Efflux of Metabolized and Nonmetabolized Fatty Acid from Canine Myocardium

Implications for Quantifying Myocardial Metabolism Tomographically

Keith A. A. Fox, Dana R. Abendschein, H. Dieter Ambos, Burton E. Sobel, and Steven R. Bergmann

SUMMARY. It has generally been assumed, from assessment of myocardial metabolism with [1-11C]palmitate and positron emission tomography, that clearance of the radiolabel from the myocardium is attributable solely to efflux of the products of oxidative metabolism. However, interpretations would differ if this assumption were unfulfilled. Furthermore, efflux of metabolized and nonmetabolized tracer has not been quantified. Accordingly, in this study, myocardium was perfused extracorporeally in 21 open-chest anesthetized dogs, and the extraction and clearance of [1-11C]palmitate were characterized under baseline conditions (normoxia, n = 21), and, again, with ischemia (n = 6), with hypoxia (n = 9), or under control conditions (n = 6). After intracoronary bolus injection of [1-11C]palmitate, myocardial time activity curves were measured with a 185-probe, and the products of oxidative metabolism (11CO2) and efflux of extracted but nonmetabolized fatty acid ('back-diffusion' of [1-11C]palmitate) were measured directly from analysis of arterial and regional coronary venous blood. Under control conditions, 45.2 ± 3.8% (mean ± SD) of initially extracted [1-11C]palmitate was metabolized to 11CO2, whereas 6.2 ± 2.6% back-diffused in unaltered form in 1–10 minutes. In contrast, with ischemia (perfusion of 26% of baseline), only 16.9 ± 9.8% of administered tracer evolved as 11CO2 (P < 0.001 compared with control) but 15.6 ± 8.9% (i.e., almost half of the total amount cleared) evolved unaltered as [1-11C]palmitate (P < 0.05). Similarly, with hypoxia, 15.1 ± 8.4% evolved as 11CO2 (P < 0.001) and 18.8 ± 11.7% back-diffused (P < 0.001). Overall, from 1–40 minutes after intracoronary injection of tracer, back-diffusion of [1-11C]palmitate contributed 40.6% of total radioactivity in the effluent with ischemia, 48.7% with hypoxia, but only 8.9% under control conditions. Despite the increased back-diffusion of [1-11C]palmitate seen with ischemia and hypoxia, the overall residue of 11C activity in myocardium increased, consistent with the diminished clearance observed in the myocardial time-activity curves and the increase in the tissue content of triglyceride and nonesterified fatty acid. Our results indicate that estimates of oxidative metabolism based upon clearance of radiolabeled fatty acid must take into account the efflux of initially extracted but nonmetabolized fatty acid. The findings apply to external determination of oxidative metabolism of the heart with any imaging modality that delineates retention and clearance of labeled fatty acids or their analogs. (Circ Res 57: 232–243, 1985)
in isolated hearts and in open-chest and intact animals (Klein et al., 1979; Lerch et al., 1981; Schon et al., 1982a, 1982b), and in hearts of patients studied with positron tomography (Ter-Pogossian et al., 1980; Schelbert et al., 1982). However, interpretation of the biological significance of tomographically detected clearance of extracted $^{11}$C-labeled fatty acid must take into account the extent to which efflux of the radiolabeled tracer can be attributed to egress of oxidative metabolites per se, on the one hand, and to nonmetabolized, but initially extracted labeled fatty acid, on the other. Egress of tracer from myocardium in the form of $^{14}$CO$_2$ has often been assumed to be the sole component responsible for net clearance of tracer. However, if a significant proportion of the extracted fatty acid back-diffuses from myocardium without being metabolized, inferences regarding the rate of oxidation based on measured overall clearance would require modification.

We have previously shown that viable but ischemic myocardium in the region of supply of a stenotic coronary artery continues to extract $^{11}$C-P (Lerch et al., 1982a) but exhibits a diminished rate of overall clearance of the tracer. The diminution of clearance delineated by residue detection has been generally interpreted to reflect decreased oxidation, and the slope of early rapid clearance phase has been interpreted as reflecting the extent of $\beta$-oxidation (Lerch et al., 1982a; Schon et al., 1982a, 1982b). However, such interpretations may be spurious in the absence of characterization of the extent to which the initially extracted fatty acid is oxidatively metabolized, the extent to which it remains within the cytosol as free or esterified fatty acid or in the neutral or phospholipid pools, and the extent to which it back-diffuses from myocardium without being metabolized at all.

Rose and Goresky, (1977), have investigated the constraints of uptake of labeled fatty acid with respect to intravascular and interstitial markers. However, their studies have not been extended to characterize residue clearance with respect to the fate of extracted tracer in myocardium and the quantitative contributions of efflux of oxidatively metabolized tracer and washout of initially extracted but not metabolized fatty acid. Interpretation of the biological implications of clearance of tracer requires such information. Accordingly, this study was undertaken to determine the quantitative significance of efflux of the components of labeled fatty acid in relation to net content of $^{11}$C activity in myocardium.

The information acquired should be applicable not only to assessment of myocardial metabolism by positron emission tomography, but also to interpretation of kinetics of tracers of fatty acid metabolism based on results obtained with any modality that externally detects the distribution of labeled substrate in myocardium.

The specific objectives were as follows: (1) quantify the respective contributions of $^{11}$CO$_2$ derived from oxidative metabolism of $^{11}$C-P and of efflux of unaltered $^{11}$C-P (back-diffusion) to the net clearance of radiolabeled fatty acid tracer from myocardium; (2) define the extent to which altered clearance of $[^{11}]$C-palmitate in normal, ischemic, and hypoxic myocardium reflects oxidative metabolism of $^{11}$C-P measured directly; (3) define the quantitative contributions from tracer in specific lipid pools within myocardium during the imaging interval in relation to efflux of $^{11}$C-P and to egress of $^{14}$CO$_2$; and (4) elucidate the extent to which the efflux of nonmetabolized fatty acid influences the interpretation of integrated positron emission tomograms or apparent clearance delineated with sequentially obtained tomograms.

**Methods**

**Animal Preparations**

In order to characterize the kinetics of fatty acid clearance from myocardium and fatty acid utilization assessed radiochemically at the same time, and to delineate rates of oxidation of fatty acid, we used open-chest, anesthetized dogs. Animals were subjected to coronary artery cannulation to permit regional perfusion at selected flows. Twenty-one mongrel dogs, weighing 26–43 kg, were premedicated with morphine sulfate, 1 mg/kg (sc), after an overnight fast. Anesthesia was induced with sodium thiopental, 12 mg/kg, iv, and was maintained with $\alpha$-chloralose, 60 mg/kg. Animals were intubated and ventilated (Harvard respiratory model 607) with room air enriched with oxygen to maintain arterial PO$_2$ greater than 90 mm Hg. A left thoracotomy was performed at the 5th intercostal space, and the heart was suspended in a pericardial cradle. The left anterior descending coronary artery (LAD) or the circumflex coronary artery (LCX) was dissected free within 1 cm from its origin. Heparin, 400 U/kg, was administered intravenously.

An extracorporeal bypass system was employed to permit independent control of coronary flow under conditions of normoxia, ischemia, or hypoxia (Lerch et al., 1982a). Blood from the femoral artery was passed through the extracorporeal bypass system (total volume, 75 ml) with the use of a calibrated roller pump (Buchler Instruments), a heat exchanger, and a Swank transfusion filter with 13-μm exclusion. Aortic and perfusion pressures were monitored continuously with Statham P23Db transducers. Perfusion pressure calibration measurements were obtained with each dog's own blood and were corrected for the small pressure gradient in the coronary cannula at the flow rates used in each experiment. The proximal LAD or LCX was cannulated with an 18-gauge polyethylene cannula, and the vessel was perfused by the extracorporeal bypass system. The native vessel proximal to the site of cannulation was ligated so that perfusion in the distribution of the cannulated vessel was derived solely from the bypass system (Lerch et al., 1982a). Interruption of blood flow through the vessel during the cannulation procedure did not exceed 1 minute. Perfusion pressure was adjusted to match aortic diastolic pressure. The epicardial electrocardiogram from the center of the zone supplied by the cannulated vessel was monitored continuously. Normal saline was infused to match the combined volumes of the extracorporeal bypass system and the total volume of samples withdrawn (approximately 150 ml). A 20-gauge polyethylene cannula was inserted into the epicardial vein.
Regional Myocardial Utilization of Substrate

Regional myocardial oxygen utilization (MVO₂) in the zone of extracorporeal perfusion was calculated based on oxygen content (including dissolved O₂), in arterial and local coronary venous blood samples and flow. Hemoglobin concentration and oxygen saturation were assayed serially with a model IL182 Instrumentation Laboratory oximeter. PO₂, PCO₂, and pH were assayed with a model 213 Instrumentation Laboratory blood gas analyzer. For calculation of unlabeled glucose, lactate, oxygen, and fatty acid utilization, perfusate and coronary venous samples were taken 10 and 40 minutes after administration of radiotracer. Plasma lactate was assayed enzymatically (Gutmann and Wahlefeld, 1984), and plasma glucose, spectrophotometrically (Kornberg and Horecker, 1955) (Calbiochem-Behring reagents). Concentrations of nonesterified fatty acid (FFA) were measured in duplicate colorimetrically (Bergmann et al., 1982). To define further the changes in individual fatty acids with each intervention, thin layer chromatography and gas chromatography were performed on lipid extracts from myocardial tissue, permitting measurement of fatty acid content and pool size of myocardial lipids. Organic extracts of tissue were spiked with an internal standard of heptadecanoic acid (C17:0), and subjected to thin layer chromatography in petroleum ether, diethyl ether, and acetic acid (97:52:3) to separate FFA and triglycerides. FFA were further characterized after elution from the silica and esterification with boron trifluoride-methanol. The resulting fatty acid methyl esters were extracted into iso-octane and analyzed by gas chromatography (HP 57904), using a 2 mm × 2 meter glass column (SP 2330 10%, Supelco). Triglyceride was quantified spectrophotometrically after elution from silica (Van Handel and Zilversmit, 1957).

In seven experiments [1-14C]palmitate was administered simultaneously with the [1-12C]palmitate to characterize the distribution of the radiotracer in the efflux. Thin layer chromatography, high pressure liquid chromatography, and liquid scintillation spectrometry were utilized to characterize the chemical form of radiotracer in the efflux from the extracorporeally perfused region of the heart. Rapidly frozen tissue samples were obtained at the end of each study and were assayed for fatty acid and triglyceride content. Gas chromatography was performed to determine the fatty acid composition of components in the lipid extract.

Preparation of Radiolabeled Palmitate and Radiolabeled Water

[1-13C]Palmitate was synthesized as described previously (Welch et al., 1985) with the use of a remotely controlled automated system in which the [1-13C]palmitate is produced from 13CO₂ obtained through the 14N[p,d]13C nuclear reaction. Cyclotron-produced 13CO₂ was reacted with the Grignard reagent, n-pentadecyl magnesium bromide diethyl ether. The palmitic acid produced was added to a 3% solution of human serum albumin in normal saline and filtered through 0.45- and 0.2-μm Millipore filters. Purity was greater than 99% as assessed by high pressure liquid chromatography.

Labeled H₂O was prepared with the fast exchange of oxygen between H₂O + CO₂ and H₂CO₃. I5O-oxygen was produced by the 14N[d,n]15O reaction (Welch et al. 1969); 99.9% of activity was present as H₂¹⁵O.

Experimental Protocol

Three groups of animals were studied. In each, baseline measurements preceded interventions. To characterize the physiological stability of the experimental animal preparation, a control group of six animals was studied initially. Analyses of tracer kinetics and substrate utilization (FFA, glucose, O₂, lactate) were repeated 90 minutes after the baseline study in the absence of any intervention in each of these animals (the control group).

In another group of six animals, ischemia was induced after baseline analysis by reduction of perfusion in the extracorporeal circuit to 10% of baseline pump flow. Thirty minutes later, tracer kinetic and substrate utilization analyses were repeated, i.e., beginning 90 minutes after the baseline injection of tracer (the ischemic group).

In a third group of nine animals, hypoxia without reduction of flow was induced after the baseline study by initiating perfusion with venous instead of arterial blood without any alteration of pump flow. Thirty minutes later, a repeat study was performed in each dog. In these animals (the hypoxic group), samples were taken from the perfusate so that the "a'-v extraction of glucose, lactate, and fatty acids could be determined.

After cannulation and bypass perfusion, a 30-minute stabilization interval was imposed for each of the dogs prior to each baseline study. To measure nutritional perfusion (including contributions from collateral supply), we injected 2-4 mCi of H₂¹⁵O, i.e., as a bolus over 1-2 seconds in a volume of 0.25 ml of blood, as described previously (Lerch et al., 1982a; Bergmann et al., 1984). Myocardial H₂¹⁵O residue time activity curves were obtained with the use of a β-probe. Five minutes after the injection of H₂¹⁵O, 4-6 mCi of [1-13C]palmitate were injected as a bolus over 1 second, i.e., in a volume of 0.25 ml, and time-activity curves were obtained for 20 minutes. Simultaneous perfusate and coronary venous blood samples were taken every minute for 10 minutes, every 2 minutes up to 20 minutes, and, subsequently, every 5 minutes up to 40 minutes. Additional perfusate and coronary venous samples were obtained 10 minutes and 40 minutes after injection of tracer for chemical analysis of fatty acid content, determination of blood gases and pH, and assay of lactate and glucose.

Immediately before the termination of each study, 5 ml of lissamine green dye were injected via the perfusion cannula, followed by intracoronary KCl to delineate the zone of perfusion and to induce cardiac arrest. Animals with branches of the draining coronary vein arising from areas that included zones of native perfusion, or animals in which the probe's area within the field of view overlapped the zone of native perfusion, were excluded (n = 2).

Analysis of Regional Myocardial Tracer Time-Activity Curves

Myocardial residue tracer time-activity curves were determined for oxygen-15-labeled water (isotope t₀ = 2.1 minutes) and [1-13C]palmitate (isotope t₀ = 20.4 minutes) with the use of a β-probe aligned between two arterial branches in the zone perfused by the extracorporeal bypass system. The high efficiency for detection of β-radiation with this system has been documented previously (Lerch et al., 1982b). The contribution of γ-photons from the heart was higher (30-35% of total activity detected) because of greater intrinsic atten-
Determination of Efflux of Oxidative Products from Efflux of Nonmetabolized Tracer

For quantitative determination of the respective contributions to total radioactivity of \(^{14}C\) and \(^{13}C\)-P in each of the arterial and coronary venous blood samples, the following procedure was employed. Two 1.0-ml aliquots of arterial and two 1.0-ml aliquots of coronary venous blood samples were placed into tubes containing 3 ml of isopropanol alcohol and 1.0 ml of 0.9 \(\mu\) sodium bicarbonate. One ml of 6 \(N\) HCl was added to one member of each pair and 1 ml of 0.1 \(N\) NaOH was added to the other. Both samples were placed in a heated (85°C) ultrasonic bath and disrupted sonically in a fume hood for 10 minutes. Radioactivity in the samples was assayed with a 7-well counter with the aid of a microcomputer. The computer corrected observed activity for physical decay of the isotope and provided a printout of regional time-activity curves.

Calculations of the myocardial turnover rate constant (k), biological half-time (t\(_b\)), and correlation coefficient (r) for characterization of the slope of specified portions of H\(_2\)O and \([^{13}C]palm\)itate time-activity curves were performed with the aid of the microcomputer, using the least squares approximation of the natural logarithm of the count rate as a function of time (Lerch et al., 1982b). Myocardial blood flow was determined based on the slope of the monoexponential H\(_2\)O washout during the interval from 15–30 seconds after injection of the tracer. Flow was calculated from the clearance rate multiplied by 0.92 (the myocardial tissue/blood partition coefficient for water), as described previously (Bergmann et al., 1984). Clearance of \(^{14}C\) activity after the early, rapid, vascular phase was determined from selected portions of the time-activity curve. Extraction fraction was determined by back extrapolation of the early component of the time activity curve (from 1–3 minutes after tracer administration) to the time of occurrence of peak counts. The extraction fraction was expressed as residue counts divided by peak counts.

We have previously shown that extraction of tracer measured in this fashion correlates closely with arteriovenous extraction measured directly (Nomura et al., 1982).

Statistical Analysis

Values are expressed as means ± SD. The \(t\)-test for paired samples was used to compare intragroup baseline values with those after interventions. Further comparisons were performed by linear regression analysis and by analysis of variance.

Results

Hemodynamics, Perfusion, and Oxygen Consumption

Hemodynamics and myocardial oxygen consumption (MVO\(_2\)) were characterized to determine...
whether cardiac work was similar under normoxic baseline conditions in the three groups, to determine whether it was consistent with perfusion maintained constant in the control group, and to characterize changes with hypoxia without reduction of flow and with ischemia.

Myocardial blood flow, MVO₂, and perfusion pressure did not differ among the three groups under baseline conditions (Table 1). There were no significant changes in heart rate or aortic systolic or diastolic pressures in the control group with ischemia or hypoxia. Furthermore, in the control group, both myocardial blood flow and myocardial oxygen consumption remained constant (Table 1).

With induction of ischemia, nutritional blood flow, determined with H₂¹⁵O (which includes the contribution of collateral supply), fell to a mean of 26 ± 12% of baseline (P < 0.005). Mean coronary perfusion pressure fell from 104 ± 25.9 mm Hg to 51.3 ± 18.2 (P < 0.05). With induction of hypoxia without reduction of pump flow, coronary perfusion pressure fell from a mean of 103 ± 6.6 to 77.1 ± 0.005. MVO₂ in the extracorporeally perfused region fell by 60.4% with ischemia (P < 0.005) and by 75.3% with hypoxia without ischemia (P < 0.005).

### Myocardial Clearance of [¹¹C]Palmitate

**Ascertainment from Residue Detection Tracer Time-Activity Curves**

Figure 1 illustrates regional myocardial residue time-activity curves from one dog in each of the experimental groups, early and late under control conditions (i.e., baseline and no intervention), ischemic perfusion, and hypoxic perfusion. The curves for [¹¹C] activity comprise three main components as characterized previously: a vascular transit component, an early phase of rapid clearance, and a late phase of slow clearance (Fig. 1). The local slope of the early phase following the vascular transit component was analyzed for the interval from 1–3 minutes. Curve stripping was not employed. The late phase during the interval from 10–20 minutes after administration of tracer exhibited a relatively slow rate of clearance. The rate constants for the early rapid phase did not differ, either between early and late baseline studies in controls, or under initial baseline conditions, in each of the three groups (Table 2). With either ischemia or hypoxia, the (tₙₐ₀ = ln 2/k) for the early rapid clearance phase (1–3 minutes) lengthened significantly (Table 2). The rate constants are shown for the early rapid phase of myocardial clearance (kₑ) and for the late phase (kₗ) for each of the three groups studied. Corresponding values are shown for the clearance of H₂¹⁵O (kₚ) for the period 15–30 seconds after administration of tracer (Table 2).

These data indicate that clearance of [¹¹C]palmitate was uninfluenced by extracorporeal coronary bypass perfusion per se. However, early clearance and late clearance of [¹¹C]palmitate were significantly reduced with ischemia or hypoxia (Table 2).

Although the early rapid phase of the myocardial residue [¹¹C]palmitate time-activity curve often has been considered to reflect oxidative metabolism of fatty acid directly (Goldstein et al., 1980; Lerch et al., 1982a; Schon et al., 1982a, 1982b), the association between kₑ and 1/MVO₂ in the early rapid phase was not close (baseline studies n = 19, r = 0.45, y = 1.01x + 2.74; for all studies n = 36, r = 0.61, y = 1.91x + 2.26). The lack of a close correlation may be attributable to back-diffusion of non-metabolized fatty acid reflected in the residue detection clearance curve. In support of this interpretation, the correlation between MVO₂ and efflux of [¹¹C]CO₂ was close (r = 0.83, n = 35, y = 2.08x + 0.124). Quantification of [¹¹C]CO₂ efflux measured di-

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>Perfusion pressure (mm Hg)</th>
<th>MVO₂ (ml O₂/100 g per min)</th>
<th>Arterial FFA extraction (μmol/100 g per min)</th>
<th>Net FFA extraction (μmol/100 g per min)</th>
<th>Lactate extraction (μmol/100 g per min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control:baseline (n = 6)</td>
<td>103 ± 8.6</td>
<td>124 ± 20.4</td>
<td>416 ± 74</td>
<td>21.7 ± 6.2</td>
<td>-35.8 ± 17.3</td>
</tr>
<tr>
<td>No intervention</td>
<td>108 ± 20.6</td>
<td>111 ± 45.6</td>
<td>438 ± 201</td>
<td>19.8 ± 13.4</td>
<td>-37.2 ± 48.5</td>
</tr>
<tr>
<td>Ischemia:baseline (n = 6)</td>
<td>104 ± 25.9</td>
<td>121 ± 43.2</td>
<td>485 ± 211</td>
<td>23.0 ± 11.3</td>
<td>-25.5 ± 13.2</td>
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<tr>
<td>Ischemia</td>
<td>51 ± 18.2</td>
<td>32 ± 16.8</td>
<td>515 ± 206</td>
<td>7.8 ± 4.3</td>
<td>-40.5 ± 39.6</td>
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<tr>
<td>Hypoxia:baseline (n = 9)</td>
<td>103 ± 6.6</td>
<td>120 ± 36</td>
<td>434 ± 213</td>
<td>21.6 ± 11.4</td>
<td>48.0 ± 97.2</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>77 ± 25.2</td>
<td>100 ± 27</td>
<td>509 ± 165</td>
<td>0.04 ± 18.3</td>
<td>-98.9 ± 69.9</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± so. P indicates significance compared with baseline studies.

* MBF = myocardial blood flow.
Figure 1. Myocardial 11C time-activity curves from one dog under baseline conditions (upper left) and, later, under control conditions with no intervention (upper right), from a separate animal with ischemic perfusion (lower left), from another animal with hypoxic perfusion (lower right). Myocardial radioactivity is corrected for isotope decay and normalized with peak counts set equal to 1.0. The histogram corresponding to each time-activity curve indicates, for the entire group, the respective proportions of the effluent constituted by 11CO2 (cross-hatched bars ± se) and 11C]palmitate (11CP, open bars) for each collection period throughout the study (controls n = 6, ischemia n = 6, hypoxia n = 9). The myocardial time-activity curve can be divided into: (1) an initial component of very rapid clearance immediately following peak counts and completed within 1 minute; (2) an early phase of rapid clearance (analyzed from 1–3 minutes); (3) a late phase of slow clearance (analyzed from 10–20 minutes). Comparison with corresponding histograms indicates the respective contributions of 11CO2 and 11CP to total radioactivity for each segment of the time-activity curve. Despite the substantially increased contribution of 11CP with ischemia or hypoxia to total radioactivity in the effluent, the rates of both early and late clearance are diminished.

TABLE 2

<table>
<thead>
<tr>
<th></th>
<th>H218O</th>
<th>[11C]Palmitate (1–3 min)</th>
<th>[11C]Palmitate (10–20 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>kH2O (per min)</td>
<td>r</td>
<td>k1 (per min)</td>
</tr>
<tr>
<td>Control:baseline</td>
<td>1.596 ± 0.463</td>
<td>0.930 ± 0.079</td>
<td>0.154 ± 0.031</td>
</tr>
<tr>
<td>No intervention</td>
<td>1.242 ± 0.542</td>
<td>NS</td>
<td>0.116 ± 0.038</td>
</tr>
<tr>
<td>Ischemia:baseline</td>
<td>1.277 ± 0.691</td>
<td>0.950 ± 0.050</td>
<td>0.178 ± 0.057</td>
</tr>
<tr>
<td>Ischemia</td>
<td>0.367 ± 0.197</td>
<td>0.880 ± 0.168</td>
<td>0.078 ± 0.034</td>
</tr>
<tr>
<td></td>
<td>&lt;0.005</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Hypoxia:baseline</td>
<td>1.374 ± 0.420</td>
<td>0.931 ± 0.078</td>
<td>0.151 ± 0.048</td>
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<tr>
<td>Hypoxia</td>
<td>1.096 ± 0.297</td>
<td>0.900 ± 0.102</td>
<td>0.084 ± 0.048</td>
</tr>
<tr>
<td></td>
<td>&lt;0.02</td>
<td>&lt;0.02</td>
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</table>

kH2O = rate of biological clearance of H218O from 15–30 seconds after bolus administration of tracer. k1 and k2 = rate constants for biological clearance of [11C]palmitate for early (k1, 1–3 min) and late (k2, 10–20 min) intervals. Values = mean ± se. Numbers for each group, see Table 1. r = correlation coefficient of the slope.
7.8%. However, net extraction, i.e., the product of residual fraction and flow, decreased significantly with both ischemia and hypoxia, as discussed below.

**Fatty Acid Metabolism Assessed Chemically and Radiochemically**

Arterial free fatty acid levels did not differ significantly under baseline conditions in the three groups; neither did they change significantly under control conditions over time (Table 1). With ischemia and with hypoxia, values were slightly but not significantly increased (Table 1).

Net free fatty acid (FFA) extraction was calculated from the product of arteriovenous FFA concentration differences and flow. Net FFA extraction did not change significantly with perfusion held constant in controls. Baseline values in all three groups were similar (Table 1). With either ischemia or hypoxia, net myocardial extraction of FFA fell significantly (Table 1).

To clarify interpretations of the data derived from analysis of efflux reflecting changes in clearance and residual 14C activity, we undertook an analysis of tissue lipid pools. Myocardial samples obtained at the completion of experiments were analyzed for total FFA, FFA of specific chain length, and triglycerides. Tissue FFA levels were higher in extracorporeally perfused zones compared with values in normally perfused zones (native perfusion) in the same animals (Table 3). With ischemia, FFA content increased in extracorporeally perfused compared with normal zones in the same animals (25.0 ± 13.7 and 15.6 ± 8.9 nmol/100 g). The levels were significantly higher than those in normal zones of control animals (14.0 ± 7.9 nmol/100 g) (P < 0.05). These findings, based upon analysis of tissue content of unlabeled lipid fractions, support the interpretation of data derived from the analysis of the 14C-P residue time-activity curves. Thus, the diminished rate of clearance of tracer with ischemia or hypoxia (Fig. 1) and the increased residue of tracer in the tissue at the end of 40 minutes (Fig. 2) are in keeping with an increase in the size of the FFA and slow turnover-lipid pools (triglyceride and phospholipid) under these conditions.

**Arteriovenous Lactate and Glucose**

To assess the possible influence of altered metabolism of glucose or lactate on overall utilization of substrate under conditions of ischemia or hypoxia, net arteriocoronary venous glucose and lactate differences were measured. Under control conditions, neither glucose nor lactate extraction changed when perfusion was held constant. With ischemia or hypoxia, arteriovenous lactate differences reflected significant increases with ischemia and with hypoxia as confirmed by the CO2 efflux data discussed below.

Triglyceride content was slightly but not significantly higher in zones of ischemia (29.4 ± 14.9 nmol/100 g) or hypoxia (26.8 ± 26.7 nmol/100 g), compared with values in normal zones in the same animals (25.0 ± 13.7 and 15.6 ± 8.9 nmol/100 g). The levels were significantly higher than those in normal zones of control animals (14.0 ± 7.9 nmol/100 g) (P < 0.05). These findings, based upon analysis of tissue content of unlabeled lipid fractions, support the interpretation of data derived from the analysis of the 14C-P residue time-activity curves. Thus, the diminished rate of clearance of tracer with ischemia or hypoxia (Fig. 1) and the increased residue of tracer in the tissue at the end of 40 minutes (Fig. 2) are in keeping with an increase in the size of the FFA and slow turnover-lipid pools (triglyceride and phospholipid) under these conditions.

**Table 3**

<table>
<thead>
<tr>
<th>Tissue Content of Fatty Acids of Specific Chain Lengths (μmol/100 g ± SD)</th>
<th>16:0*</th>
<th>16:1</th>
<th>18:0</th>
<th>18:1</th>
<th>18:2</th>
<th>20:4</th>
<th>Total FFA</th>
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<tbody>
<tr>
<td>Control (n = 6)</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Native-perfused</td>
<td>3.37 ± 2.09</td>
<td>0.45 ± 0.22</td>
<td>3.23 ± 1.13</td>
<td>4.13 ± 1.92</td>
<td>2.53 ± 1.54</td>
<td>0.40 ± 0.94</td>
<td>14.2 ± 5.7</td>
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<tr>
<td>ECB-perfused†</td>
<td>6.46 ± 3.05</td>
<td>1.06 ± 1.12</td>
<td>5.73 ± 1.29</td>
<td>9.65 ± 4.30</td>
<td>4.65 ± 3.46</td>
<td>0.76 ± 1.90</td>
<td>27.8 ± 9.1</td>
</tr>
<tr>
<td>Ischemia (n = 6)</td>
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<tr>
<td>Native-perfused</td>
<td>3.33 ± 0.36</td>
<td>0.65 ± 0.22</td>
<td>2.91 ± 0.72</td>
<td>4.87 ± 1.82</td>
<td>2.47 ± 0.48</td>
<td>0</td>
<td>14.2 ± 1.9</td>
</tr>
<tr>
<td>ECB-perfused†</td>
<td>9.23 ± 2.66</td>
<td>2.04 ± 0.31</td>
<td>9.71 ± 6.91</td>
<td>18.19 ± 6.31</td>
<td>3.13 ± 1.92</td>
<td>0</td>
<td>42.3 ± 14.2</td>
</tr>
<tr>
<td>Hypoxia (n = 9)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Native-perfused</td>
<td>3.94 ± 1.41</td>
<td>0.84 ± 0.90</td>
<td>3.28 ± 1.05</td>
<td>5.42 ± 3.30</td>
<td>2.20 ± 1.32</td>
<td>0</td>
<td>15.2 ± 6.03</td>
</tr>
<tr>
<td>ECB-perfused†</td>
<td>8.84 ± 8.19</td>
<td>2.52 ± 1.71</td>
<td>5.26 ± 4.62</td>
<td>14.20 ± 7.41</td>
<td>2.14 ± 1.29</td>
<td>0</td>
<td>33.8 ± 21.0</td>
</tr>
</tbody>
</table>

The tissue content of individual fatty acids in the zones subjected to extracorporeal perfusion (ECB-perfused) is compared with that of myocardium supplied by the uninterrupted left anterior descending coronary artery (native perfusion). P indicates significance compared with native perfusion.

* 16:0 = palmitic acid, 16:1 = palmitoleic acid; 18:0 = stearic acid, 18:1 = oleic acid, 18:2 = linoleic acid; 20:4 = arachidonic acid.
† Extracorporeally bypass-perfused.
with control perfusion (70.4 ± 74.4 μmol/100 ml for baseline studies; 71.0 ± 34.5 μmol/100 ml for control studies). With ischemia or hypoxia, extraction of glucose tended to increase (103 ± 27.6 μmol/100 ml with ischemia and 223 ± 84 μmol/100 ml with hypoxia, control n = 3, ischemia n = 5, hypoxia n = 3). Thus, despite the marked dog-to-dog variation with respect to glucose and lactate, ischemia or hypoxia tended to increase efflux of lactate and extraction of glucose.

The Contribution of Removal of Oxidative Metabolites of [14C]Palmitate to Total Clearance of Tracer

Judging from assays of the [14CO2] and [14C]palmitate arteriovenous differences in samples obtained throughout the data collection intervals, [14CO2] comprised 87.8 ± 6.2% of radioactivity in the effluent from 1–20 minutes after injection of tracer in control studies. No differences (88.4 ± 3.1%) were seen in the late studies in the absence of an intervention (Table 4). However, with ischemia, the mean percentage of [14CO2] with respect to total radioactivity in the effluent fell to 59.4 ± 11.0% (P < 0.01). It fell to 51.3 ± 16.2% with hypoxia (P < 0.005).

Analyses of the lipid extracts of the coronary venous effluent were performed in the seven animals in which [1-14C]CP and [1-14C]CP were administered simultaneously. [14C] activity was present only in the FFA band following thin layer chromatography, and high pressure liquid chromatography (HPLC) analysis confirmed a single peak of activity corresponding with that of 16:0 fatty acid standard. These results confirm that the tracer in the effluent in these studies was in the form of unmetabolized radiolabeled palmitate.

Figure 1 shows the results of sequential analyses of radioactivity in effluent throughout the data collection interval in animals in each of the three groups and with respect to the myocardial residue time-activity curve. From Table 4, it can be seen that maximal [14CO2] production occurred during the 1- to 10-minute interval corresponding to the phase of rapid early clearance. During the late phase of the curve (10–40 minutes), the proportion of [14CO2] in the effluent remained stable (Fig. 1). With ischemia or hypoxia, back-diffusion of nonmetabolized fatty acid constituted a mean of 40.6% and 48.7% of activity in the effluent, respectively, compared to only 8.9% with no intervention (control). As indicated in Figure 1, the proportions of radioactivity in the effluent constituted by [14CO2] and [14C]palmitate were maintained constant throughout the latter part of the data collection interval (i.e., from 10–40 minutes after injection of tracer). Most [14CO2] production occurred in the early phase of clearance (1–10 minutes) (Table 4). With hypoxia, the contribution of [14CO2] to total efflux is relatively greater during this
of tracer averaged 49 ± 12% for baseline studies in all groups and was not significantly different, averaging 45.2 ± 4.8%, for the late studies in the control group. Values in the intervals from 10–20 or from 20–40 minutes after injection of tracer were similar under control conditions (Fig. 3). However, with ischemia, the percentage of myocardial activity present in the tissue 1 minute after injection of tracer and subsequently appearing as $^{11}$CO$_2$ fell significantly ($P < 0.001, 1–10$ minutes) (Fig. 3). Similarly, with hypoxia, it fell significantly during the same interval ($P < 0.0001$ compared with baseline values) (Fig. 3). In addition, $^{11}$CO$_2$ appearing in the intervals of 10–20 and 20–40 minutes expressed as a percentage of myocardial activity 1 minute after injection of tracer was reduced with ischemia or hypoxia (Table 4).

Efflux of nonmetabolized $[^{14}$C]palmitate during the interval from 1–40 minutes after injection of tracer constituted $9.0 ± 6.0\%$ of initially extracted $[^{14}$C]palmitate under baseline conditions. It remained constant under control conditions ($8.9 ± 7.2\%$). Efflux of nonmetabolized $[^{11}$C-P increased to $19.5 ± 9.4\%$ with ischemia and to $27.4 ± 10.5\%$ with hypoxia ($P$ for both compared with baseline values < 0.05). Similarly, with ischemia or hypoxia, the efflux of nonmetabolized fatty acid was greater than that under control conditions during the intervals from 10–20 or 20–40 minutes after administration of tracer (Table 4).

The distribution of the injected dose of $[^{11}$C-P was calculated for each time period, and the nonextracted component, the retained components, $[^{11}$C-P efflux, and $[^{11}$CO$_2$ efflux were analyzed (Fig. 4, A and B). It can be seen that after vascular transit, total clearance is diminished with ischemia, but $^{11}$C-P
Efflux of Labeled Fatty Acid Components

Discussion

The results of this study indicate that during the early phase of myocardial time-activity curves, clearance of administered [11C]palmitate is directly related to oxidative metabolism of fatty acids reflected by efflux of 11CO2. However, because of back-diffusion of nonmetabolized fatty acid, the relationship between clearance of total myocardial radioactivity and MVO2 is not close. During the later phases of the residue detection time-activity curve from 10–40 minutes after administration of tracer, results are consistent with incorporation of the extracted fatty acid into phospholipid and triglyceride and with the measured rate of turnover of the combined, slowly turning over pools (Neely et al., 1972; Opie, 1976).

Although the contribution of back-diffusion of nonmetabolized fatty acids to net clearance of myocardial radioactivity is modest under normoxic conditions, it becomes substantial with ischemia or hypoxia. Findings in this study indicate that unadjusted estimates of myocardial metabolism based solely upon residue detection indexes of clearance would overestimate oxidative metabolism of fatty acids in ischemic or hypoxic myocardium.
perfusion is reduced to values as low as 7.1 with or without a concomitant increase in CO₂ (Opie, 1965).

Kinetics of Myocardial Clearance Evaluated with Residue Detection Curves

As previously demonstrated by our group and by others, the myocardial residue time-activity curve after intracoronary injection of [¹¹C]palmitate can be deconvoluted into: (1) a very early phase of rapid vascular clearance, (2) an early phase of rapid clearance, and (3) late phase of slow clearance (Weiss et al., 1976; Lerch et al., 1982a, b; Schon et al., 1982a, 1982b). The present findings provide direct evidence based upon simultaneous kinetic and biochemical analyses that, under normoxic conditions, the phases of rapid clearance of initially extracted fatty acids after intracoronary injection of tracer reflects oxidative metabolism of fatty acid.

In the present study, production of ¹⁷⁷CO₂ and efflux of [¹¹C]palmitate were determined directly, as was net extracted [¹¹C]palmitate 1 minute after injection of tracer. Results of the efflux studies demonstrated that only a small portion of extracted [¹¹C] palmitate was metabolized to ¹⁷⁷CO₂ within the first minute after the tracer was administered (1.3 ± 3.6%). They demonstrated, also, that under normoxic conditions, more than 85% of the total radioactivity in the effluent during the interval from 1-10 minutes after injection of tracer was present in the form of oxidation products of fatty acid. However, with ischemia or hypoxia, approximately 50% reflected back-diffusion of nonmetabolized fatty acid. With ischemia, the fraction of initially extracted [¹¹C]palmitate that appeared in the effluent as ¹⁷⁷CO₂ was markedly decreased from the 60.1% seen with normoxic perfusion to 16.9%. It was decreased to 15.1% with hypoxia in the corresponding 1- to 10-minute interval. Similarly, from 10-40 minutes, the fraction of extracted [¹¹C]palmitate appearing as ¹⁷⁷CO₂ decreased from 21.2% with normoxic perfusion to 16.2% with ischemia and 13.0% with hypoxia. However, with either ischemia or hypoxia, the fraction of [¹¹C]palmitate radioactivity that appeared as back-diffused nonmetabolized fatty acid were significantly increased, particularly during the interval from 1-10 minutes (6.2% with normoxic perfusion, 15.6% with ischemia, 18.8% with hypoxia).

The net retention of [¹¹C] activity in the tissue 40 minutes after administration of tracer was 19% under control conditions. However, 47% of initially extracted tracer remained in the tissue with ischemia and 44% with hypoxia (Fig. 2). The increased retention is consistent with the reduced rate of residue clearance observed in the [¹¹C]palmitate time-activity curve (Fig. 1 and Fig. 4, A and B). Nevertheless, clearance of initially extracted carbon-11-labeled fatty acids assessed by positron tomography or with any imaging modality based upon residue detection of labeled fatty acid will be significantly influenced by back-diffusion of nonmetabolized fatty acids throughout the data collection period. Thus, images integrating data obtained relatively late, from 20-40 minutes after administration of tracer, will exhibit relatively higher ratios of tracer in ischemic compared with normal zones, in contrast to images integrating data obtained early. This apparent paradox is the consequence of faster overall clearance of [¹¹C]palmitate from normoxic, well-perfused zones because of oxidative metabolism, in contrast to the slower overall clearance from ischemic or hypoxic zones attributable to both oxidation and back-diffusion of unaltered [¹¹C]palmitate. Analyses of lipid extracts corroborated this interpretation. Whereas most of the initially extracted fatty acid was oxidatively metabolized under normoxic conditions, substantial portions back-diffused or were retained as free or as esterified fatty acid under conditions of hypoxia or ischemia.

The results obtained indicate that estimates of metabolic activity based upon residue clearance of radiolabeled fatty acid underestimate the extent of the defect in oxidative metabolism. This effect is especially marked with ischemia or hypoxia when less than 60% of clearance may be accounted for by oxidative metabolism. The extent of the efflux of nonmetabolized tracer may be dependent not only upon tissue oxygenation but, also, upon numerous variables known to influence oxidative capacity and cellular integrity. Thus, the results of this study demonstrate that external assessments of oxidative metabolism of fatty acid based on analyses of residual clearance require consideration of the efflux of nonmetabolized tracer. Although the contributions of back-diffusion remained consistent within each of these study groups with defined conditions, the extent of back diffusion under more diverse circumstances requires further elucidation.

The following approaches may be useful in providing unambiguous remote interpretation of biological implications of observed rates of clearance of tracer: 1) Positron-emission tomography, permitting rapid data acquisition, and correction for partial volume effects, spillover, and gating to the cardiac cycle should enhance the accuracy of determinations of net fatty acid extraction during brief intervals of data acquisition early after administration of tracer. This will have the effect of minimizing the cumulative effects of back-diffusion and disparate rates of clearance from ischemic compared with normal zones; 2) With instrumentation which permits sufficient temporal resolution, extraction may be ascertainable from the measured input function of the tracer; and 3) Comparisons of extraction and clearance of [¹¹C]palmitate, and extraction of [¹¹C]palmitate analogs trapped by myocardium without undergoing oxidation during the data acquisition interval may provide independent indexes of the extent of back-diffusion under specific conditions.

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References


Rose CP, Goresky CA (1977) Constraints on the uptake of labeled palmitate by the heart. The barriers at the capillary and sarcolemmal surfaces and the control of intracellular sequestration. Circ Res 41: 534–545


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K A Fox, D R Abendschein, H D Ambos, B E Sobel and S R Bergmann

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