Effect of Increasing Degrees of Ischemic Injury on Myocardial Oxidative Metabolism Early After Reperfusion in Isolated Rat Hearts

Günter Görge, Pascal Chatelain, Jutta Schaper, and René Lerch

The present investigation studied the effect of increasing severities of ischemic injury on recovery of oxidative metabolism after reperfusion in isolated rat hearts perfused retrogradely with erythrocyte-containing medium. Hearts subjected to 60 minutes of low-flow ischemia (5% of control perfusion) exhibited delayed but sustained recovery of left ventricular pressure development during reperfusion and preservation of ultrastructure delineated with electron microscopy. Immediately after reperfusion, myocardial oxygen consumption returned to control values, well before left ventricular pressure development recovered. Early after reperfusion release of $^{14}\text{CO}_2$ from [1-$^{14}\text{C}$]palmitate was reduced (−53%, $p<0.01$). Conversely, release of $^{14}\text{CO}_2$ from [U-$^{14}\text{C}$]glucose was increased (+131%, $p<0.05$). After 60 minutes of reperfusion $^{14}\text{CO}_2$ release had completely returned to normal for both labeled substrates. Pulse-labeling experiments indicated that during transient depression of [1-$^{14}\text{C}$]palmitate oxidation more tracer was incorporated into myocardial lipid esters, primarily triglycerides. In contrast to hearts subjected to low-flow ischemia, hearts subjected to 60 minutes of no-flow ischemia exhibited poor recovery of contractile function during the reperfusion period. Electron microscopic examination of reperfused hearts showed advanced myocyte damage consistent with irreversible injury. Interestingly, myocardial oxygen consumption in this group also recovered to control values. The substrate pattern during the early reperfusion period was similar to that of hearts subjected to low-flow ischemia. After 120 minutes of no-flow ischemia, recovery of oxidative metabolism was virtually absent. The results indicate a pronounced dissociation between recovery of oxidative metabolism and of contractile function in reperfused myocardium. The oxidative metabolic rate was disproportionally high compared with contractile function, not only in reversibly “stunned” hearts, but also in severely damaged hearts exhibiting signs of irreversible injury. (Circulation Research 1991;68:1681–1692)

Following coronary thrombolysis during evolving myocardial infarction, resumption of oxidative substrate metabolism in the reperfused region is a prerequisite for recovery of contractile function. In normal myocardium the overall rate of oxidative metabolism is closely related to the contractile performance. Although the myocardium is capable of using a variety of substrates for oxidative metabolism, in normal myocardium fatty acids represent the predominant fuel in most instances.

Recent experiments suggest that in reperfused postischemic myocardium, both the relation between oxygen consumption and mechanical function as well as the pattern of substrates used for oxidative metabolism may be altered.

Myocardial injury induced by ischemia followed by reperfusion has been found to be associated with reduced or increased oxygen consumption. In isolated ferret hearts subjected to 60 minutes of no-flow ischemia oxygen consumption was reduced to 41% after 40 minutes of reperfusion. However, the rate-pressure product was reduced to only 9% of control, indicating that oxygen utilization was higher than one would expect from the reduction of contractile function. In dogs subjected to ten 5-minute coronary occlusions alternating with 10-minute reperfusion periods Stahl et al observed that after the last reperfusion oxygen consumption was increased by

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13% compared with control myocardium, despite a decrease of systolic shortening by 97%. In a similar model with 1 hour of reperfusion after three 10-minute occlusions, a reduction of regional shortening by 63% was associated with an increase of oxygen consumption by 8%.5

Indirect evidence of altered myocardial substrate metabolism after postsischemic reperfusion has emerged from studies in which positron emission tomography and radiolabeled substrates were used. In a dog model with transient coronary occlusion Schwaiger et al6,7 observed that myocardial extraction of [1-13C]palmitate was preserved after reperfusion but that clearance of radioactivity from the myocardium was delayed for up to 3 hours after a 20-minute occlusion6 and for up to 7 days after a 3-hour occlusion.7 The reduced rate of clearance was interpreted as impairment of fatty acid oxidation. This interpretation has been supported by Myeas et al,8 who measured myocardial 14CO2 production in dogs after intracoronary injection of [1-14C]palmitate given 10 minutes after reperfusion following 60 minutes of coronary occlusion. In addition to a reduction of fatty acid oxidation these authors observed an accelerated rate of glucose oxidation. In contrast, Lopaschuk et al9 observed that, 20 minutes after the onset of reperfusion following 25 minutes of low-flow ischemia, oxidation of both [1-14C]palmitate and [U-14C]glucose did not differ from control in isolated working rat hearts. Furthermore, from experiments in pig hearts subjected to 45 minutes of low-flow ischemia (40% of control), Liedtke et al10 recently reported rapid recovery of palmitate oxidation to even supranormal values within 15 minutes of reperfusion accompanied by the return of increased glucose oxidation to baseline.11

The extent of recovery of oxidative substrate metabolism as well as the pattern of substrate use in reperfused myocardium is likely to be related to the degree of ischemic tissue injury. It has been proposed that recovery of oxidative metabolism may be confined to reversibly injured myocardium and may presage later recovery of contractile function.12 However, no investigation has delineated the relation between the severity of ischemic injury and the recovery of oxidative metabolism.

The objectives of the present study were 1) to examine the effect of increasing degrees of ischemic injury on recovery of oxidative metabolism after reperfusion and 2) to determine whether the relative contribution of palmitate and glucose to oxidative metabolism is altered.

Materials and Methods

Isolated Perfused Rat Heart

Adult male ZUR:SIV rats (Department of Veterinary Medicine, University of Zurich) (200–300 g) were fasted for 24 hours and anesthetized by diethyl ether inhalation. One minute after intravenous injection of 1,000 IU heparin the heart was rapidly excised and immersed in ice-cold saline. After complete cessation of contractions the caval and pulmonary veins were ligated and the heart was weighed. The aorta was then mounted onto a stainless steel cannula, and perfusion was initiated. Hearts were perfused retrogradely at constant flow by a roller pump (model IPS4, Ismatec SA, Zurich) in a nonrecirculating system. All perfusates were warmed to 37°C and equilibrated with 95% O2–5% CO2. Erythrocyte-enriched media (see below) were passed through a transfusion filter with a pore size of 10 µm (MF 10, Biotest, Dreieich, FRG). Hearts were paced at 280 beats/min by electrodes attached to the right atrium. A fluid-filled latex balloon connected to a Statham P23ID transducer (Gould Inc., Oxnard, Calif.) was introduced into the left ventricular cavity via the left atrium, and maximum systolic and minimum diastolic pressures were continuously monitored on a strip-chart recorder (type 2400S, Gould Instruments, Cleveland, Ohio). The pulmonary artery was cannulated for anaerobic collection of the coronary effluent.

Perfusates and Radioactive Tracers

During surgical preparation the hearts were perfused at a flow rate of 12 ml/min·g−1 wet wt with Krebs-Henseleit (KH) buffer containing glucose (11 mM) and insulin (70 milliunits/l). The KH buffer consisted of (meq/l) Na+ 143, K+ 5.4, Cl− 128, Ca2+ 2.5, Mg2+ 1.2, SO4 2− 1.2, HCO3− 25, and H2PO4− 1.4. After preparation perfusion was changed to the experimental medium consisting of KH buffer containing glucose (11 mM) and palmitate (0.4 mM) complexed to albumin (0.4 mM) (Cohn Fraction V, Sigma Chemical Co., St. Louis), insulin (70 milliunits/l), and trace amounts of [1-14C]palmitate (40 µCi/l), [U-14C]glucose (40 µCi/l), or [1-14C]acetate (20 µCi/l) (Amersham Corp., Arlington Heights, Ill.). The experimental medium was supplemented with washed human erythrocytes at a hematocrit of 0.30. The rationale for the inclusion of erythrocytes was to permit sufficient oxygenation at a physiological flow rate of 2 ml/min·g−1. Conventional erythrocyte-free perfusion requires high flow rates resulting in small and difficult to measure arteriovenous differences of labeled substrates and metabolites.13

Protocol and Experimental Groups

At the onset of perfusion with the experimental medium, systolic pressure was adjusted to 90 mm Hg by changing the volume of the latex balloon. The volume was then kept constant throughout the experiment. Before induction of ischemia all hearts were equilibrated for 20 minutes with the experimental medium at control flow (2 ml/min·g−1). At the end of the equilibration period samples of the perfusate (withdrawn just above the aortic cannula) and the pulmonary effluent were collected for the determination of baseline values of metabolic parameters. Subsequently, four different protocols were followed.

Control group. The first group of hearts (n=19) was perfused aerobically without intervention for 140 minutes. Samples of the perfusate and the pulmonary effluent were collected again 85, 95, 110, and 140
minutes after onset of perfusion with the experimental medium (corresponding to 5, 15, 30, and 60 minutes of reperfusion in the groups with 60 minutes of ischemia). At the end of the experiment the heart was rapidly frozen with Wollenberger clamps precooled in liquid nitrogen.

**Group with 60 minutes of low-flow ischemia.** The second group of hearts (n=22) was subjected to 60 minutes of low-flow ischemia with a coronary flow reduction to 5% of control (0.1 ml-min⁻¹·g⁻¹) followed by 60 minutes of reperfusion at control flow. During ischemia, temperature was maintained at 37°C by a heated jacket. Perfusate and coronary effluent samples were collected 5, 15, 30, and 60 minutes after the onset of reperfusion. At the end of the experiments the hearts were freeze-clamped.

**Group with 60 minutes of no-flow ischemia.** The protocol of the third group of hearts (n=15) differed from the preceding group in that myocardial blood flow was completely stopped for 60 minutes followed by 60 minutes of reperfusion. To avoid intravascular erythrocyte aggregation during cessation of flow the coronary circulation was filled with KH buffer during the ischemic period.

**Group with 120 minutes of no-flow ischemia.** The fourth group of hearts (n=11) was subjected to 120 minutes of no-flow ischemia. In this group the hearts were frozen after 30 minutes of reperfusion because in preliminary experiments no further improvement of functional and metabolic parameters was observed for an additional 30 minutes.

**Analytical Procedures and Calculations.**

Hemoglobin content and oxygen saturation of the perfusate and coronary effluent were measured with a laboratory oximeter (CO oximeter 282, Instrumentation Laboratory, Lexington, Ky.). PO₂, PCO₂, and pH were determined with a blood gas analyzer (type IL 213, Instrumentation Laboratory). Myocardial oxygen consumption was calculated by multiplying the perfusate–coronary effluent difference of total (hemoglobin-bound and dissolved) oxygen content with myocardial blood flow per gram tissue.

Myocardial oxidation of continuously supplied [1-¹⁴C]palmitate, [U-¹⁴C]glucose, and [¹⁴C]-acetate was determined by measurement of [¹⁴C]CO₂ release into the pulmonary effluent. For this purpose 1 ml anaerobically collected coronary effluent was added to 2 ml of 2N NaOH in a sealed 25-ml Erlenmeyer flask. [¹⁴C]CO₂ was released by injection of 3 ml of 2N H₂SO₄ and trapped on a 3-cm² piece of filter paper (Whatman No. 1, Whatman Ltd., Maidstone, UK) soaked with 250 µl hyamine hydroxine (NCS, Amersham). The filter paper was removed after 12 hours and counted by β spectrometry (type LS 75000, Beckman Instruments, Inc., Fullerton, Calif.). Recovery estimated from six H₂[¹⁴CO₂] standards was 93.0±1.3% (mean±SEM).

Myocardial oxidation (in nmol·min⁻¹·g⁻¹) of palmitate and glucose was calculated by multiplying the fraction of arterial [¹⁴C]substrate radioactivity appearing as [¹⁴C]CO₂ in the coronary effluent (coronary-venous [¹⁴C]CO₂ radioactivity [dpm/ml]/arterial [¹⁴C]substrate radioactivity [dpm/ml]) with the arterial substrate concentration (nmol/ml) and myocardial perfusion (ml·min⁻¹·g⁻¹). The extraction fraction of palmitate was calculated by dividing the arteriovenous difference of palmitate-bound [¹⁴C]activity by the arterial [¹⁴C]palmitate radioactivity. To measure the palmitate-bound [¹⁴C]activity, samples of the perfusate and the coronary effluent were centrifuged and 1 ml erythrocyte-free supernatant was placed into tubes containing 2 ml isopropyl alcohol. After the addition of 1 ml of 6N HCl, the samples were warmed in a water bath (85°C) for 10 minutes and radioactivity was measured by β spectrometry. Acid-treated samples of H₂[¹⁴CO₂] standards consistently released more than 99% of radioactivity (n=10).

The frozen myocardium was homogenized in 0.6N ice-cold perchloric acid. The supernatant was neutralized to pH 7.5 with NaOH and analyzed for ATP and creatine phosphate (CP) by enzymatic assays.16,17

**Pulse-Labeling Experiments With [¹⁴C]Palmitate.**

To determine the metabolic fate of extracted [¹⁴C]palmitate during the early reperfusion period, in additional experiments [¹⁴C]palmitate was not continuously delivered, as in the main protocol, but rather injected as a pulse early after reperfusion, and the distribution of retained [¹⁴C]activity between the aqueous fraction and the lipid-soluble subfractions was measured. For this purpose, hearts (n=7) were subjected to 40 minutes of low-flow ischemia (0.1 ml·min⁻¹·g⁻¹) followed by reperfusion at control flow. Five minutes after the onset of reperfusion 10 µCi of [¹⁴C]palmitate complexed to 1 ml of 0.4 mM albumin containing 0.4 mM palmitate in KH buffer was infused into the aortic root within 15 seconds. The injected radioactivity was determined by counting a small volume. The coronary effluent was collected from the onset of tracer infusion. Two minutes after tracer administration the hearts were rapidly frozen. Five hearts with a pulse injection of [¹⁴C]palmitate after 65 minutes of perfusion at control flow rate served as controls.

Cumulative myocardial release of [¹⁴CO₂] (expressed as a percentage of injected activity) was determined as described above by analyzing a sample of the collected coronary effluent. Myocardial uptake of [¹⁴C]palmitate (as a percentage of injected activity) was estimated by summing the [¹⁴C] activity retained in the myocardium and that released in the form of [¹⁴CO₂]. The myocardium was homogenized, and lipid extraction was performed with the Bligh and Dyer procedure.18 Radioactivity in the aqueous, lipid, and solid phases was determined by β spectrometry. Recovery of radioactivity averaged 98±8%. Aliquots of the lipid phase were concentrated under N₂ and further fractionated by thin-layer chromatography by using plates precoated with Silicagel 60 (Merck, Darmstadt, FRG) developed in chloroform/methanol/acetic acid 95:5:1 (vol/vol/vol).19 Results are expressed as a
percentage of total recovered radioactivity in the myocardial homogenate.

**Electron Microscopy**

Hearts were perfusion-fixed 10 minutes after the onset of reperfusion following either 60 minutes of low-flow ischemia (n=5), 60 minutes of no-flow ischemia (n=4), or 120 minutes of no-flow ischemia (n=3). Control hearts were perfusion-fixed after 140 minutes of perfusion at control flow (n=3). For fixation hearts were perfused with 2.5% glutaraldehyde buffered in 0.1 M cacodylate (pH 7.4) for 5 minutes at room temperature.20 The hearts were then removed and immersed in the same solution for 24 hours. Hearts were rinsed with 0.1 M cacodylate buffer plus 7.5% sucrose. Small blocks were selected from the anterior, lateral, and posterior walls of the left ventricle and subdivided into subepicardial and subendocardial samples. These were postfixed with osmium tetroxide and embedded in Epon. Ultrathin sections were stained with uranyl acetate and lead citrate and examined electron microscopically (CM 10, Philips, Eindhoven, The Netherlands) by one of us (J.S.). Classification of the degree of ischemic injury was carried out using a semiquantitative scoring system as described previously.21 This evaluation system using ultrastructural criteria is based on a study comparing functional, biochemical, and morphological data of ischemic hearts.21 The criteria have proven useful to distinguish between normal, reversibly injured, and irreversibly injured myocardium.21

**Statistical Analysis**

Data are expressed as mean±SEM. A one-way analysis of variance was first performed to test for differences among mean values measured at a given time point after reperfusion in the four groups. If a difference was indicated, an unpaired t test was used to compare the different groups. Critical values for multiple comparisons were determined by the Bonferroni method.22 In the pulse-labeling experiments values of control and reperfused hearts were compared by an unpaired t test. Differences were considered significant when p<0.05.

**Results**

**Effect of Ischemia and Reperfusion on Contractile Function, High Energy Phosphate Content, and Ultrastructure**

*Left ventricular function.* Before induction of ischemia left ventricular function was not different among the experimental groups (Figure 1). In control hearts there was a slight deterioration of cardiac function between 20 and 140 minutes of perfusion with an increase of left ventricular diastolic pressure by 1.5±0.5 mm Hg and a decrease of left ventricular systolic pressure by 8.7±2.3 mm Hg. Left ventricular pressure development (LVPD, difference between left ventricular systolic and diastolic pressure) decreased by 9.8±2.4 mm Hg.

In hearts subjected to 60 minutes of low-flow ischemia, early (5 minutes) after the onset of reperfusion, average LVPD was moderately reduced to 59.4% of the corresponding value of control hearts (p<0.01 compared with control hearts). Sixty minutes after the onset of reperfusion LVPD improved to 81.9% of control (p=NS).

Hearts subjected to 60 minutes of no-flow ischemia exhibited poor recovery of contractile function. Average LVPD was only 7.9% of the value of control hearts at 5 minutes (p<0.01) and 25.6% at 60
minutes after the onset of reperfusion \((p<0.01)\). There was marked elevation of left ventricular diastolic pressure, indicating myocardial contracture. Hearts subjected to 120 minutes of no-flow ischemia exhibited no recovery of contractile function during reperfusion.

Thus, 60 minutes of low-flow ischemia resulted in depression of contractile function early after reperfusion that was mostly reversible. In contrast, after no-flow ischemia for either 60 or 120 minutes contractile function remained severely depressed during the reperfusion period.

**Myocardial ATP and CP content.** Table 1 summarizes myocardial ATP and CP content after 140 minutes of control perfusion and after 60 minutes of reperfusion following 60 minutes of either no-flow or low-flow ischemia. After 60 minutes of reperfusion following 60 minutes of low-flow ischemia myocardial ATP content was moderately reduced to 73\% \((p<0.05)\) of the control value. Myocardial CP content was only slightly reduced to 86\% \((p=NS)\). At the end of the reperfusion period after 60 minutes of no-flow ischemia, ATP and CP values were severely depressed to 24\% \((p<0.01)\) and 13\% \((p<0.01)\) of control values, respectively.

**Myocardial ultrastructure.** Figure 2 shows typical electron micrographs of the myocardium in the four groups of hearts. Ultrastructural findings are summarized in Table 2. Hearts perfused at control flow exhibited normal ultrastructure. Ten minutes after reperfusion following 60 minutes of low-flow ischemia, the ultrastructural appearance of the myocardium was only slightly different from normal myocardium, with slight swelling of mitochondria and moderate loss of dense granules. The sarcomeres were relaxed, nuclear chromatin was evenly distributed, and the sarcoplasmic reticulum was intact. Hearts subjected to 60 minutes of no-flow ischemia and 10 minutes of reperfusion exhibited marked alterations of mitochondrial structure with clearing of the matrix, destruction of the cristae, and large amorphous densities in a majority of these organelles. Nuclear shrinkage was evident, and contraction bands were common. Based on previous observations, the advanced changes in this latter group indicate irreversible damage.

### Table 1. Effect of 60 Minutes of Low-Flow Ischemia or No-Flow Ischemia on Myocardial Content of ATP and Creatine Phosphate

<table>
<thead>
<tr>
<th>Ischemia protocol</th>
<th>ATP ((\mu mol/g\ dry\ wt))</th>
<th>CP ((\mu mol/g\ dry\ wt))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>24.3±2.0</td>
</tr>
<tr>
<td>60-min low-flow</td>
<td>10</td>
<td>17.7±1.4*</td>
</tr>
<tr>
<td>60-min no-flow</td>
<td>8</td>
<td>5.8±0.9†</td>
</tr>
</tbody>
</table>

Values are mean±SEM. Hearts of the control group were freeze-clamped after 140 minutes of perfusion at control flow rate. Hearts of the 60-minute low-flow group and the 60-minute no-flow group were freeze-clamped after 60 minutes of reperfusion at control flow rate. CP, creatine phosphate.

\*\(p<0.01\) vs. control.

\†\(p<0.01\) vs. low-flow ischemia.

Figure 3 depicts myocardial oxygen consumption in the four experimental groups. In hearts subjected to 60 minutes of low-flow ischemia oxygen consumption during reperfusion was virtually identical to that measured in control hearts at each time point, even at 5 minutes after the onset of reperfusion, when contractile function was significantly depressed. Surprisingly, average myocardial oxygen consumption was also identical to control hearts after 5 minutes of reperfusion following 60 minutes of no-flow ischemia, when LVPD was less than 10\% of control. Thereafter, oxygen consumption decreased, but the average value was still 63\% of the corresponding value measured in control hearts at 60 minutes following the onset of reperfusion. In contrast, oxygen consumption was severely depressed following reperfusion after 120 minutes of no-flow ischemia.

Thus, during the early reperfusion period oxygen consumption was exceedingly high compared with contractile function, both in moderately injured hearts that subsequently recovered contractile function and in hearts that were severely injured after 60 minutes of no-flow ischemia as evidenced by poor recovery of contractile function, severe depletion of high energy phosphate stores, and ultrastructural damage.

**Myocardial Palmitate Oxidation of Reperfused Myocardium**

Palmitate extraction and palmitate oxidation, estimated from release of \(^{14}\text{CO}_2\), is depicted in Figure 4. In hearts subjected to 60 minutes of low-flow ischemia, extraction of \([1-^{14}\text{C}]\text{palmitate}\) (Figure 4, top panel) was identical to that of control hearts during the entire reperfusion period. However, release of \(^{14}\text{CO}_2\) (Figure 4, bottom panel) was reduced 5 minutes after the onset of reperfusion to 47\% of control \((p<0.01)\). Subsequently, the release of \(^{14}\text{CO}_2\) increased in this group to 91\% of control \((p=NS)\) after 60 minutes of reperfusion.

In hearts subjected to 60 minutes of no-flow ischemia the behavior of palmitate extraction and oxidation was similar to that of the group with 60 minutes of low-flow ischemia. Palmitate extraction did not differ significantly from control hearts during the entire reperfusion period. However, the release of \(^{14}\text{CO}_2\) was transiently depressed to 69\% of control \((p<0.05)\) at 5 minutes after reperfusion but subsequently recovered almost completely to 94\% of control at 60 minutes, despite poor recovery of contractile function.

In contrast, hearts reperfused after 120 minutes of no-flow ischemia exhibited severe and persistent depression of palmitate extraction and oxidation.

Table 3 summarizes the results of the experiments using pulse labeling with \([1-^{14}\text{C}]\text{palmitate}\) early after reperfusion following 40 minutes of low-flow ischemia. At the moment of tracer injection (5 minutes after the
onset of reperfusion) LVPD averaged 92±8 mm Hg in the control group but was reduced to 40±4 mm Hg in the reperfused group. In these hearts average myocardial uptake of [I-14C]palmitate was slightly reduced to 78% of control (p<0.05). Production of 14CO2 was markedly reduced to 36% of the value measured in the control group (p<0.05). 14C activity in the aqueous phase of the tissue extract, containing low molecular weight products of oxidative metabolism, was significantly reduced, consistent with reduced oxidation of labeled palmitate. In contrast, more 14C label was recovered in tissue lipids, primarily in triglycerides.

TABLE 2. Semiquantitative Evaluation of Ultrastructural Changes in Control Hearts and Hearts Subjected to Different Ischemia Protocols Followed by 10 Minutes of Reperfusion

<table>
<thead>
<tr>
<th>Ischemia protocol</th>
<th>n</th>
<th>Normal granules</th>
<th>Clearing of matrix</th>
<th>Amorphous densities</th>
<th>Cristae broken</th>
<th>Nucleus (clearing, chr clumping)</th>
<th>Myofilaments (contraction bands)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>60-min low-flow</td>
<td>5</td>
<td>(+)</td>
<td>(+)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>60-min no-flow</td>
<td>4</td>
<td>-</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>120-min no-flow</td>
<td>3</td>
<td>-</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Control hearts were perfusion-fixed after 140 minutes of perfusion at control flow. Postischemic hearts were perfusion-fixed 10 minutes after the onset of reperfusion.
Chr, chromatin; -, absent; +, present.
Thus, early after reperfusion, the reperfused myocardium exhibits a shift from palmitate oxidation to incorporation into tissue lipids.

**Myocardial Oxidation of [1-14C]Acetate After Reperfusion**

To evaluate whether inhibition of mitochondrial carnitine-dependent fatty acid transfer was responsible for the transient reduction of release of $^{14}$CO$_2$ from [1-14C]palmitate early after reperfusion, production of $^{14}$CO$_2$ from continuously supplied [1-14C]acetate was measured in hearts subjected to 60 minutes of low-flow ischemia followed by reperfusion. Mitochondrial transfer of acetate is independent of carnitine. Similar to the experiments with [1-14C]palmitate, there was a reduction of myocardial release of $^{14}$CO$_2$ during the early reperfusion period. Average release of $^{14}$CO$_2$ was 63% ($p<0.01$) of the corresponding value measured in control hearts at 5 minutes after the onset of reperfusion and 74% ($p<0.05$) at 15 minutes (Table 4). Comparable to the experiments with [1-14C]palmitate, there was complete recovery of [1-14C] acetate oxidation at 30 minutes.

**Myocardial Glucose Oxidation of Reperfused Myocardium**

Myocardial oxidation of glucose, estimated from release of $^{14}$CO$_2$ during continuous labeling with [U-14C]glucose, is depicted in Figure 5. In hearts subjected to either 60 minutes of low-flow ischemia or 60 minutes of no-flow ischemia, release of $^{14}$CO$_2$ rapidly recovered and tended to be transiently increased during the initial 15–30 minutes after the onset of reperfusion.

**Discussion**

The main purpose of this study was to relate oxidative metabolism in reperfused myocardium to the severity of ischemic injury. The results indicate that 1) overall oxidative metabolism recovers rapidly to preischemic levels in reversibly injured myocardium, well before recovery of contractile function; 2) oxidative metabolism may also recover to preischemic levels in myocardium that is irreversibly injured after reperfusion according to currently applied histological and biochemical criteria; and 3) the normal substrate pattern is restored during reperfusion, with only a transient decrease of fatty acid oxidation and an increase of glucose oxidation early after reperfusion.

**Methodological Considerations**

The retrogradely perfused isolated heart preparation was selected to study defined degrees of ischemic injury, because it allows the control of a number of variables that influence the severity of ischemic damage in vivo. These variables include myocardial perfusion, work load, substrate composition of the perfusate, neurohumoral influences, and other factors.

A shortcoming of isolated heart preparations is that definitive distinction between reversible and irreversible postischemic injury may be difficult because the observation period is limited. In the present study 60 minutes of low-flow ischemia resulted in incomplete recovery of developed pressure to only 59% at 5 minutes after reperfusion. Although some irreversible damage cannot be excluded, the majority of the myocardium was most likely reversibly injured in this group, as evidenced by the preservation of ultrastructure and recovery of both developed pressure to 82% and CP to 86% after 60 minutes of
reperfusion. In contrast, after 60 minutes of normothermic no-flow ischemia, recovery of left ventricular pressure development was poor (26% of control) during the 60-minute reperfusion interval. Depression of contractile function may reflect reversible “stunning” of viable myocardium. However, the extremely low values of high energy phosphate content\(^\text{25}\) and the advanced ultrastructural damage in hearts processed for electron microscopy\(^\text{21}\) suggest that most of the myocytes in this group and the 120-minute ischemia group were irreversibly injured at the end of the experiment.

Irreversible damage of myocytes that are reversibly injured at the end of the ischemic period may occur early after reperfusion.\(^\text{26}\) The experimental protocol of the present study does not permit the distinction between irreversible damage occurring during ischemia and that arising during the early reperfusion period.

Continuous perfusion of hearts with labeled substrate was used for repeated estimation of the rate of substrate oxidation. This approach is valid provided that myocardial pools of the substrate and the metabolic intermediates are equilibrated with the tracer-containing medium. At the moment of induction of ischemia, production of \(^{14}\)CO\(_2\) from \([1-^{14}\text{C}]\)palmitate and \([\text{U}-^{14}\text{C}]\)glucose has reached 87% and 78% of the value measured at 85 minutes in the control hearts. This suggests that labeling of fatty acid pools with high turnover was close to equilibrium at the moment of induction of ischemia. However, a degree of caution may be warranted in interpreting values of

**FIGURE 4.** Myocardial extraction (top panel) and oxidation (bottom panel) of palmitate in the four experimental groups of hearts. The graphs show the value immediately before induction of ischemia (B, baseline) and those measured during the postischemic reperfusion period. The extraction fraction was calculated from the arteriocoronary venous difference of \([1-^{14}\text{C}]\)palmitate radioactivity. Oxidation of palmitate was estimated from myocardial release of \(^{14}\text{CO}_2\). For the ischemia groups the range of 2 SEM of the values of the control group is indicated by the hatched area. Palmitate oxidation was initially depressed in all three ischemia groups. However, oxidation completely recovered after both 60 minutes of low-flow ischemia and 60 minutes of no-flow ischemia. Values are mean±SEM. **\(p<0.01\) vs. control group; ***\(p<0.05\) vs. 60-minute low-flow ischemia group; \(\text{**}p<0.01\) vs. 60-minute no-flow ischemia group.
Table 3. Effect of 40 Minutes of Low-Flow Ischemia Followed by 5 Minutes of Reperfusion on Myocardial Uptake of [1-14C]Palmitate, Release of 14CO2, and Distribution of 14C Activity in Subfractions of Myocardial Homogenate After Pulse Labeling

<table>
<thead>
<tr>
<th></th>
<th>Control (n=7)</th>
<th>Reperfused (n=5)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uptake of [1-14C]palmitate (% of injected activity)</td>
<td>19.3±0.5</td>
<td>15.0±1.6</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Production of 14CO2 (% of injected activity)</td>
<td>2.2±0.2</td>
<td>0.8±0.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Activity in tissue homogenate (% of total recovered activity)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aqueous phase</td>
<td>71.0±2.4</td>
<td>51.4±3.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lipid phase</td>
<td>24.9±2.3</td>
<td>42.0±4.8</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Free fatty acid fraction</td>
<td>2.5±0.5</td>
<td>5.2±0.5</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Triglyceride fraction</td>
<td>15.0±2.3</td>
<td>24.5±3.8</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Polar lipid fraction</td>
<td>3.0±0.4</td>
<td>5.6±5.6</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are mean±SEM. [1-14C]Palmitate was injected as a bolus 5 minutes after the onset of reperfusion following 40 minutes of low-flow ischemia (5% of control perfusion). Hearts were frozen for analysis 2 minutes after tracer injection. [1-14C]Palmitate uptake was estimated by summing myocardial activity and activity released in the form of 14CO2 14CO2 was measured in the coronary effluent collected during 2 minutes after tracer injection.

14CO2 release measured early after reperfusion, before a new steady state is reached. During ischemia, lowering of specific activity of intermediate pools by afflux of unlabeled molecules from interrelated pathways may occur, potentially leading to underestimation of the oxidative metabolic rate early after reperfusion. On the other hand, washout of labeled metabolites that have accumulated during ischemia may cause overestimation.

Overall Oxidative Substrate Metabolism After Reperfusion

Under normoxic conditions myocardial oxygen consumption in isolated rat hearts is closely related to cardiac work.27 This relation appears to be disrupted in reperfused postischemic myocardium. Normal regional oxygen consumption in stunned myocardium has recently been reported in anesthetized4 and awake5 dogs with regional ischemia produced by coronary occlusion followed by reperfusion. The present study with an in vitro model of global ischemia and reperfusion provides supportive evidence for this observation. In the hearts that exhibited reversible contractile dysfunction after 5 minutes of reperfusion following 60 minutes of low-flow ischemia, myocardial oxygen consumption was identical to that of control hearts, despite a significant reduction of developed pressure to 60% of control.

Our results suggest further that oxygen consumption may persist at almost normal rates not only in reversibly stunned myocardium, but also in myocardium that exhibits signs of irreversible injury after reperfusion. Hearts subjected to 60 minutes of no-flow ischemia showed almost complete recovery of oxygen consumption to 94% of control at 5 minutes after the onset of reperfusion. Although oxygen consumption subsequently slowly declined, it still averaged 63% at the end of the 60-minute reperfusion period. Thus, there exists resumption of oxidative metabolism in myocardium that is irreversibly injured according to currently used criteria. However, resumption of metabolic activity appears to be limited to early stages of irreversible damage, because oxygen consumption was severely depressed in hearts damaged by 120 minutes of no-flow ischemia.

Oxidation of [1-14C]Palmitate and of [U-14C]Glucose in Reperfused Myocardium

In both groups of hearts that exhibited recovery of oxygen consumption, either after reversible stunning

Figure 5. Myocardial oxidation of glucose in the four groups of hearts estimated from myocardial release of 14CO2 during perfusion with medium containing [U-14C]glucose. The graphs show the value immediately before induction of ischemia (B, baseline) and those measured during the posts ischemic reperfusion period. For the ischemia groups the range of 2 SEM of the values of the control group is indicated by the hatched area. In hearts subjected to 60 minutes of low-flow ischemia or 60 minutes of no-flow ischemia oxidation of glucose was transiently increased during the reperfusion period. Values are mean±SEM. **p<0.01, *p<0.05 vs. control group; ††p<0.01, †p<0.05 vs. 60-minute low-flow ischemia group; ‡‡p<0.01 vs. 60-minute no-flow ischemia group.
by 60 minutes of low-flow ischemia or after induction of advanced injury by 60 minutes of no-flow ischemia, the rate of oxidation of both [1-14C]palmitate and [U-14C]glucose returned to normal levels during the reperfusion period. Complete restoration of oxidation of continuously delivered [14C]glucose and [14C]palmitate after reperfusion has been previously observed by Lopaschuk et al in an isolated working rat heart preparation and by Liedtke et al and Renstrom et al in an extracorporeally perfused working swine heart preparation. In the study by Liedtke et al oxidation of [14C]palmitate was even higher at 15 minutes after the onset of reperfusion despite persistent depression of contractile performance.

The preservation of oxygen consumption and oxidation of both palmitate and glucose in presumably irreversibly injured myocardium in the present study argues against the hypothesis that failure of mitochondrial substrate metabolism is an early event in ischemic injury. The subcellular events underlying the apparent dissociation between oxidative metabolism and contractile function in reperfused myocardium have yet to be clarified. Possible mechanisms include utilization of ATP at contractile elements to maintain wall tension, uncoupling of oxidative phosphorylation, and utilization of respiratory chain-derived energy for mitochondrial calcium transport.

Several previous reports have suggested a change of the substrate pattern after postischemic reperfusion. Meyers et al observed a shift from oxidation of palmitate to oxidation of glucose in open-chest dogs subjected to 1 hour of coronary occlusion followed by 1 hour of reperfusion. Enhanced oxidation of glucose has been observed in dogs as late as 24 hours after reperfusion following a 3-hour coronary occlusion. However, in contrast to the experiments in dogs, in the present study and in several previous reports, the normal substrate pattern for oxidative metabolism was rapidly restored within 15–30 minutes of reperfusion.

Although in hearts subjected to 60 minutes of either low-flow or no-flow ischemia oxidation of [1-14C]palmitate and [U-14C]glucose returned to values similar to those measured in control hearts, the time course of recovery of release of 14CO2 differed for the two substrates. In hearts perfused with [U-14C]glucose-containing medium, release of 14CO2 increased rapidly after reperfusion to even supranormal values. On the other hand, in hearts perfused with [1-14C]palmitate-containing medium release of 14CO2 recovered more gradually, early after the onset of reperfusion. Furthermore, in the experiments with administration of [1-14C]palmitate as a pulse at 5 minutes after the onset of reperfusion, more tracer was incorporated into tissue lipids, with the highest proportion recovered in triglycerides. These observations may reflect a transient modification of the relative contribution of palmitate and glucose to oxidative metabolism immediately after reperfusion. However, quantitative estimation of the rate of substrate oxidation early after reperfusion, before a new steady state of 14CO2 release is reached, may be compromised by methodological limitations as outlined above.

Pauly and McMillin-Wood observed impairment of the transfer of long-chain fatty acids across the inner membrane of mitochondria isolated from a myocardial region subjected to 60 minutes of ischemia followed by 20 minutes of reperfusion. In the present study, oxidation of [1-14C]acetate was reduced to a similar extent compared with the oxidation of [1-14C]palmitate. This observation suggests that mechanisms other than inhibition of mitochondrial transport of palmitoyl coenzyme A may be involved in the transient reduction of release of 14CO2 from [1-14C]palmitate early during reperfusion.

**Implications for Myocardial Imaging With Radiolabeled Metabolic Substrates**

Assessment of myocardial substrate metabolism by new imaging modalities such as positron emission tomography has been proposed for the delineation and characterization of myocardial injury after reperfusion procedures. In dogs subjected to coronary occlusion followed by reperfusion, Schwaiger et al observed that clearance of [1-11C]palmitate radioactivity from the myocardium was delayed and uptake of [18F]2-fluoro-2-deoxyglucose was increased in the reperfused region. The results of the present study are compatible with transient depression of fatty acid oxidation and enhancement of glucose utilization in reperfused myocardium. However, in the isolated rat heart the substrate pattern was almost completely normalized after 30 minutes of reperfusion, whereas altered kinetics of [1-13C]palmitate and [18F]2-fluoro-
2-deoxyglucose in dogs in vivo persisted for days. The explanation for the apparent difference in the time course of normalization of substrate metabolism is not clear. Differences in the animal species and the severity of the ischemic insult may account for the discrepancy. Furthermore, externally derived kinetics of labeled substrates are influenced by numerous factors in addition to the oxidative metabolic rate, which include the size and turnover of the labeled intracellular pools, washout of labeled metabolites, and backdiffusion of nonmetabolized tracer.

Recovery of regional extraction of metabolic substrates documented after reperfusion by positron emission tomography is considered to indicate tissue viability. The results of the present study suggest that residual metabolism may not be restricted to reversibly injured parts of the myocardium, but also may persist in regions that are already irreversibly injured. This observation warrants some caution for the interpretation of metabolic parameters in terms of reversibility of the ischemic injury, at least for the early reperfusion period.

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References


**KEY WORDS** • metabolism • fatty acids • rat heart • reperfusion • glucose
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