Fasting In Vivo Delays Myocardial Cell Damage After Brief Periods of Ischemia in the Isolated Working Rat Heart

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To assess the effects of fasting on recovery of function and exogenous glucose metabolism after 15 minutes of total ischemia, we perfused isolated working rat hearts from fed and fasted animals. Hearts were perfused in a recirculating system with bicarbonate buffer containing glucose (10 mM). Mechanical performance, release of marker proteins for ischemic membrane damage (lactate dehydrogenase, myoglobin, citrate synthase), and the concentrations of lactate and glucose in the perfusion medium were measured serially. Tissue metabolites were also measured. Fasting raised the myocardial glycogen content by 25%. Cardiac performance of perfused hearts from fed and fasted animals was the same during the preischemic and the postischemic period. The time of return of function to preischemic values was significantly less in hearts from fasted rats (2.3 versus 7.8 minutes, \( p < 0.025 \)). The release of cytosolic and mitochondrial marker proteins was significantly lower in hearts from fasted rats than in hearts from fed rats. Glucose metabolic rates during control and reperfusion were unchanged for hearts from fasted rats, but decreased for hearts from fed rats during reperfusion. Conversely, lactate utilization was significantly increased in hearts from fed rats during reperfusion. The adenine nucleotide content at the end of ischemia was higher in hearts from fasted animals than in hearts from fed animals. We conclude that increasing glycogen levels prior to ischemia improves recovery of function, lessens membrane damage, and prevents loss of adenine nucleotides. (Circulation Research 1991;68:1045–1050)

Very little is known about the effects of fasting on the resistance of the myocardium against ischemic stress. Among the many hormonal and metabolic changes in the fasting organism, changes in endogenous substrate supply are of particular interest for the ischemic heart.

Fasting is known to increase the myocardial glycogen content in rats through inhibition of the glycolytic pathway by enhanced oxidation of fatty acids. Although data by Neely et al suggest that lactate from glycogen breakdown during ischemia has deleterious effects on the postischemic cardiac performance and that lowering of the myocardial glycogen content protects the rat heart during ischemia, data by Scheuer and Stezoski and by others have suggested that an increased myocardial glycogen content protects the heart during periods of oxygen deprivation. Moreover, we have recently reported that reducing the myocardial glycogen content before ischemia had no protective effect after hypothermic ischemic arrest in the isolated perfused rabbit heart.

In the present study we examined the effects of endogenous substrates, chiefly glycogen, on myocardial function and metabolism after brief periods of total ischemia. We focused on glycogen, because it is the major endogenous substrate that the heart can utilize for energy production during ischemia, since it has been previously demonstrated that even after 90 minutes of global ischemia the total tissue content of triacylglycerols and phospholipids remains unchanged in rat heart.

To assess beneficial or harmful effects of fasting on the ischemic myocardium, we compared perfused hearts from rats that had free access to food and water with rats that were fasted for 16 hours. We found that fasting of animals in vivo improves postischemic function of rat hearts in vitro as assessed by contractile and biochemical parameters, but that this improvement is not due to enhanced glycogenolysis during ischemia.
Materials and Methods

Materials

All chemicals were obtained from Fisher Scientific, Lexington, Mass., or Sigma Chemical Co., St. Louis. Enzymes and cofactors were obtained from Boehringer Mannheim, Indianapolis, Ind., unless indicated otherwise.

Animals

Male Sprague-Dawley rats (300–350 g) were obtained from Sasco Corp., Houston, and were either fed ad libitum or fasted overnight (for 16 hours) with free access to water.

Perfusion Apparatus

We used the isolated working heart perfusion apparatus of Neely et al. as modified by Taегtmeyer et al. and measured the physiological performance of the heart (heart rate, aortic systolic and diastolic pressure, and cardiac output), assessed glucose and lactate metabolic rates, and estimated the release of marker proteins from the myocardium.

Working Heart Preparation

The working heart preparation has been described in detail earlier. Briefly, rats were anesthetized with sodium pentobarbital (10 mg/100 g body wt i.p.). After injection of heparin (200 IU) into the inferior vena cava, the heart was rapidly removed and mounted on the aortic cannula. A brief period of retrograde perfusion (approximately 5 minutes) was necessary to wash out any blood from the heart and to perform the left atrial cannulation. Hearts were then perfused at 37°C with recirculating Krebs-Henseleit bicarbonate buffer (200 ml) containing glucose (10 mM) as a substrate. The perfusion medium was gassed with 95% O2–5% CO2.

All experiments were carried out with an afterload of 100 cm H2O and a preload of 15 cm H2O. Aortic flow and coronary flow were measured every 10 minutes. Cardiac output was calculated as the sum of aortic and coronary flow. Heart rate and systolic and diastolic pressure were continuously measured with a Hewlett-Packard transducer and recording system.

Perfusion Protocol

The perfusion protocol consisted of three parts. Hearts were perfused under normoxic conditions for 30 minutes. This initial perfusion period was followed by 15 minutes of total global ischemia and then by reperfusion for another 30 minutes. Ischemia was induced by clamping both the aortic and the atrial lines, and reperfusion was accomplished by opening the same. At the end of the experiments, hearts were frozen with a pair of aluminum tongs cooled to the temperature of liquid nitrogen. In selected experiments, hearts were freeze-clamped at the end of the ischemic period. The frozen tissue was stored at −70°C until extraction of the tissue metabolites.

Biochemical Methods

Perfusate samples. At specified times during the experiment, small (1 ml) samples of the coronary effluent and the perfusion medium were withdrawn to measure glucose and lactate as well as lactate dehydrogenase, myoglobin, and citrate synthase, which we considered cellular marker proteins of sarcolemmal and mitochondrial ischemic damage.

Glucose and lactate assays. Glucose and lactate were measured with a glucose/lactate analyzer (2300 STAT, YSI Inc., Yellow Springs, Ohio). Rates of glucose utilization and lactate production and utilization were calculated by disappearance or appearance of glucose or lactate in the perfusion medium as described earlier.

Assays of lactate dehydrogenase, myoglobin, and citrate synthase. Lactate dehydrogenase was determined spectrophotometrically using a kit purchased from Sigma Diagnostics, St. Louis. Results are expressed as units per gram dry weight.

Myoglobin was determined by measuring the absorbance of myoglobin at 410 nm at 25°C. Crystallized horse heart myoglobin (Sigma) served as standard. Results are expressed as milliunits per gram dry weight.

Citrate synthase was assayed by the method of Srere using 5,5'-dithiobis-(2-nitro-benzoate). The results are expressed as milliunits per gram dry weight.

All readings were made on a Gilford Spectrophotometer Model 2600 (Gilford Instruments, Oberlin, Ohio). Areas under the curve were calculated as the integral of each enzyme-activity curve using a BASIC program (areas under the curve, without units). Peak activity of the proteins is the maximum of activity during reperfusion.

Tissue extraction and metabolite assays. Tissue was extracted with 6% perchloric acid as described earlier. ATP, ADP, AMP, phosphocreatine, glucose 6-phosphate, pyruvate, alanine, and citrate were measured as described in Bergmeyer. Glycogen was determined by the method of Walaas and Walaas using amyloglucosidase. Triacylglycerols were extracted with chloroform/ethanol (2:1, vol/vol) and determined by the method of Eggstein et al.

A small portion of the pulverized tissue was used to obtain the wet weight/dry weight ratio. Tissue was weighed (wet weight) and dried in an oven (60°C) for at least 36 hours and weighed again (dry weight). Tissue metabolites data are presented as micromoles per gram dry weight, glycogen as micromoles glucose per gram dry weight.

Statistical Analysis

All data are presented as mean±SEM. A two-tailed t test (paired and unpaired) was used to compare the preischemic and postischemic period from hearts of fed and fasted rats. Differences were considered statistically significant when p<0.05.
Results

Glycogen Content In Vivo, Performance In Vitro, and Time to Recovery of Function

Before perfusion (in vivo), the glycogen content of hearts from fed rats was significantly lower than of hearts from fasted rats (89±5 versus 125±5 μmol/g dry wt, p<0.002). The triacylglycerol content in vivo also increased with fasting (from 109±14 versus 151±10 μmol/g dry, p<0.05).

While the time to full recovery of preischemic function (heart rate, cardiac output, and systolic and diastolic pressure) after ischemia was significantly longer in hearts from fed rats than in hearts from fasted rats (Figure 1), steady-state cardiac performance during the preischemic and postischemic period was the same in hearts from fed and fasted rats (range, 222–249 ml/min g dry wt, not significant; data not shown). It should be noted that we did not observe any prolonged myocardial dysfunction ("stunning") after 15 minutes of ischemia.

Glucose and Lactate Metabolic Rates

We also measured rates of glucose utilization and lactate metabolism before and after ischemia. The results are presented in Table 1. In the preischemic period, hearts from both groups used exogenous glucose as substrate and produced lactate. During the postischemic period, glucose utilization by hearts from fed animals was markedly reduced (from −978 to −305 μmol/g dry/hr) with simultaneous increased lactate utilization (from +278 to −976 μmol/g dry/hr). In hearts from fasted animals, no change in glucose utilization was seen. The lactate utilization was less and the glucose utilization significantly greater than in hearts from fed animals (Table 2). These data have been published earlier16 and are included here for clarity.

Markers of the Ischemic Cell Damage

Figure 2 shows release of lactate dehydrogenase (MW 140,000) from the perfused hearts. In both groups a small but consistent amount of enzyme was released during the preischemic period. Hearts from fed animals demonstrated a significant release of lactate dehydrogenase in the early postischemic period, while hearts from fasted animals did not show any difference in the release of enzyme between the preischemic and postischemic period. To compare the total amount of lactate dehydrogenase released we also analyzed areas under the curve during the preischemic and postischemic period in hearts from fed and fasted animals (Table 2). While there were no significant differences during the control period, total release of lactate dehydrogenase was significantly greater during the postischemic period in hearts from fed than from fasted animals. Even more important, there was no significant difference of the lactate dehydrogenase release between the preischemic and postischemic period in hearts of fasted animals.

We also examined the release of myoglobin, a helical protein (MW 17,500) that has been used as a marker for myocardial cell damage.11 Results for peak release and total release (areas under the curve) are summarized in Table 3. There was signif-

![Figure 1. Mean time to return of function after 15 minutes of total ischemia in hearts from fed and fasted rats. Values are mean±SEM of 10 (fed) and five (fasted) rats.](http://circres.ahajournals.org/)

![Figure 2. Lactate dehydrogenase release from the myocardium into the coronary effluent of hearts from fed and fasted rats before and after 15 minutes of total ischemia. Note the significantly higher postischemic release of lactate dehydrogenase by hearts from fed rats. Values are mean±SEM of 10 (fed) and five (fasted) experiments at each time point. *p<0.05.](http://circres.ahajournals.org/)
significantly less myoglobin release in hearts from fasted rats during the postischemic period. The total release of the marker enzyme for the inner mitochondrial membrane, citrate synthase (MW 98,000), was also significantly less in hearts from fasted rats. The data are presented in Table 4.

**Tissue metabolites.** To determine whether the energy state of the myocardium at the end of ischemia differed between hearts from fed and fasted animals, we also measured tissue metabolites at the end of ischemia (Table 5). Compared with hearts from fed animals, hearts from fasted animals contained significantly higher amounts of ADP, total adenine nucleotides, glycogen, glucose 6-phosphate, and citrate, while the contents of ATP, AMP, pyruvate, and alanine were not different.

**Discussion**

The present study demonstrates that fasting and alterations of endogenous substrate supply in rat hearts by glycogen loading preserve the adenine nucleotide content during ischemia, shorten recovery time, normalize postischemic glucose utilization, and lessen ischemic membrane damage after brief periods of ischemia. These findings are somewhat surprising in the context of reports on accelerated glycogen breakdown and the resultant lactate production during ischemia, exerting deleterious effects on the function of both ischemic and postischemic myocardium. The findings are, however, consistent with other earlier observations of a protective effect of glycogen loading in rabbit hearts subjected to hypothermic ischemic arrest for up to 12 hours. In these experiments, glycogen loading was achieved by the simultaneous injection of glucose and insulin. The present results now seem to suggest that the nutritional state of the animal before ischemia (and not the amount of glycogen broken down during ischemia) is an important determinant for the outcome of the postischemic myocardium: hearts from fed and fasted rats used approximately the same amount of glycogen during ischemia and also produced approximately the same amount of lactate (Table 5). Enhanced glycogenolysis during ischemia can therefore not explain the beneficial effects of fasting. These findings are similar to earlier findings by Kilgour et al. and Tani and Neely, who also found no difference in tissue lactate content between glycogen-rich diabetic rat hearts and control hearts after ischemia.

A surprising finding was that the net loss of adenine nucleotides in heart muscle, a well-known phenomenon already observed after very brief periods of ischemia, was retarded in glycogen-rich hearts of fasted rats. The significantly higher ADP content at the end of ischemia in hearts from fasted rats may provide a readily available substrate for repophosphorylation during reperfusion and hence may be an important contributor to the faster return of function. In contrast, hearts from fed rats would be required to replenish some of their adenine nucleotides by de novo synthesis. Since de novo synthesis of adenine nucleotides is a very slow process compared with the de novo synthesis of other intermediary metabolites, it is unlikely that hearts of fed animals replenished their adenine nucleotide stores during 30 minutes of reperfusion. Thus, it is reasonable to assume that intracellular ADP concentration fulfills an important role in the rate of recovery of function on reoxygenation of reversibly ischemic myocardium. Furthermore, the breakdown of adenine nucleotides generates protons and is ultimately linked to the production of oxygen-derived free radicals, which may initiate peroxidation of membranes may lead to changes in membrane fluidity, and opens ion channels like the calcium channel. The acidification of the cell during ischemia and the rise in intracellul-

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### Table 2. Peak Activity and Areas Under the Curve for Lactate Dehydrogenase

<table>
<thead>
<tr>
<th></th>
<th>Preischemic</th>
<th>Postischemic</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Peak</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fed</td>
<td>10</td>
<td>20.1±6.9</td>
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<tr>
<td>Fasted</td>
<td>5</td>
<td>3.4±1.8*</td>
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<tr>
<td><strong>AUC</strong></td>
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<td></td>
</tr>
<tr>
<td>Fed</td>
<td>10</td>
<td>76.5±20.4</td>
</tr>
<tr>
<td>Fasted</td>
<td>5</td>
<td>35.2±14.5</td>
</tr>
</tbody>
</table>

Values are mean±SEM. Peak, unit/g dry wt; AUC, areas under the curve.

*tp<0.02 vs. fed.

† tp<0.05 vs. preischemic fed.

‡ tp<0.05 vs. postischemic fed.

### Table 3. Peak Activity and Areas Under the Curve for Myoglobin

<table>
<thead>
<tr>
<th></th>
<th>Preischemic</th>
<th>Postischemic</th>
</tr>
</thead>
<tbody>
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<td><strong>Peak</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fed</td>
<td>6</td>
<td>5±1</td>
</tr>
<tr>
<td>Fasted</td>
<td>5</td>
<td>0.8±0.4*</td>
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<tr>
<td><strong>AUC</strong></td>
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<td></td>
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<tr>
<td>Fed</td>
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<td>75±17</td>
</tr>
<tr>
<td>Fasted</td>
<td>5</td>
<td>34±15</td>
</tr>
</tbody>
</table>

Values are mean±SEM. Peak, mg/g dry wt; AUC, areas under the curve.

*tp<0.004 vs. postischemic fed.

† tp<0.01 vs. postischemic fed.

### Table 4. Peak Activity and Areas Under the Curve for Citrate Synthase

<table>
<thead>
<tr>
<th></th>
<th>Preischemic</th>
<th>Postischemic</th>
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</thead>
<tbody>
<tr>
<td><strong>Peak</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fed</td>
<td>6</td>
<td>10.5±2.4</td>
</tr>
<tr>
<td>Fasted</td>
<td>5</td>
<td>4.8±1.1*</td>
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<tr>
<td><strong>AUC</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fed</td>
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<td>123±13</td>
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<tr>
<td>Fasted</td>
<td>5</td>
<td>130±82</td>
</tr>
</tbody>
</table>

Values are mean±SEM. Peak, milliunits/g dry wt; AUC, areas under the curve.

*tp<0.05 vs. postischemic fed.
lar calcium can activate phospholipases and proteases,28 which in turn may lead to an increase in membrane permeability as suggested in our experiments by the loss of marker proteins. Thus, according to this hypothesis, brief periods of ischemia are priming the myocardium for the deleterious effects of reperfusion. We wish to propose now that fasting and glycogen loading modify this “priming process” and protect the myocardium against reperfusion injury by yet unknown mechanisms.

A third unexpected finding of our study was the decreased glucose utilization on reperfusion of hearts from fed rats. A possible explanation may be that hearts from fed rats with a lower adenine nucleotide content were not able to phosphorylate enough glucose to glycogen 6-phosphate to keep up with the increased postischemic ATP demand and switched to lactate as the preferred substrate for energy production. The decrease in glucose utilization was not only associated with an increased lactate utilization but correlated also with a significantly greater release of marker proteins and a delay in the time to recovery of function. These observations are in principal agreement with earlier findings by Bricknell and Opie,29 who showed that hearts perfused with pyruvate as substrate lost significantly more lactate dehydrogenase during reperfusion than hearts perfused with glucose. It has been proposed that ATP derived from the Embden-Meyerhof pathway is preferentially used for ion pumps and membrane functions.30,31 The small difference between the amount of ATP derived from glucose (fasted hearts) and preferentially from lactate/pyruvate (fed hearts) may be crucial for defense and clearance mechanisms in the early reperfusion period after ischemia.

A possible reason for the protective effect of fasting could be found in a physiological role for glycogen, as it relates to cell homeostasis; glycogen is stored in the cardiac cell close to the sarcoplasmic reticulum and a glycogen–sarcoplasmic reticulum complex has been described by Entman et al.32 The central role that the sarcoplasmic reticulum plays in Ca2+ metabolism and contractile coupling and the close connection between glycogen and sarcoplasmic reticulum makes it likely that an increased glycogen content protects the sarcoplasmic reticulum directly, although there is no direct proof for this hypothesis. Such a protective effect could also help to explain the unexpected findings reported by Tani et al21 that diabetic, glycogen-rich hearts preserve their ability to maintain intracellular [Ca2+]i better than control hearts during ischemia. However, we cannot exclude that other metabolic and hormonal changes contribute to the protective effect in hearts of fasted animals. Even after short periods of fasting the plasma-insulin levels fall, plasma fatty acid levels increase, and the tissue content not only of glycogen but also of citrate, glucose 6-phosphate, coenzyme A, and triacylglycerols increases.8 Although the total tissue content of triacylglycerols and phospholipids remains largely unchanged during ischemia and reperfusion, it has been demonstrated that the ATP-consuming triacylglycerol-esterification fatty acid cycle is activated during ischemia.8 The decreased adenine nucleotide content in hearts of fed rats might therefore lead to impaired reesterification of nonesterified fatty acids, which have as amphiphiles deleterious effects on the ischemic and reperfused myocardium.33

Although we are aware that most of the above scenario is still speculative, we have provided evidence for the beneficial effects of fasting on the ischemic myocardium. It is most likely that the increased myocardial glycogen content after fasting protects the heart, although the mechanisms are still unknown.

### References

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KEY WORDS • glycogen • reperfusion injury • adenine nucleotides • marker proteins
Fasting in vivo delays myocardial cell damage after brief periods of ischemia in the isolated working rat heart.
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doi: 10.1161/01.RES.68.4.1045

*Circulation Research* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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