Validation of [1-\(^{11}\)C]Acetate as a Tracer for Noninvasive Assessment of Oxidative Metabolism With Positron Emission Tomography in Normal, Ischemic, Postischemic, and Hyperemic Canine Myocardium

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Extraction and clearance kinetics of [1-\(^{11}\)C]acetate were examined in 65 experiments in 30 open-chest dogs. Twenty-nine studies were performed at control, 13 during ischemia, eight after reperfusion, 13 during dipyridamole-induced hyperemia, and two during alteration of cardiac workload. [1-\(^{11}\)C]Acetate was injected directly into the left anterior descending coronary artery, and myocardial tissue-time activity curves were recorded with a gamma probe. The single-pass extraction fraction averaged 64.2±9.7% in control, 65.3±9.1% in ischemia, 70.0±4.4% in reperfusion, and 46.5±7.4% in dipyridamole-induced hyperemia groups. \(^{11}\)C clearance was biexponential in all cases. The rate constant k\(_4\), for the first rapid clearance phase correlated closely with myocardial oxygen consumption (r=0.94) in control, ischemia, reperfusion, and dipyridamole-induced hyperemia groups. Monoeponential fitting of only the first linear part of the clearance curve yielded the rate constant k\(_{mono}\), which also correlated with myocardial oxygen consumption (r=0.96). Arterial lactate concentrations and the amount of free fatty acid oxygen equivalents consumed by the myocardium were shown to have a small but statistically significant impact on the relation between [1-\(^{11}\)C]acetate clearance rate constants and myocardial oxygen consumption. The fraction of \(^{14}\)CO\(_2\) activity contributing to overall \(^{11}\)C activity leaving the myocardium after simultaneous injection of [1-\(^{11}\)C]acetate (n=24) was relatively high in all cases (97.4±2.5% in control, 89±2.6% in ischemia, 94.1±3.5% in reperfusion, and >99% in dipyridamole groups), indicating that externally measured \(^{11}\)C clearance corresponds to CO\(_2\) production and thus to tricarboxylic acid cycle activity. In conclusion, the results validate the use of [1-\(^{11}\)C]acetate as a tracer of oxidative myocardial metabolism for use with positron emission tomography. (Circulation 1990;81:1594–1605)

Several studies have examined the usefulness of [1-\(^{11}\)C]acetate as a tracer of myocardial oxidative metabolism. Early work with positron emission tomography (PET) and [1-\(^{11}\)C]acetate in open-chest dogs\(^1\) and humans\(^2\) revealed monoexponential clearance of \(^{11}\)C activity from myocardium after intravenous injection of [1-\(^{11}\)C]acetate. Increased cardiac workload was associated with an increase in the \(^{11}\)C clearance rate constant, suggesting a correlation between myocardial oxygen consumption and \(^{11}\)C washout after [1-\(^{11}\)C]acetate injection. In addition, tracer clearance was delayed in myocardial regions with electrocardiographic evidence of acute ischemia in patients with coronary artery disease.
A recent study in our laboratory in isolated, perfused rat hearts demonstrated biexponential $^{14}$C clearance after $[1-^{14}$C]acetate administration. There was a close correlation between the slope of the rapid clearance phase and the tricarboxylic acid (TCA) cycle flux under varying workload conditions. In contrast to results with labeled palmitate, changes in substrate supply did not alter the tissue kinetics of $[1-^{14}$C]acetate except after administration of acetate in nontracer amounts, equivalent to nonphysiologically high acetate serum levels. Similar results were published by Brown et al., who found biexponential $^{14}$C clearance from normoxic, perfused, isolated rabbit hearts and monoeponential clearance in ischemia after administration of $[1-^{14}$C] and $[1-^{14}$C]acetate. Rate constants for myocardial $[1-^{14}$C]acetate clearance correlated well with oxygen consumption.

Recent studies in our group with PET after intravenous injection of $[1-^{14}$C]acetate confirmed the close correlation between myocardial $^{14}$C tracer clearance and oxygen consumption in normal myocardium in intact animals. These findings were similar to those obtained by Brown et al with $[1^{14}$C]acetate and PET in closed-chest dogs. Furthermore, dynamic PET imaging in canine experiments revealed regional alterations of $^{14}$C washout in reperfused myocardium after intravenous $[1-^{14}$C]acetate administration, indicating suppression of oxidative metabolism in postischemic myocardium.

The present study was designed to assess $[1-^{14}$C]acetate clearance kinetics in the more controlled setting of the open-chest dog under various blood flow and metabolic conditions: at control, during ischemia, after reperfusion, and in dipyridamole-induced hyperemia. Intracoronary tracer injection was performed to obtain a primary tissue clearance curve and to minimize tracer recirculation. With a previously described method, the single-pass extraction fraction of $[1-^{14}$C]acetate was calculated from the recorded tissue-time activity curves. Invasive measurements of regional myocardial blood flow, oxygen, and substrate consumption were obtained to examine whether any of these parameters affect extraction fraction or $[1-^{14}$C]acetate tissue clearance kinetics. Data were acquired with a gamma probe to obtain better counting statistics at higher-resolution sampling rates than possible with PET. Furthermore, total $^{14}$C activity and $^{14}$CO$_2$ were measured in the coronary sinus or coronary vein effluent after intracoronary coinjection of $[1-^{14}$C]acetate to determine the extent to which washout of $^{14}$C activity after $[1-^{14}$C]acetate injection reflects CO$_2$ production and TCA cycle activity. The aim of this study was thus to define more accurately the tissue kinetics of $[1-^{14}$C]acetate as a tracer of myocardial oxidative metabolism and to delineate factors that might alter the tissue kinetics of this tracer.

Methods

Surgical Preparation

Thirty mongrel dogs, weighing 18.5–27 kg (mean weight, 21.3±2.2 kg), were anesthetized with thiamic-
Subsequently, a bolus of 80–150 μCi [1-13C]acetate, diluted with saline 0.9% to a volume of 0.2–0.3 ml, was loaded into the tubing attached to the LAD needle and then flushed rapidly into the LAD with 1 ml saline. Myocardial 13C activity was recorded with a lead shielded and collimated NaI (TI) scintillation detector. The diameter of the detector was 5.5 cm, and the opening of the lead shielding, which was 8 cm below the detector, was 7.5 cm in diameter and led to a field of view at the myocardium of approximately 11 cm in diameter. The field of detection encompassed the myocardium supplied by the LAD, but it excluded the site of tracer administration. Activity counting was performed in 100-msec intervals for a period of 40 minutes, the data being transferred to a digital computer (Microwav, Digital Equipment Corp, Maynard, Massachusetts). Subsequently, one or two injections were repeated at intervals of at least 1 hour, allowing background activity to return to levels less than 5% of peak activity because of the short physical (20.4 minutes) and biological half-life of [1-13C]acetate.

Study Subgroups

Group 1. Eleven experiments were performed in nine anesthetized dogs without any kind of intervention. To extend the range of cardiac work and thus myocardial oxygen consumption, atrial pacing with frequencies between 160 and 220 beats/min or infusion of methoxamine hydrochloride 20 mg i.v. (Vasoxyl, Burroughs Wellcome, Research Triangle Park, North Carolina) were performed in four studies in four dogs. In another 11 experiments in four dogs, dobutamine (Dobutrex, Eli Lilly Industries, Indianapolis, Indiana) was infused intravenously at a rate of 5–10 μg/kg/min. Cardiac workload was reduced in three experiments in three dogs by intramuscular administration of morphine (30–60 mg) and intravenous propranolol (2–3 mg). These group 1 studies with no interventions other than changes in cardiac workload, which were maintained throughout the study period, will subsequently be referred to as the control group.

Group 2. To evaluate selectively the effect of myocardial blood flow on acetate kinetics, intravenous dipryridamole (0.15 mg/min/kg; persantine, Boehringer Ingelheim Pharmaceuticals, Ridgefield, Connecticut) was infused continuously throughout the experiments in 13 studies in six dogs, thus uncoupling myocardial blood flow and MV02. The signal from the electromagnetic flow probe indicated steady-state flow conditions throughout all experiments with a mean of 9.0±7.1% for the entire study period.

Group 3. The validity of [1-13C]acetate as a tracer of oxidative metabolism in myocardial ischemia has not yet been explored in situ in large animals. Therefore, [1-13C]acetate kinetics were evaluated in 13 studies in six dogs after partial occlusion of the proximal LAD. Reductions in myocardial blood flow in the affected myocardial regions were verified by microsphere measurements. Flow in the ischemic segments was reduced to 33.1±19.1% compared with the surrounding normally perfused myocardium. When several studies were performed in one dog, the partial coronary occlusion was maintained during all experiments.

Group 4. To determine the usefulness of [1-13C] acetate to measure MV02 in the setting of reperfusion, the tissue kinetics of [1-13C]acetate were evaluated in eight studies in three dogs after release of LAD occlusion. Serial acetate injections were performed after one 30-minute occlusion period. Heart rate and systemic blood pressure remained stable throughout each experiment.

To assess the impact of sudden changes in cardiac workload on acetate clearance, cardiac pacing was initiated after 7 minutes at a heart rate of 200 beats/min in one experiment. In another experiment, [1-13C]acetate was injected during pacing, and cardiac workload decreased suddenly by cessation of pacing and return to sinus rhythm after 6 minutes.

Before the dogs were killed, lissamine green was injected into the LAD in all experiments to delineate the myocardium supplied by the LAD. The dogs were killed by injection of pentobarbital and highly concentrated potassium chloride solution, and the hearts were excised rapidly. Last, tissue samples were obtained for microsphere activity measurements, with a well counter (Auto gamma well counter Micrad, Inc, Harriman, Tennessee) to determine myocardial blood flow values after weighing the samples.

[1-13C]Acetate Synthesis

[1-13C]Acetate was obtained by a modified version5 of the procedure initially described by Pike et al.2

Data Processing

The recorded time activity data were transferred to a VAX 780 computer (Digital Equipment Corp) and corrected for physical decay of 13C. Tissue-time activity curves were subsequently generated in different time resolution modes (Figure 1A and B) and fitted with a multieponential curve-fitting routine according to the following equation:

\[ y(x) = ae^{-k_1} + ce^{-k_2} \]  

(1)

In all experiments, a delay in 13C washout preceded the biexponential clearance of 13C activity and resulted in a characteristic hump on the clearance phase, subsequently called "hump phase" (Figure 1A). This hump phase was not accounted for by the biexponential curve fitting and needed separate fitting.

Fitting was initiated at the end of the delay in 13C washout (hump phase, Figure 1A), myocardial clearance thereafter following a biexponential pattern in all experiments. Parameters k1 and k2 in Equation 1 define the rate constants (k=ln(2/T1/2)) for the two myocardial clearance components. Separate rate constants (kinit) for the initial delay in 13C clearance were obtained by fitting only the first 160 seconds of
the myocardial tissue-time activity curve with a higher (1 point/sec) time resolution display (Figure 1B). First-pass extraction fraction of [1-13C]acetate, that is, the fraction of activity retained in the myocardium after one passage of the tracer through the coronary vascular bed, was calculated by extrapolating the initial part (hump phase) of the myocardial clearance curve to the time of maximum activity. The activity value hereby obtained (B) is given as percentage of the maximum activity (A), which is proportional to the entire amount of the injected activity:

\[ E(\%) = 100 \times \frac{B}{A} \]  

The relative amount of 13C activity entering each of the two major clearance phases was determined by extrapolating the monoexponential curve fit of each component back to the time of maximum activity (Figure 1A).

**Assessment of Myocardial CO2 Production With [1-14C]Acetate**

In 24 of the experiments (groups 1–4), 20 μCi [1-14C]acetate was injected into the LAD with [1-13C] acetate. Two-milliliter blood samples were taken simultaneously from the coronary sinus, or a coronary vein draining the myocardium in the region of intervention supplied by the LAD, and an arterial line. Sampling was initiated 1–2 minutes after injection, depending on ease of blood withdrawal, and continued at 1-minute intervals up to 10 minutes after [1-14C]acetate injection and at 3-minute intervals for the next 15 minutes. Total 14C activity and 14CO2 activity in each of the 2-ml blood samples were measured by the method described by Buffington et al.15 The samples were transferred to a flask containing a small plastic vial with phenylethylamine (0.3 ml) and acidified with 1 ml of 6N HCl. The 14CO2 released by this procedure was trapped in phenylethylamine and quantified by liquid scintillation counting. The remaining 14C activity in the flask, representing non-CO2 14C, was also determined by liquid scintillation counting of an aliquot. Arteriovenous differences for non-CO2 14C and 14CO2 were calculated, and the contribution of 14CO2 to total 14C activity released from the myocardium was determined. The 14CO2 clearance from the myocardium was plotted against time, and multiexponential curve fitting was performed with a VAX 780 computer.

**Measurement of Myocardial Blood Flow**

Myocardial blood flow was determined by injection of radiolabeled microspheres into the left atrium and by the arterial reference sampling technique.14 Approximately 2×10^5 microspheres (diameter, 16.5±1 mm) labeled with 113Sn, 103Ru, 95Nb, 55Co, or 46Sc (NEN-TRAC microspheres, Du Pont, North Billerica, Massachusetts) were injected into the left atrium. Arterial blood was withdrawn with a Harvard infusion pump at a rate of 7.8 ml/min for 2 minutes. Blood and tissue sample activities were counted in a NaI (TI) well counter after the end of the experiment. Regional myocardial blood flow in the myocardial segments supplied by the LAD was estimated from the area of anterior wall delineated by lissamine green staining.

**Metabolic Measurements**

Hemoglobin, hematocrit, blood gases, and serum levels of glucose, lactate, and free fatty acids were determined twice, once at the beginning and at the end of each experiment. Serum blood gases, glucose, lactate, and free fatty acid levels were measured as described previously.5 Myocardial oxygen, glucose, lactate, and free fatty acid consumptions were subsequently calculated from the measured arteriovenous differences, hematocrit levels and myocardial blood flow determined with microspheres and with the mean of the two determinations.

**Statistical Analysis**

Values are mean±SD. Comparison of results between experimental and control groups was performed by analysis of variance (ANOVA), followed by Dunnett's t test16 if an F test on the ANOVA indicated significant intergroup differences. Comparisons between two groups only were made by paired or unpaired t test as appropriate. Differences with a p value less than 0.05 were considered significant. Regression analysis was assumed to be sufficiently rigorous to make negligible the technical violation of

**Table I. Hemodynamic Parameters for Various Study Groups**

<table>
<thead>
<tr>
<th>Condition</th>
<th>n</th>
<th>Heart rate (beats/min)</th>
<th>Systolic pressure (mm Hg)</th>
<th>Rate-pressure product (10^3 beats/mm Hg/min)</th>
<th>Flow (ml/min/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No intervention</td>
<td>11</td>
<td>148±24</td>
<td>130±25</td>
<td>20.6±5.1</td>
<td>100±39</td>
</tr>
<tr>
<td>Pacing with methoxamine</td>
<td>4</td>
<td>168±56</td>
<td>143±30</td>
<td>23.7±8.0</td>
<td>91±22</td>
</tr>
<tr>
<td>Morphine with propranolol</td>
<td>3</td>
<td>117±25</td>
<td>105±5</td>
<td>12.3±2.7</td>
<td>76±19.0</td>
</tr>
<tr>
<td>Dobutamine</td>
<td>11</td>
<td>195±43†</td>
<td>180±39†</td>
<td>34.6±10.2†</td>
<td>161±67*</td>
</tr>
<tr>
<td>Dipyridamole</td>
<td>13</td>
<td>145±18</td>
<td>117±14</td>
<td>16.8±24</td>
<td>447±91†</td>
</tr>
<tr>
<td>Ischemia</td>
<td>13</td>
<td>148±22.4</td>
<td>130±208</td>
<td>19.1±3.2</td>
<td>41±14†</td>
</tr>
<tr>
<td>Reperfusion</td>
<td>8</td>
<td>150±35</td>
<td>103±33*</td>
<td>15.8±8.3</td>
<td>56±31</td>
</tr>
</tbody>
</table>

Values are mean±SD; n, number of experiments. *p<0.05 and †p<0.01 vs. nonintervention group.
independence due to performing two or three repeated experiments in the same animal.

**Results**

**Hemodynamic and Metabolic Parameters**

Hemodynamic parameters for the experimental groups are indicated in Table 1. Dobutamine was found to be more effective than methoxamine and pacing, allowing stable increases in heart rate, systolic blood pressure, and rate-pressure product to be achieved. Although the pacing with methoxamine and morphine with propranolol groups did not produce significant hemodynamic changes by unpaired comparison with the nontreated group, intradog comparisons showed that pacing and methoxamine increased the rate-pressure product significantly compared with control conditions (p<0.03 by paired t test). Similarly, by paired t test, morphine and propranolol reduced the rate-pressure product significantly compared with control conditions (p<0.001). Myocardial blood flow was increased significantly by dobutamine and by dipyridamole and was decreased in ischemia compared with flow in studies without intervention. Regional myocardial blood flow in ischemic segments averaged 33.1±19.1% of flow in the surrounding nonischemic tissue (40.5±14.4 ml/100 g/min in the ischemic LAD territories and 135.7±40.9 ml/100 g/min in control myocardium). Systolic pressure tended to be lower in reperfusion experiments; the decrease in myocardial blood flow for all reperfusion experiments did not reach statistical significance.

Metabolic parameters are shown in Table 2. The percentage of oxygen equivalents that could be accounted for by free fatty acid consumption was quite low, less than 40%, probably reflecting the arterial plasma concentration of fatty acids, which was also low. The exception to this were the dobutamine experiments, where the arterial plasma free fatty acid concentration, free fatty acid consumption, and percentage of oxygen equivalents consumed as free fatty acid were all increased compared with the experiments without intervention (baseline). Lactate consumption was decreased in ischemia and reperfusion, and net lactate production resulted in eight of the ischemic studies and one reperfusion study. The total percentage of oxygen equivalents consumed as free fatty acid and carbohydrate was not significantly different from 100% except for the percentage in the dipyridamole group, in which substrate uptake accounted for 190±109% of oxygen equivalents consumed because of a large significant increase in glucose uptake. An explanation for this effect might be increased glycogen storage during vasodilator treatment.

**Myocardial 11C Tissue-Time Activity Curves After Intracoronary [1-11C]Acetate Injection**

Figure 1 depicts a representative 11C time-activity curve in different time resolution modes after intracoronary [1-11C]acetate injection. Figure 1B (temporal resolution 1 point/sec) demonstrates peak activity at 5 seconds (A), representing the entire amount of [1-11C] injected. Subsequently, a sharp decline in activity occurs, because of clearing of nonextracted [1-11C]acetate from the myocardial vascular bed. This phase is therefore subsequently referred to as vascular phase. The activity present after the end of the vascular component represents [1-11C] acetate retained by the myocardium. This activity clears from the myocardium in a biexponential pattern (Figure 1) with a rapid (short T1/2) first and a slow (long T1/2) second phase. Before the rapid clearance phase, there is a delay of approximately 200 seconds in myocardial 11C washout before phase 1 reaches steady state. This hump phase is believed to represent equilibrium of [1-11C]acetate and its metabolites with intracellular pools. The rate constant (k1init) for this phase ranged from 0.03 to 0.33/min for all study subgroups.

Rate constants k1 and k2 for the two clearance phases were determined by biexponential fitting of time-activity curves; k1 ranged from 0.06 to 0.69/min, whereas k2 ranged from 0.0004 to 0.02/min for all...
A study subgroups. After injection of $^{14}$C acetate, $^{14}$CO$_2$ accounted for 97.4±2.5% of total myocardial $^{14}$C efflux in the control group. Thus, the rapid phase of tracer clearance represented almost exclusively direct oxidation of [1-$^{14}$C]acetate.

The [1-$^{14}$C]acetate clearance slope was determined not only by the cardiac workload at the time of tracer injection but also by cardiac work throughout each study. An increase in cardiac workload during one experiment by sudden onset of cardiac pacing led to a steepening of the clearance curve (Figure 2). Conversely, starting an experiment with pacing and then ceasing electrical stimulation of the heart decreased the steepness of the slope markedly (results not shown).

Figure 1. Panel A: Myocardial tissue-time activity curve after intracoronary [1-$^{14}$C]acetate injection. Rate constants $k_1$ and $k_2$ were calculated as the slopes of the two clearance phases. In addition, a monoexponential rate constant, $k_{\text{mono}}$, was determined from the initial linear part of the clearance curve (temporal resolution, 1 point/15 sec). Panel B: Plot of temporal high-resolution display of the first 160 seconds. Method used for extraction fraction measurement (temporal resolution 1 point/sec) is also shown. Cts, counts; $E$, single-pass extraction fraction; $B$, activity extrapolated from the initial part of the myocardial clearance curve; $A$, maximum activity.

[1-$^{14}$C]Acetate Extraction Fraction

The myocardial [1-$^{14}$C]acetate extraction fraction was determined for each experimental group (Figure 3A). Extraction fraction was 64.2±9.7% for the 29 control experiments (group 1). In the control group, an inverse relation between flow and extraction fraction was observed ($y=76.5-0.10x$, $r=0.52$, $p<0.005$). No significant alterations in acetate extraction were found in ischemia experiments (group 3) (65.3±9.1%, $n=13$) or reperfusion experiments (group 4) (70±4.4%, $n=8$) (Figure 3A). Myocardial blood flow and MVO$_2$ were linearly related in the control studies; therefore, to dissociate the effects of blood flow and oxygen consumption on acetate extraction, we uncoupled myocardial blood flow and MVO$_2$ in 13 experiments in six dogs by intravenous dipyridamole infusion (group 2). Dipyridamole increased myocardial blood flow to 482±78 ml/100 g/min, whereas MVO$_2$ was only 367±54 μmol/100 g/min. Figure 3 shows that [1-$^{14}$C]acetate extraction was significantly reduced in group 2 (dipyridamole infusion). When the dipyridamole experiments were compared with 10 control experiments with a similar range of MVO$_2$ (287-421 μmol/100 g/min; mean, 367±45 μmol/100 g/min), the difference between control (65.3±9.1%; $n=10$) and dipyridamole (46.5±7.4%, $n=13$) extraction was also significant ($p<0.001$), confirming that the extraction fraction is decreased in dipyridamole-induced hyperemia. The inverse relation between acetate extraction and myocardial blood flow in control and dipyridamole experiments is indicated in Figure 3B. An inverse relation was found with a simple linear fit to the data (extraction fraction $=70.5-0.0048x$, $r=0.66$, $p<0.001$) or by fitting to the Renkin-Crone equation (extraction fraction $=100-58e^{-0.44MBF}$, $r=0.59$, $p<0.001$). No significant correlations between myocardial substrate consumptions and extraction fraction were found in these experiments.

Relation Between Myocardial $^{14}$C Clearance and Metabolism

The rate constant $k_1$ for the rapid phase was found to be linearly related to MVO$_2$ throughout a wide range of cardiac workload in 29 control experiments (Figure 4), in agreement with results obtained in vitro and in vivo throughout a less extensive range of oxygen consumption. For ischemia and reperfusion experiments, significant linear relations were found between $k_1$ and MVO$_2$; correlation coefficients of 0.68 ($p<0.02$) and 0.76 ($p<0.025$) were obtained in ischemic and reperfused myocardium, respectively. For dipyridamole experiments, the relation between $k_1$ and MVO$_2$ was not significant ($r=0.32$); however, this probably reflects the very small range of MVO$_2$ (309-479 μmol/100 g/min) encompassed by this group.

In addition to biexponential fitting, tissue-time activity curves were also fitted monoexponentially, with a short linear section of the rapid phase (Figure 1A). A linear relation ($p<0.001$) between $k_{\text{mono}}$ and
Figure 2. Plot of effect of abrupt changes in cardiac workload on the myocardial tissue-time activity curve. Clearance curve of the experiment started at baseline conditions; pacing at a heart rate of 200 beats/min began 7 minutes after [1-1C]acetate injection. Cts, counts.

Figure 3. Panel A: Bar graph of first-pass extraction fraction at control and after various interventions. Panel B: Plot of extraction fraction in dipyridamole experiments compared with control experiments.

Figure 4. Regression plot of correlation between rate constants for the rapid [1-1C]acetate clearance phase ($k_1$) and $MVO_2$ in all study subgroups.

Figure 5. Regression plot of correlation of the monoexponential rate constant ($k_{mono}$) of the rapid linear clearance phase and $MVO_2$. 
myocardial oxygen consumption was obtained for all experiments (Figure 5); significant correlations were also obtained for control (r=0.95, p<0.001), ischemia (r=0.80, p<0.002), and reperfusion experiments (r=0.79, p<0.02).

To examine the relation between k1 and MVO2 more closely, the k1/MVO2 ratio was determined and compared between the various groups. Because of the linear relation between k1 and MVO2, and because the regression line goes practically through zero, one would expect that the k1/MVO2 ratio should remain constant for each study if this relation was not offset by metabolic or interventional factors. Within each group, there were no significant differences in the k1/MVO2 ratio between high and low MVO2 experiments. Figure 6 demonstrates that, although similar to control in hyperemia and reperfusion, the k1/MVO2 ratio was increased in the ischemia group (p<0.05). A similar increase in the k Mono/MVO2 ratio in ischemia compared with the control group was also observed (p<0.05). When the ischemia experiments were compared to only those control experiments with MVO2 less than 450 μmol/100 g/min, there was still a tendency for k1/MVO2 and k Mono/MVO2 to be higher in the ischemia than in the control group; however, that trend was no longer significant. This may reflect the smaller number of experiments in the control group with MVO2 less than 10 ml/100 g/min (n=12). There was no significant trend to higher k/MVO2 ratios with lower MVO2 in the control group that might have explained this phenomenon.

Because MVO2 was linearly related to blood flow, the linear relation between 11C clearance after acetate injection and MVO2 could theoretically also be influenced by effects of flow on clearance kinetics. To determine the effect of flow changes on k1 independent of changes in work load, the experiments in which flow and MVO2 were uncoupled with dipyridamole were compared with control experiments at similar MVO2. In the 13 dipyridamole experiments, k1/MVO2 was 0.021±0.003 compared with 0.019±0.004 in the 10 control experiments, showing no significant difference despite a sixfold increase in flow with dipyridamole (482±78 vs. 78±12 ml/100 g/min, p<0.001).

The dipyridamole experiments (group 2) demonstrated that k1 was determined by MVO2 and not by myocardial blood flow; in experiments in which MVO2 was not uncoupled from myocardial blood flow (control group), k1 was found to correlate (p<0.001) with myocardial blood flow (Figure 7), reflecting the close relation between blood flow and MVO2 in normal myocardium. In contrast, with dipyridamole, the relation of k1 to flow was abolished.

To determine the effects of myocardial substrate selection on acetate kinetics, the k1/MVO2 ratio was plotted as a function of plasma substrate levels and myocardial substrate utilization, expressed as a percentage of MVO2. By comparison with the control dogs only, weak but significant correlations were found with arterial lactate concentration (y=0.00058x+0.36, r=0.45, p<0.02) and myocardial free fatty acid consumption (y=0.47–0.00012x, r=0.40, p<0.03). No other significant substrate effects were found.

Because the rapid 11C clearance phase represents direct oxidation of [1-11C]acetate, the relative amount

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**Armbrecht [1-11C]Acetate As a Tracer for MVO2**

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**FIGURE 6.** Bar graph of the k1/MVO2 ratio for all study subgroups.

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**FIGURE 7.** Regression plot of correlation of rate constants for the first rapid clearance phase (k1) and myocardial blood flow.
of \(^{11}\)C activity entering the rapid phase might be influenced by MVO\(_2\). For the control dogs, there was a significant relation between the relative size of the rapid phase and MVO\(_2\) (\(r=0.55, p<0.002\)). A significant relation was also found when all experimental groups were considered (\(r=0.46, p<0.001\)). Conversely, the relative amount of tracer entering the slower clearance phase was inversely correlated to MVO\(_2\) (\(p<0.003\)). The slope \(k_2\) of the second clearance phase ranged from 0.0004 to 0.02/min and was not found to be related significantly to MVO\(_2\) or metabolic parameters. Of note, accurate determination of \(k_2\) is hindered by low residual myocardial activity after 10–20 minutes and by interference of radioactivity from previous microsphere injections with the low count rate.

Rate constants obtained exclusively for the initial hump phase (\(k_{\text{init}}\)) also correlated well with MVO\(_2\) in 29 control experiments (\(r=0.90\), Figure 8A). Furthermore, there was a close correlation between \(k_{\text{init}}\) and the maximum of myocardial CO\(_2\) production after injection of \([1-^{14}]\)acetate (Figure 8B, \(r=0.94\)).

Clearance of \(^{14}\)CO\(_2\) and Total \(^{14}\)C After Simultaneous Intracoronary Injection of \([1-^{11}]\)C and \([1-^{14}]\)C Acetate

Measurement of the arteriovenous \(^{14}\)CO\(_2\) and \(^{14}\)C differences across the LAD perfusion bed after simultaneous intracoronary injection of \([1-^{11}]\)C and \([1-^{14}]\)Cacetate demonstrated biexponential clearance of \(^{14}\)C activity, similar to the externally detected \(^{14}\)C myocardial tissue-time activity curves. The contribution of \(^{14}\)CO\(_2\) to total effluent \(^{14}\)C activity between 5 and 15 minutes after injection is summarized in Figure 9. This period was chosen because the major fitting parameters are determined within this time frame. After injection of \([1-^{11}]\)Cacetate, \(^{14}\)CO\(_2\) accounted for 97.4±2.5% of total myocardial \(^{14}\)C efflux in the control group, 89.1±2.6% in ischemia (\(p<0.002\) vs. control group), 94.1±3.5% after reperfusion (\(p=\text{NS}\) vs. control group) and more than 99% with dipyridamole (\(p=\text{NS}\) vs. control group). Of note, at high myocardial blood flows (with dipyridamole and dobutamine), the arteriovenous difference for non-CO\(_2\) \(^{14}\)C activity was very small, precluding accurate measurement. In the first measurements 1–2 minutes after injection of \([1-^{14}]\)Cacetate (mean, 1.8±1.0 minutes), the amount of non-CO\(_2\) \(^{14}\)C activity was considerably higher (4.8±6.4% in control, 50±12% in ischemia, and 26±23% in reperfusion groups; negligible in the dipyridamole and dobutamine groups). When biexponential fitting was

**FIGURE 8.** Panel A: Regression plot of the rate constant for the hump phase \(k_{\text{init}}\) and MVO\(_2\). Panel B: Plot of correlation of the hump phase \(k_{\text{init}}\) and time of maximum of CO\(_2\) efflux from myocardium after \([1-^{14}]\)acetate injection.

**FIGURE 9.** Bar graph of contribution of \(^{14}\)Co\(_2\) to total \(^{14}\)C efflux from the myocardium after intracoronary injection of \([1-^{14}]\)acetate. Measurements were made between 5 and 15 minutes after injection.
used to determine the rate constant for $^{14}$CO$_2$ clearance from myocardium, it was found to correlate closely with $k_1$ determined noninvasively and simultaneously with $[1-^{14}$C]acetate (Figure 10). Visual inspection of the linear fit suggested a possible small deviation from linearity at low $k_1$; reanalysis of the data with a binominal fit also gave a good correlation ($r=0.98$) with a decreased residual sum of squares (0.02 vs. 0.023), but the improvement in fit did not reach statistical significance ($F$ test). When the control experiments were considered alone, a linear fit ($r=0.99$) was obtained.

**Discussion**

This study confirms the usefulness of $[1-^{14}$C]acetate as a tracer of oxygen consumption in myocardium not only during control conditions but also during ischemia, reperfusion, and dipyridamole-induced hyperemia. Experiments with $[1-^{14}$C]acetate demonstrate that the rapid acetate clearance phase represents nearly exclusively TCA cycle turnover of the tracer.

External measurement of $[1-^{14}$C]acetate kinetics after intracoronary tracer injection allows fast high-resolution recording of myocardial tissue-time activity curves. Tracer recirculation, which complicates studies after intravenous injections, is practically abolished with this approach. Thus, measurement of $[1-^{14}$C]acetate extraction fraction is possible in this study setup with a previously validated approach. By extrapolation of the myocardial clearance curve back to the time of maximum activity and by division of this value by the maximum activity, a fraction of the tracer retained in the myocardium during a single passage through the coronary vascular bed was calculated. This value was defined as the single-pass extraction fraction. Of note, this value differs from the steady-state extraction fraction, which represents forward and reverse transport of substrate. In the control dogs, there was an inverse relation between myocardial blood flow and extraction fraction, a finding that was confirmed throughout a range of extended flow by infusion of dipyridamole. However, there was considerable variability in the extraction fraction, particularly at normal physiological myocardial blood flow rates. Myocardial oxygen and substrate consumption did not have an impact on the extraction of $[1-^{14}$C]acetate. Extraction fraction did not increase with a decrease in myocardial blood flow in ischemia compared with control conditions (65.3±9.1% vs. 64.2±9.7%), although this would be expected from the inverse relation between extraction fraction and myocardial blood flow. Elevated intracellular concentrations of acetyl coenzyme A (CoA) in ischemia might decrease cellular uptake of acetate, which is thought to be driven mainly by diffusion. Another possible explanation for this finding is increased back diffusion, which might lead to an underestimation of true unidirectional single-pass extraction fraction with the method used.

Once acetate has been esterified to acetyl-CoA, most of it undergoes oxidation in the TCA cycle to CO$_2$. Clearance of $[1-^{14}$C]acetate was biexponential in all experiments. A delay of $^{14}$C washout from the myocardium before biexponential tissue clearance begins was consistently seen in all experiments and was termed the hump phase. The effect may be due to two different mechanisms. First, equilibration of label into TCA cycle intermediates may require several minutes, depending on the speed of TCA cycle turnover. Randle et al. demonstrated in rat hearts that labeling of the small acetyl-CoA pool requires only about 10 seconds, whereas the labeling of all the TCA cycle intermediates required several minutes in most instances. This hypothesis would be consistent with our findings that the rate constant for the hump phase ($k_{init}$) correlated with $\text{MV}O_2$ and that $k_{init}$ was also inversely correlated with the time to peak myocardial CO$_2$ production. Thus, with higher $\text{MV}O_2$ and TCA cycle turnover, labeling of the TCA cycle intermediates would be expected to take place faster, leading to an earlier production of $^{14}$C-labeled CO$_2$. A second possible pool represented by the hump phase might be a hypothetical CO$_2$ pool, through which the $^{14}$C-labeled CO$_2$ must pass before maximum $^{14}$C clearance can begin. A similar effect was also found in earlier studies with palmitic acid, which could also reflect the two aforementioned mechanisms.

The experiments with simultaneous injection of $[^{14}$C]acetate revealed that clearance of radioactivity from myocardium after injection of labeled acetate was almost exclusively in the form of labeled CO$_2$, which is in agreement with results obtained in vitro in isolated, perfused hearts. In contrast to results obtained with $[1-^{14}$C]palmitate, in which ischemia leads to large increases in back diffusion of nonmetabolized $[1-^{14}$C]palmitate, we observed with $[1-^{14}$C]acetate only a small increase in efflux of non-CO$_2$ tracer in ischemia to approximately 10% of total activity leaving myocardium during the period of curve fitting. Thus, the rate constant $k_1$ determined externally with $^{11}$C correlated well with the rate constant for clearance of $^{14}$CO$_2$ measured in blood.
The possible slight trend toward nonlinearity at low values of \(k_1\) may reflect the small increase in non-CO\(_2\) activity in ischemia.

Further evidence that recirculation does not play a major role in \([1-^{13}C]\)acetate kinetics is the fact that slopes for the relation between \(k_1\) and MVo\(_2\) were nearly identical after intracoronary injection in this study (\(y=0.005+0.00041x\)) and after intravenous tracer injection in two recent studies with \(^{13}\)C acetate and PET (Buxton et al; \(y=0.0393+0.00035x\); Brown et al; \(y=0.02+0.00039x\)). Thus, \([1-^{13}C]\)acetate washout represented myocardial CO\(_2\) production and thus TCA cycle turnover in the same way after intracoronary and intravenous injections.

Clearance kinetics after intracoronary \([1-^{13}C]\)acetate injection are also largely insensitive to changes in myocardial substrate supply and selection. Because the proportion of oxygen consumed by the TCA cycle oxidations is similar for all major substrates (2% for glucose and lactate, 19% for palmitate, and 19% for oleate), this result is not unexpected. In the control dogs, an increase in the percentage of oxygen equivalents accounted for by fatty acid uptake was accompanied by a small decrease in the \(k/MV_{O2}\) ratio. Similar results were obtained in closed-chest dogs with noninvasive measurement of acetate tissue kinetics by PET. Several studies have demonstrated increased oxygen consumption with increasing free fatty acid utilization despite constant hemodynamic parameters, although others have been unable to reproduce this "oxygen wasting" effect. A decrease in myocardial efficiency when utilizing free fatty acid leading to a higher MV\(_{O2}\) for a given cardiac workload and TCA cycle turnover would account for the changes in the \(k/MV_{O2}\) ratio observed in this study. The quantitative impact of free fatty acid metabolism on the \(k/MV_{O2}\) ratio is small; however, a shift in fatty acid consumption from 25% to 75% of MV\(_{O2}\) would lead to a decrease in the \(k/MV_{O2}\) ratio of about 12%. Because high arterial lactate levels inhibit myocardial free fatty acid oxidation, the effects of high arterial lactate in increasing the \(k/MV_{O2}\) ratio might be attributable to this mechanism. However, arterial lactate levels did not correlate significantly with free fatty acid consumption, expressed as a percentage of myocardial oxygen consumption in this study (\(r=0.21,\) NS), and hence, the mechanism of action of lactate is unclear. An increase in arterial lactate concentration from low (0.5 mM) to high (1.4 mM) resting physiological plasma levels would increase the \(k/MV_{O2}\) ratio by about 20%. The effect that high concentrations of lactate resulting from exercise would have on acetate kinetics is unknown. However, in another study testing \([1-^{13}C]\)acetate with PET in human myocardium, no such dependence of \([1^{13}C]\) acetate washout on substrate levels was found.

The relation between \(k_1\) (and \(k_{\text{mono}}\)) and MV\(_{O2}\) was unaltered by dipyridamole-induced hyperemia and in myocardium reperfused after a 30-minute occlusion of the LAD. However, in ischemic myocardium, an increase of approximately 15–20% was observed in the \(k/MV_{O2}\) ratio. One possible mechanism contributing to this increase would be the small increase in non-CO\(_2\) tracer material observed in ischemia, which may wash out more rapidly than the labeled CO\(_2\). Another contributory factor might be increased carbohydrate utilization in ischemic myocardium, which has been shown to increase the \(k/MV_{O2}\) ratio. Mean carbohydrate utilization (as a percentage of oxygen equivalents) was greater in the ischemia than in the control group (72±15% vs. 51±28%), although the difference was not significant. It should also be noted that determination of MV\(_{O2}\) in the ischemic zone would be expected to be less accurate than for the other groups, because inhomogeneity of flow and oxygen consumption are likely to occur and because difficulties in determination of the LAD perfusion bed may also introduce errors. Increased collateral flow is also likely in ischemia, which would lead to an underestimation of MV\(_{O2}\) and hence an overestimation of the \(k/MV_{O2}\) ratio. Such errors may also contribute to the larger standard deviation of the \(k/MV_{O2}\) ratio for the ischemic group. Thus, systematic errors in determination of MV\(_{O2}\) could contribute to the apparent change in the \(k/MV_{O2}\) ratio.

Accurate biexponential fitting of \([1-^{13}C]\)acetate tissue-time activity curves requires relatively long acquisition times. Because the rate-pressure product is generally lower in humans than in anesthetized dogs, this situation will be exacerbated, particularly in ischemic segments where MV\(_{O2}\) is reduced. With the short (20-minute) physical half-life of \(^{13}\)C, obtaining adequate counting statistics at later times may become limiting. In addition, long acquisition times are undesirable, particularly in critically ill patients. Because changes in myocardial work load affect the acetate clearance kinetics, MV\(_{O2}\) must also be maintained constant throughout the study. The demonstration that \(k_{\text{mono}}\) which was obtained by monoeponential fitting of the initial linear portion of the clearance curve, traces myocardial oxidative metabolism as well as \(k_1\) suggests that short acquisitions with monoeponential fitting of tissue-time activity curves will be of value in patient studies.

Results obtained in vitro and in vivo support the hypothesis that the rapid phase of acetate clearance represents direct oxidation of labeled acetate by the TCA cycle. The fraction of the injected amount of \([1-^{13}C]\)acetate entering the rapid clearance phase with rising MV\(_{O2}\), demonstrating that increased workload leads to a greater fraction of acetate undergoing direct oxidation. The slower phase has not been characterized but is likely to represent entry of label into amino acid pools in equilibrium with TCA cycle intermediates. The major alternative to direct oxidation of acetate has been shown to be conversion to glutamate (by transamination of 2-oxoglutarate) and to aspartate (by transamination of oxaloacetate). The results are thus consistent with elevated TCA cycle flux that causes increased competition for tracer between the TCA cycle reactions and transamination reactions,
resulting in less activity entering the amino acid pools.

One limitation of the present study is the use of anesthetized dogs and consequent changes in metabolic and hormonal regulatory mechanisms. Although substrate utilization and catecholamine stimulation appear to have minimal effects on the relation between acetate kinetics and \( \text{MVO}_2 \), studies in conscious animals would be of merit to confirm that results are similar.

In conclusion, this study extends previous findings in vitro and in vivo by supporting the validity of \([1-^{13}C]\)acetate for the noninvasive measurement of regional myocardial oxygen consumption in vivo with PET. The relation between rate constants for the early rapid phase of tracer clearance after \([1-^{13}C]\)acetate administration and \( \text{MVO}_2 \) was unchanged in hyperemic and posts ischemic myocardium, whereas only a small increase in the \( k_{\text{r}}/\text{MVO}_2 \) ratio was observed in ischemia. Myocardial fuel supply and selection have only minimal effects on \([1-^{13}C]\)acetate kinetics. Quantitative measurement of regional \( \text{MVO}_2 \) with \([1-^{13}C]\)acetate and PET may be of value for assessing residual oxidative metabolism in jeopardized myocardium, which can assist in clinical decision making in patients with coronary artery disease.

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References


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