difference of group means. A value of p<0.05 was considered significant. All data are presented as mean±SD.

**Results**

**Effects of Carnitine Treatment on Myocardial Total Carnitine Content and Esters**

The effects of a 60-minute aerobic baseline perfusion on total intracellular myocardial carnitine content in the presence of 10 mM L-carnitine is shown in Table 1. Carnitine content was 4,804±358 nmol/g dry wt in control hearts, whereas in hearts perfused with carnitine, total content was 9,692±2,090 nmol/g dry wt. In these hearts, an increase in all carnitine esters was observed, including the short-chain fraction that contains acetyl carnitine. This increase in total intracellular carnitine is consistent with our previous work.15

The concentration of carnitine esters was also measured in hearts after the 40-minute period of reperfusion after ischemia (Table 1). Total myocardial carnitine content in control hearts was not altered after ischemia and reperfusion. In carnitine-treated hearts, however, carnitine content was lower compared with values seen immediately before ischemia. Carnitine...
content in these hearts, however, remained significantly elevated compared with control hearts.

Effects of Carnitine Treatment on Reperfusion Recovery of Ischemic Hearts

The effects of carnitine treatment on mechanical function in hearts subjected to a 35-minute period of ischemia is shown in Figure 1 and Table 2. Control hearts subjected to 35 minutes of no-flow ischemia recovered only 44% of preischemic heart function. This was reflected by decreases in the recovery of both heart rate and peak systolic pressure. In carnitine-treated hearts, a significant and dramatic improvement in post-ischemic mechanical recovery of both heart rate and peak systolic pressure was seen. In these hearts, a 71% recovery of preischemic heart function was seen. In this study, heart rate was not controlled during the recovery period, because pacing can impose a high metabolic demand in the immediate reperfusion period and can also lead to tachycardia or fibrillation. During early reperfusion, heart rate recovered more quickly in carnitine-treated heart (at 5 minutes of reperfusion, heart rates were 110±80 and 185±75 beats per minute in control and carnitine-treated hearts, respectively). Heart rate in the carnitine-treated hearts remained significantly elevated throughout the reperfusion period (Table 2).

### Table 2. Effects of Carnitine Treatment on Mechanical Function in Nonischemic and Reperfused Ischemic Hearts

<table>
<thead>
<tr>
<th>Perfusion condition</th>
<th>HR (bpm)</th>
<th>PSP (mm Hg)</th>
<th>HR×PSP (×10⁻³)</th>
<th>ΔP (mm Hg)</th>
<th>HR×ΔP (×10⁻³)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aerobic perfusion</strong></td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Control</td>
<td>248±41</td>
<td>108.4±14.0</td>
<td>27.1±6.7</td>
<td>43.9±23.5</td>
<td>11.5±6.9</td>
</tr>
<tr>
<td>Carnitine-treated</td>
<td>258±28</td>
<td>103.4±13.6</td>
<td>27.2±3.9</td>
<td>40.6±17.0</td>
<td>10.4±4.4</td>
</tr>
<tr>
<td>At 10 minutes of reperfusion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>75±66</td>
<td>53.3±41.1</td>
<td>6.1±6.7</td>
<td>26.5±23.3</td>
<td>2.8±2.5</td>
</tr>
<tr>
<td>Carnitine-treated</td>
<td>185±66*</td>
<td>80.8±12.4*</td>
<td>15.2±6.5*</td>
<td>34.8±13.5</td>
<td>5.9±1.9*</td>
</tr>
<tr>
<td>At 40 minutes of reperfusion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>158±69</td>
<td>67.8±29.9</td>
<td>12.1±7.4</td>
<td>26.6±13.6</td>
<td>4.3±2.8</td>
</tr>
<tr>
<td>Carnitine-treated</td>
<td>230±43*</td>
<td>82.9±13.6</td>
<td>19.2±4.7*</td>
<td>29.7±13.2</td>
<td>6.6±2.7*</td>
</tr>
</tbody>
</table>

HR, heart rate; PSP, peak systolic pressure; ΔP, developed pressure; bpm, beats per minute. Values are mean±SD for 13 and 18 hearts in control and carnitine-treated groups, respectively. Hearts were perfused as described in "Methods." Carnitine, when present, was added to the perfusate at a concentration of 10 mM for a period of 60 minutes before ischemia. Hearts were reperfused after a 35-minute period of global ischemia.

*Significant compared with control hearts under the same perfusion conditions.

Effects of Carnitine Treatment on Myocardial Glucose Oxidation and Glycolysis in Nonischemic and Ischemic Reperfused Hearts

Measurements of glucose oxidation were obtained during the initial aerobic perfusion and during aerobic reperfusion of hearts after ischemia. Figure 2 shows cumulative glucose oxidation during the 40-minute period after ischemia. Glucose oxidation rates in both control and carnitine-treated hearts were found to be linear between 10 and 40 minutes. Steady-state rates of glucose oxidation in nonischemic hearts and in hearts reperfused after ischemia are shown in Figure 3. In control hearts, glucose oxidation recovered to preischemic rates. Carnitine treatment resulted in a significant increase in glucose oxidation rates in nonischemic hearts. During reperfusion of ischemic hearts, carnitine treatment also stimulated glucose oxidation rates.

Rates of glycolysis were also measured in both nonischemic and reperfused ischemic hearts. Figure 4 shows cumulative glycolysis during the aerobic reperfusion of control and carnitine-treated hearts. Between 10 and 40 minutes, glycolytic rates were linear in both groups. Figure 5 shows the steady-state glycolytic rates in both nonischemic and ischemic reperfused hearts. Glycolytic rates were substantially greater than glucose oxidation rates in hearts perfused with high concentrations of fatty acids. Carnitine treatment did not dramatically alter glycolytic rates in either nonischemic or reperfused ischemic hearts. This suggests that in the intact heart, carnitine is a much more potent regulator.
of the pyruvate dehydrogenase complex activity than of phosphofructokinase.

Since work performed by the heart is a key determinant in overall energy substrate use, we also normalized glycolysis and glucose oxidation rates for differences in work performed by hearts after ischemia (Table 3). In the first 10 minutes of reperfusion, glucose oxidation rates normalized for work were higher than seen in aerobic hearts, suggesting that recovery of glucose oxidation precedes recovery of heart function. In car

![Figure 2](image1)

**Figure 2.** Graph showing effect of carnitine treatment on total $^{14}$CO$_2$ production from glucose in hearts reperfused after a period of ischemia. Hearts perfused with 11 mM [ul-$^{14}$C]glucose, 1.2 mM palmitate, and 100 μU/ml insulin at a left atrial filling pressure of 11.5 mm Hg and hydrostatic afterload pressure of 80 mm Hg for a 60-minute period of aerobic perfusion were subjected to a 35-minute period of global no-flow ischemia. Total $^{14}$CO$_2$ production from [ul-$^{14}$C]glucose was measured between 0 and 40 minutes of reperfusion. Carnitine, when present, was added to the perfusate at a concentration of 10 mM at the initiation of the aerobic baseline perfusion. Values are reported as mean±SD for seven hearts in each group. *Significantly different from control hearts.

![Figure 3](image2)

**Figure 3.** Bar graph showing effect of carnitine treatment on steady-state rates of glucose oxidation in nonischemic and reperfused ischemic hearts. Steady-state rates of glucose oxidation were determined in hearts perfused with 11 mM [ul-$^{14}$C]glucose, 1.2 mM palmitate, and 100 μU/ml insulin at a left atrial filling pressure of 11.5 mm Hg and hydrostatic afterload pressure of 80 mm Hg. Global no-flow ischemia was produced for a 35-minute period as described in “Methods.” $^{14}$CO$_2$ production from [ul-$^{14}$C]glucose was measured between 40 and 60 minutes of perfusion in nonischemic hearts and between 20 and 40 minutes of reperfusion in hearts subjected to 35 minutes of global ischemia. Carnitine, when present, was added to the perfusate at a concentration of 10 mM at the initiation of the aerobic baseline perfusion. Values are reported as mean±SD for seven hearts in each group. *Significantly different from control hearts.

![Figure 4](image3)

**Figure 4.** Graph showing effect of carnitine on total tritiated water ($^3$H$_2$O) production from glucose in hearts reperfused after a period of ischemia. Hearts perfused with 11 mM [5-$^3$H]glucose, 1.2 mM palmitate, and 100 μU/ml insulin at a left atrial filling pressure of 11.5 mm Hg and hydrostatic afterload pressure of 80 mm Hg for a 60-minute period of aerobic perfusion were subjected to a 35-minute period of global no-flow ischemia. Total tritiated water production from [5-$^3$H]glucose was measured between 0 and 40 minutes of reperfusion. Carnitine, when present, was added to the perfusate at a concentration of 10 mM at the initiation of the aerobic baseline perfusion. Values are reported as mean±SD for seven hearts in each group.

![Figure 5](image4)

**Figure 5.** Bar graph showing effect of carnitine on steady-state rates of glycolysis in nonischemic and reperfused ischemic hearts. Steady-state rates of glycolysis were determined in hearts perfused with 11 mM [5-$^3$H]glucose, 1.2 mM palmitate, and 100 μU/ml insulin at a left atrial filling pressure of 11.5 mm Hg and hydrostatic afterload pressure of 80 mm Hg. Global no-flow ischemia was produced for a 35-minute period as described in “Methods.” $^3$H$_2$O production from [5-$^3$H]glucose was measured between 40 and 60 minutes of perfusion in nonischemic hearts and between 20 and 40 minutes of reperfusion in hearts subjected to 35 minutes of global ischemia. Carnitine, when present, was added to the perfusate at a concentration of 10 mM at the initiation of the aerobic baseline perfusion. Values are reported as mean±SD for seven hearts in each group.
Carnitine Stimulation of Glucose Oxidation in Reperfusion

Table 3. Effect of Carnitine Treatment on Steady-State Rates of Glycolysis and Glucose Oxidation Normalized for Work-Load Differences in Hearts Perfused Under Aerobic Conditions and During Reperfusion of Previously Ischemic Hearts

<table>
<thead>
<tr>
<th>Perfusion condition</th>
<th>Glycolysis rates (nmol [1-14C]glucose/min · HR·PSP×10⁻³)</th>
<th>Glucose oxidation rates (nmol [1-14C]glucose/min · HR·PSP×10⁻³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerobic perfusion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>12.94±8.19</td>
<td>1.30±0.57</td>
</tr>
<tr>
<td>Carnitine-treated</td>
<td>15.93±6.02</td>
<td>3.89±1.59*</td>
</tr>
<tr>
<td>Reperfusion after ischemia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>At 10 minutes of reperfusion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>228.67±134.77</td>
<td>6.63±4.63</td>
</tr>
<tr>
<td>Carnitine-treated</td>
<td>255.50±137.09</td>
<td>12.30±3.90*</td>
</tr>
<tr>
<td>At 40 minutes of reperfusion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>24.50±10.53</td>
<td>2.08±1.14</td>
</tr>
<tr>
<td>Carnitine-treated</td>
<td>26.43±21.08</td>
<td>4.93±2.56*</td>
</tr>
</tbody>
</table>

HR, heart rate (beats per minute); PSP, peak systolic pressure. Values are mean±SD for seven hearts in each group. Glycolysis and glucose oxidation rates are presented as rates corrected for work performed by the heart. Carnitine was added to the perfusate at a concentration of 10 mM for a period of 60 minutes.

*Significant compared with control hearts under the same perfusion conditions.

Nitine-treated hearts, a significant increase in glucose oxidation rates was seen during this period compared with control hearts. By 40 minutes of reperfusion, rates of glucose oxidation normalized for work were similar to rates seen in nonischemic hearts. Again, however, a significant increase in glucose oxidation was seen in carnitine-treated hearts.

Effects of Carnitine Treatment on Myocardial Levels of Long-Chain Acyl-CoA, ATP, and Lactate

The beneficial effects of carnitine in the ischemic heart have often been suggested to occur secondary to a lowering of myocardial levels of long-chain acyl-CoA. Table 4 shows the effects of carnitine treatment on levels of long-chain acyl-CoA, ATP, creatine phosphate, and lactate in nonischemic and reperfused ischemic hearts. In carnitine-treated hearts, a significant increase in myocardial long-chain acyl-CoA content was observed in nonischemic hearts. This was accompanied by a slight decrease in the levels of high-energy phosphates. After perfusion with hearts with carnitine, a 5-minute Langendorff drip-out was initiated to remove any carnitine present in the extracellular space. It should be recognized that this perfusion could potentially alter the redistribution of carnitine and CoA esters. Under these conditions, a decrease in the acyl-acyl-CoA levels of these esters would be expected. Whether this would actually underestimate the values for these esters is uncertain.

After ischemia, carnitine treatment resulted in lower myocardial long-chain acyl-CoA levels compared with the control hearts. This was not, however, associated with any increase in myocardial ATP content. In fact, after reperfusion of previously ischemic hearts, high-energy phosphate content in carnitine-treated hearts was similar to that assayed in control hearts (Table 4). Lactate levels were also measured in aerobic and reperfused ischemic hearts. At the end of the aerobic perfusion, lactate levels were low in both groups. During ischemia, these levels increased to values >120 μmol/g dry wt. As expected, during reperfusion, lactate decreased in both groups. Interestingly, in carnitine-treated hearts, lactate levels were significantly lower than those seen in control hearts.

Discussion

Circulating levels of fatty acids can be markedly elevated during and after a myocardial infarction or coronary bypass surgery. Myocardial glucose oxidation and glycolysis are markedly depressed in the presence of high levels of fatty acids, primarily because of an inhibition of pyruvate dehydrogenase.

Table 4. Effects of Carnitine Treatment on Myocardial Levels of Long-Chain Acyl-CoA, ATP, Phosphocreatine, and Lactate in Nonischemic and Reperfused Ischemic Hearts

<table>
<thead>
<tr>
<th>Perfusion condition</th>
<th>Long-chain acyl-CoA (nmol/g dry wt)</th>
<th>ATP (μmol/g dry wt)</th>
<th>Phosphocreatine (μmol/g dry wt)</th>
<th>Lactate (μmol/g dry wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>After initial aerobic perfusion</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>169.2±28.2</td>
<td>19.8±2.2</td>
<td>40.2±4.9</td>
<td>10.4±7.7</td>
</tr>
<tr>
<td>Carnitine-treated</td>
<td>307.1±66.6*</td>
<td>16.7±5.6</td>
<td>33.4±12.6</td>
<td>4.8±2.1*</td>
</tr>
<tr>
<td>After reperfusion after ischemia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>226.5±59.9</td>
<td>13.8±4.0</td>
<td>36.0±10.8</td>
<td>30.2±28.9</td>
</tr>
<tr>
<td>Carnitine-treated</td>
<td>116.8±37.4*</td>
<td>13.8±1.8</td>
<td>31.9±5.3</td>
<td>11.5±6.5</td>
</tr>
</tbody>
</table>

CoA, coenzyme A. Values are mean±SD for seven hearts in each group. Carnitine, when used, was added to the perfusate at a concentration of 10 mM for a 60-minute period.

*Significant compared with control hearts under the same perfusion conditions.
complex activity and phosphofructokinase, respectively.45,46,48 Paralleling our previous work, we show that in the presence of high concentrations of fatty acids, glycolytic rates during reperfusion are substantially higher than glucose oxidation rates.49 This is because fatty acids are much more potent inhibitors of glucose oxidation than glycolysis. During aerobic reperfusion of ischemic hearts, inhibition of glucose oxidation by high levels of fatty acids impairs recovery of mechanical function.5,6,12,13 If glucose oxidation is stimulated by pharmacological intervention, an enhanced degree of functional recovery will occur.5,7,12,13 For instance, etomoxir, a carnitine palmitoyltransferase I inhibitor, can improve mechanical recovery of ischemic hearts by partially overcoming fatty acid inhibition of glucose oxidation.5,7,13 Direct stimulation of glucose oxidation by dichloroacetate also improves mechanical recovery of hearts during reperfusion.12 Recently, we demonstrated that carnitine also stimulates glucose oxidation in hearts perfused in the presence of high concentrations of fatty acids.15 In the present study, we extend these observations by demonstrating that carnitine also stimulates glucose oxidation during reperfusion of previously ischemic hearts. Carnitine treatment also significantly improved functional recovery of ischemic hearts. This suggests that overcoming fatty acid inhibition of glucose oxidation could explain the beneficial effects of carnitine seen in experimental models of ischemia.

The key site at which fatty acids inhibit glucose oxidation is at the level of the pyruvate dehydrogenase complex.45,48 The myocardial pyruvate dehydrogenase complex is composed of catalytic enzymes and two regulatory enzymes (pyruvate dehydrogenase kinase and pyruvate dehydrogenase phosphatase) and protein X, which is considered to have an important role in the interactions of the catalytic components of pyruvate dehydrogenase complex. The catalytic efficiency of pyruvate dehydrogenase complex is regulated by a phosphorylation–dephosphorylation cycle, with a pyruvate dehydrogenase kinase inhibiting the pyruvate dehydrogenase complex activity and a pyruvate dehydrogenase phosphatase stimulating the pyruvate dehydrogenase complex. The pyruvate dehydrogenase kinase is activated by an increase in the intramitochondrial acetyl-CoA/CoA ratio, resulting in the dramatic decrease in glucose oxidation observed in the intact heart.6,12,14,15 We recently demonstrated that carnitine stimulates glucose oxidation, probably by a mechanism secondary to a decrease in the intramitochondrial acetyl-CoA/CoA ratio, resulting in a stimulation of pyruvate dehydrogenase complex activity. Carnitine accomplishes this by binding intramitochondrial acetyl residues in the matrix, where carnitine acyltransferase is present.35 Acetyl carnitine efflux from the matrix is then stimulated, and the acetyl group of acetyl carnitine is released in the cytosol. This frees CoA in the matrix, the net effect of which is to decrease the intramitochondrial acetyl-CoA/CoA ratio.36 By this mechanism, increasing the availability of carnitine increases the use of glucose and suppresses the contribution of fatty acids as substrate.15 In so doing, there is a greater clearance of pyruvate from the glycolytic pathway and into the tricarboxylic acid cycle as acetyl-CoA.

A number of studies have shown that carnitine and its derivatives exert a beneficial effect in ischemic hearts.16–28 The mechanism by which this occurs, however, has not been delineated. It is commonly proposed that carnitine acts by preventing the loss of myocardial carnitine stores caused by esterification or membrane leakage from the ischemic tissue. Cell membrane leakage of carnitine has been suggested to lead to a disruption of the carnitine acyltransferase–translocase system, leading to adverse consequences of local accumulation of long-chain acyl-CoA and tissue free fatty acids. Another mechanism by which carnitine is suggested to be beneficial is by stimulating fatty acid oxidation, which should facilitate the removal of fatty acid intermediates that accumulate during ischemia. Increased levels of long-chain acyl-CoA and long-chain acylcarnitine have been suggested to exert detrimental effects on cell membrane structure and function.29–31 Long-chain acyl-CoA is also thought to be a potent inhibitor of the mitochondrial adenine nucleotide translocase.29,30 However, in the intact aerobic and postischemic heart, we and others have provided evidence to suggest that no relation exists between the accumulation of fatty acid intermediates and ATP content.6,7,15,32–34 Furthermore, the fact that fatty acid oxidation is not impaired in the reperfused ischemic heart does not support the concept that carnitine exerts its beneficial effect by facilitating fatty acid oxidation.6 In fact, we have recently demonstrated that carnitine decreases fatty acid oxidation in hearts perfused with high concentrations of fatty acid secondary to an increase in glucose oxidation.15

Fatty acid inhibition of glucose oxidation during reperfusion of ischemic hearts is detrimental to mechanical recovery of function.5–7,13 The reason why low glucose oxidation rates impaire recovery has yet to be determined. One possible mechanism may be related to the rate of return of oxidation metabolism during reperfusion. Studies by Bunger et al.16 have shown that pyruvate can stimulate pyruvate dehydrogenase complex activity in postischemic hearts, resulting in an enhanced phosphorylation potential and contractile function during reperfusion. These authors suggest that the increased rate of myocardial respiration by pyruvate occurs by a shift in the mitochondrial redox state toward reduction, with a resultant increase in thermodynamic driving force. Zimmer and others13 recently demonstrated in postischemic rat hearts that stimulating pyruvate dehydrogenase complex activity with pyruvate allowed ADP to drive mitochondrial respiration. As a result, an increased movement of pyruvate through pyruvate dehydrogenase complex in early reperfusion could improve the rate of recovery of oxidative metabolism. If this concept is correct, then high concentrations of fatty acids should have an opposite effect by inhibiting pyruvate dehydrogenase complex activity and thereby delaying the rate of return of oxidative metabolism. It was interesting to note that, compared with control hearts, carnitine induced a greater increase in the total 14CO2 production from [u-14C]glucose by the heart during the
first minutes of reperfusion (Figure 2). A significantly better mechanical function was also observed during this period.

When oxidative metabolism ceases during ischemia, glycolysis becomes a major source of ATP production.47 Therefore, experimental approaches aimed at stimulating glycolysis during ischemia should be beneficial in salvaging the ischemic myocardium. However, consideration of coronary flow is critical in determining the importance of maintaining glycolysis during ischemia as well as the outcome of the ischemic injury. During low-flow ischemia, stimulating glycolysis delays the onset of ischemic contracture and improves recovery of function during aerobic reperfusion.52,53 During severe (no-flow) ischemia, on the other hand, ATP derived from glycolysis is a major contributor of H+ ion production, which can contribute to injury.54,55 In the setting of reperfusion of previously ischemic hearts, however, the importance of glycolysis during recovery has received very little attention. Separating the benefits of stimulating glycolysis by carnitine during ischemia and/or reperfusion was not the focus of this study. Our results demonstrate, nonetheless, that carnitine does not exert any dramatic effects on glycolysis in either normoxic or reperfused ischemic hearts.

Limitations of Study

In this study we used a recirculating system to measure steady-state rates of glucose oxidation and glycolysis. As a result, the possibility exists that noxious metabolites released during or immediately after ischemia may be responsible for the depressed function in the control hearts. However, we do not believe this to be the case, since a relatively large recirculating volume was used in these hearts. A second reason is that we have previously shown that if fatty acids are absent during reperfusion, a complete recovery of function occurs in hearts perfused under similar conditions.7 Furthermore, even in the presence of fatty acids, addition of Etomoxir or dichloroacetate during the reperfusion period will overcome the depression of heart function in fatty acid-perfused hearts.6,12 During the actual ischemic period, conditions were identical in these hearts. Therefore, it is unlikely that noxious metabolite release during ischemia was causing the depression of heart function during reperfusion. Like carnitine, both of these agents overcome the fatty acid inhibition of glucose oxidation. If release of noxious metabolites during ischemia was in fact the reason for the depressed functional recovery in hearts, it would have to be occurring only in untreated hearts, because functional recovery of carnitine-treated hearts was almost complete during reperfusion. Although we cannot rule out that carnitine pretreatment may have been preventing the release of noxious metabolites during ischemia, our results are consistent with the theory that the beneficial effects of carnitine are the result of a stimulation of glucose oxidation during reperfusion.

It is possible that carnitine may have exerted its beneficial effect by increasing coronary flow during reperfusion. Unfortunately, the experimental protocol did not allow for the measurement of coronary flow. However, in a previous study,7 we demonstrated that a depressed recovery of coronary flow is unlikely to be a key factor in the depressed recovery of mechanical function and that after global no-flow ischemia, recovery of coronary flow occurs in parallel with recovery of mechanical function.

In carnitine-treated hearts, a significant improvement in heart rate was seen during reperfusion (Table 2). It is possible that this increase in heart rate in the carnitine-treated hearts could evoke the “Bowditch phenomenon,” also known as the rate treppe and the staircase effect. The force–frequency relation of the Bowditch phenomenon is defined as the increase in contractile strength that accompanies frequency of stimulation. Although this can readily be seen in tissues such as ventricular trabeculae, septal preparations, and papillary muscles, it is best seen under conditions of low frequency of stimulation. In papillary muscle, an increase in force development is seen at rates of stimulation between six and 95 beats per minute, whereas at higher rates of contraction, the phenomenon is no longer observed.56 In addition, as rats grow older, they become less sensitive to the staircase effect. Ventricular trabeculae and papillary muscles from neonatal rat hearts 6 and 13 days old show positive staircase responses, with increments in stimulation of 30–90 beats per minute.57 However, if these tissues are obtained from 22-day-old rat hearts, the effect no longer persists with the same increments in stimulation. In fact, in these rats, a slight negative staircase response is seen with higher frequency of stimulation,57 indicating that the adult rat heart demonstrates an atypical force–frequency relation, with a tendency for a decrease in contractile strength to occur with increasing frequency.56 Our earliest measurements on reperfusion recovery were obtained at 5 minutes into reperfusion, a time in which heart rate in both groups was already well above the rates at which the Bowditch phenomenon is normally seen. During this period, heart rates were 110±80 and 185±75 beats per minute for control and carnitine-treated hearts, respectively. As a result, it is unlikely that the Bowditch phenomenon is an important factor in the improved recovery of carnitine-treated hearts seen in this study.

Although the beneficial effects of carnitine on functional recovery are accompanied by a stimulation of glucose oxidation, it cannot be ruled out that it may also be acting by overcoming long-chain acyl-CoA inhibition of ATP translocase.29,30 Although ATP levels after reperfusion were similar in control and carnitine-treated hearts, it is possible that a compartmentalization of ATP may be occurring. Unfortunately, this could not be determined in this study.

Conclusions

In conclusion, we demonstrate that the beneficial effect of carnitine on functional recovery of previously ischemic hearts perfused in the presence of fatty acids is accompanied by a stimulation in glucose oxidation. The effect of carnitine on long-chain acyl-CoA could not be correlated with high-energy phosphate content of the reperfused ischemic myocardium, thereby contradicting the commonly proposed theory of action of carnitine. Instead, we believe that the well-documented beneficial effects of carnitine on ischemic hearts is better correlated with its ability to overcome fatty acid inhibition of glucose oxidation during reperfusion.
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Beneficial effect of carnitine on mechanical recovery of rat hearts reperfused after a transient period of global ischemia is accompanied by a stimulation of glucose oxidation.

T L Broderick, H A Quinney, C C Barker and G D Lopaschuk

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