Validity of Estimates of Myocardial Oxidative Metabolism with Carbon-11 Acetate and Positron Emission Tomography Despite Altered Patterns of Substrate Utilization

Michael A. Brown, Donald W. Myears and Steven R. Bergmann
Cardiovascular Division, Washington University School of Medicine, St. Louis, Missouri

We recently demonstrated that the myocardial turnover rate constant (k) measured noninvasively with positron emission tomography (PET) after intravenous administration of [11C]acetate provides a reliable index of myocardial oxidative metabolism (MVO2) theoretically independent of the pattern of myocardial substrate use. However, because estimates of metabolism with other metabolic tracers are sensitive to substrate use, we measured k in 12 dogs during baseline conditions and again after infusion of either glucose (n = 8) or Intralipid (n = 4), interventions that raised arterial glucose or fatty acids by more than fivefold with concomitant changes in myocardial substrate use. Following glucose administration k increased, but no difference was detected after compensation for changes in hemodynamics and myocardial work induced by the infusion (0.18 ± 0.03 min⁻¹ (t½ = 3.9 min) at baseline compared with 0.22 ± 0.06 min⁻¹ (t½ = 3.2 min, p = N.S.), k was not affected by Intralipid infusion (k = 0.15 ± 0.06 min⁻¹ at baseline and 0.14 ± 0.04 min⁻¹ during infusion), and correlated closely with MVO2 measured directly (n = 19 comparisons, r = 0.89). The results indicate that estimates of MVO2 using [11C]acetate and PET are valid despite changes in the pattern of myocardial substrate utilization.


Noninvasive assessment of regional oxidative metabolism in patients with cardiac disease would be useful to define the natural history of cardiac dysfunction at a metabolic level and to evaluate its response to therapeutic interventions. We initially demonstrated in isolated rabbit hearts that carbon-11(11C) acetate is predominantly metabolized to labeled CO2 and that the externally detected rate of clearance reflects the rate of oxidation of [11C]acetate (1). Since oxidation of acetate occurs in the mitochondria via the tricarboxylic acid cycle, and since this cycle is tightly coupled to oxidative phosphorylation, clearance of [11C]CO2 from the myocardium reflecting oxidation of acetate correlates closely with overall myocardial oxygen consumption (MVO2) over a wide range of flow and metabolic states (1). We recently extended these observations to intact dogs in which the turnover rate constant of 11C radioactivity from the myocardium, measured using positron emission tomography, was shown to reflect oxidation of acetate to labeled CO2, and correlated with directly measured myocardial oxygen consumption over a wide range of metabolic states induced by sympathetic stimulation or blockade (2).

In the heart, oxidation of acetate is predominantly mitochondrial (3—4). Prior to mitochondrial oxidation, acetate is converted to acetyl-CoA by a synthase. Acetyl-CoA, on the other hand, is the final common pathway of multiple biochemical processes (including glycolysis, and lipid and amino acid catabolism among others) prior to oxidation in the mitochondria. Theoretically, oxidation of radiolabeled acetate should not be affected by the substrate serving as the source of acetyl-CoA if the size of the acetyl-CoA pool is small compared with its turnover rate and if pool size is not altered considerably in relation to turnover (valid assumptions based on previous studies (5—8)). Although radiolabeled acetate can be incorporated into lipids, amino acids, and ketones, as we and others have demonstrated, the mag-
plitude of these biochemical pathways appears to be minor under most circumstances (1,5—8), and thus does not appear to mitigate the use of this tracer for estimates of MVO₂ (1,2). Nonetheless, because altered patterns of myocardial substrate use induced by changes in plasma substrate concentrations profoundly influence estimates of myocardial metabolism by the other putative metabolic tracers [¹¹C]palmitate (9,10) and fluorine-18 fluorodeoxyglucose (11—13), and because during conditions such as ischemia and reperfusion the pattern of myocardial substrate use changes rapidly (14), the purpose of the present study was to examine the effects of alterations of myocardial substrate use on the rate of oxidation of [¹¹C]acetate and on estimates of myocardial oxygen consumption using positron emission tomography.

METHODS

Twelve mongrel dogs, weighing 20 to 31 kg, were premedicated after an overnight fast with subcutaneous morphine (1 mg/kg) and anesthetized with intravenous thiopental (12.5 mg/kg) and alpha-chloralose (60 mg/kg). Catheters were placed under fluoroscopic guidance into the coronary sinus for coronary venous sampling, and into the descending aorta and inferior vena cava. Aortic pressure and heart rate were monitored continuously. The rate-pressure product, an index of cardiac work, was calculated from the product of systolic blood pressure and heart rate.

Experimental Protocol

Assessment of oxidation of [¹¹C]acetate was attempted twice in each dog. Initial measurements were performed under baseline conditions and a second measurement was then made after either an intravenous infusion of glucose (n = 8) (50 g glucose, 100 U regular insulin and 20 mmol KCI in 100 ml sterile H₂O per hour) designed to raise plasma glucose and decrease plasma fatty acid concentrations, or after an intravenous infusion of Intralipid (n = 4) (0.6 mg lipid/kg/60 min of a 20% solution of neutral triglycerides consisting predominantly of linoleic, oleic, palmitic and linolenic acids, Kabivitrum, Inc., Alameda, CA) designed to raise plasma fatty acid concentrations. Since the metabolic effects of glucose or Intralipid infusion are prolonged, randomization of the order of baseline and infusion studies was not performed.

Each study consisted of an oxygen-15 (¹⁵O) labeled carbon monoxide (CO) scan to delineate blood pool, an ¹⁵O water (H₂O) scan to measure myocardial blood flow and [¹¹C]acetate scan. An interval of 10 min following ¹⁵O administration (t₀ = 2.1 min) and 100 min after ¹⁵C administration (t₀ = 20.4 min) was allowed for decay of the previously injected tracer prior to subsequent tracer administration. Infusion of either glucose or Intralipid was commenced following completion of the baseline study. After ~40 min of glucose or Intralipid infusion the [¹⁵O]CO and [¹⁵O]H₂O scans were repeated followed by repeat [¹¹C]acetate administration ~60 min after the start of the glucose or lipid infusion.

PET Procedure

Animals were placed in a Plexiglas shell designed to fit within the tomographic unit, PETT VI (15). The position of the apex of the heart was initially marked during fluoroscopy, and a low power laser subsequently used to position the animal at the appropriate level. Tissue attenuation of the thorax was measured with a ring source of the positron emitter germanium-68/gallium-68. The [¹⁵O]CO scan was obtained after administration of 50 mCi via the endotracheal tube, and data acquired for 5 min. After return of radioactivity to baseline, a bolus of 40 mCi of [¹⁵O]H₂O was administered, and data collected dynamically in 5 second frames for 90 sec following injection. For the purposes of image display only, [¹⁵O]H₂O images were reconstructed from a composite of 15—90 sec. Subsequently, a bolus of [¹¹C]acetate (~0.8 mCi/kg) was administered intravenously, and data acquired dynamically in 90 second frames for a total of 30 min.

For analysis, 2-3 midventricular tomographic slices were selected and a large region of interest was placed within the myocardium as defined by the [¹¹C]acetate scan. The same regions of interest were used for analysis of the [¹⁵O]H₂O data. An additional region of interest was placed in the center of the left ventricular cavity defined in the [¹⁵O]CO scan, allowing measurement of the ¹⁵C radioactivity content of left ventricular cavity blood. Data from the myocardial wall and blood pool were corrected for physical decay, partial volume and spillover effects as previously described (2,16) and results of individual regions were averaged. Cardiac dimensions were assumed to have a left ventricular cavity radius of 15 mm and wall thickness of 10 mm based on measurements obtained in postmortem studies from our laboratory and consistent with similar measurements by others (17). Clearance of ¹¹C radioactivity from myocardium was biexponential, and a multieponential curve fitting routine was used to calculate the parameters of the two phases. The half-time of each phase was calculated from t₀ = ln2/k, where k = turnover rate constant.

Myocardial blood flow in absolute terms was calculated using a one-compartment model as previously described (2,16,18).

Substrate Utilization

Aortic and coronary sinus blood samples were taken at the beginning, middle and end of each [¹¹C]acetate scan. Oxygen tension, oxygen saturation and hemoglobin were measured in each sample (Instrumentation Laboratory model 282 Co-oximeter, Waltham, MA) and oxygen content calculated for each sample. Oxygen extraction per ml of blood was calculated from the arteriovenous difference, and the mean of the three measurements per study calculated. Myocardial oxygen consumption (MVO₂) (µmol/g/min) was calculated from the product of flow and oxygen extraction.

Arterial and coronary venous samples obtained during each [¹¹C]acetate study were analyzed for fatty acid, glucose, lactate, and acetate. Substrate utilization was calculated from the product of blood flow and substrate extraction fraction.

Fatty acid in plasma was assayed with a colorimetric assay described previously (19). Lactate and glucose were assayed using commercially available enzymatic kits (Behring Diagnostics, La Jolla, CA), as was acetate (Boehringer Mannheim Biochemicals, Indianapolis, IN).

Synthesis of Radiotracers

Carbon-11 acetate was prepared from the reaction between [¹¹C]CO₂ produced in the Washington University Medical Center Cyclotron, and methylmagnesium bromide. Details of
Glucose infusion, presumably because of osmotic effects. Minor increases in heart rate and decreases in systolic blood pressure and rate pressure product were observed during glucose infusion. The turnover rate constant could not be determined in one control and one glucose infusion study because of computer failures; and directly measured MVO$_2$ could not be assessed in two dogs in which the coronary sinus catheter slipped out prior to tracer administration (suspected based on oxygen measurements and confirmed by angiography at the completion of the study).

RESULTS

Hemodynamics

Infusion of glucose tended to decrease heart rate, and increase blood pressure, blood flow and MVO$_2$, whereas infusion of Intralipid tended to increase heart rate and MVO$_2$ while decreasing blood pressure (Table 1). However, none of the changes were statistically significant.

Substrate Utilization

In the glucose group, a mean of 59 g of glucose were infused by the commencement of the second [¹³C]acetate study. At this time arterial glucose levels had increased fivefold and myocardial glucose extraction increased (Table 2). Fatty acid arterial concentration, myocardial fatty acid extraction and arteriovenous difference fell. Little difference was found in arterial levels or arteriovenous extraction of lactate or acetate during the glucose infusion.

PET Data

All Carbon-11 acetate images were of high quality (Figure 2). Clearance of $^{11}$C radioactivity from myocardium was biexponential in all studies, consisting of a major rapidly clearing phase and slowly clearing minor phase (Fig. 2). The calculated rate constant of the minor phase was essentially zero in all but three studies, indicating that over the period of measurement no clearance could be measured from this phase. The relative distribution of the $^{11}$C label was estimated from the relative size of the major and minor phases. No significant change was present with glucose infusion (baseline: major phase 82 ± 2%; post-glucose: 79 ± 4%) or with lipid infusion (baseline: 83 ± 1%; postlipid: 83 ± 1%).

After infusion of glucose, a mild increase in the rate constant of the major phase was found, indicating more rapid clearance of tracer (0.17 ± 0.06 to 0.21 ± 0.04 min$^{-1}$, p < 0.05; $t_0$ clearance of 4.4 ± 1.7 and 3.5 ± 0.8 min, p < 0.05). Clearance of this phase has previously been found to correlate closely with both oxygen consumption and rate-pressure product in an identical model over a wide range of cardiac workloads (2). After normalizing the turnover rate constant to a constant rate-pressure product of 20,000 mm Hg x bpm thereby correcting for the minor hemodynamic alterations induced by the glucose infusion, no significant differences between control and glucose studies was found (0.18 ± 0.03 compared to 0.22 ± 0.06 min$^{-1}$, $t_0$ = 3.9 ± 0.6 and 3.4 ± 0.8 min, respectively). Normalization of the turnover rate constant to a constant oxygen consumption of 4 μmol/g/min showed no significant difference between groups (0.15 ± 0.02 compared to 0.17 ± 0.03 min$^{-1}$). Comparison of the regression relationships between clearance rates and oxygen

| TABLE 1 |
| Effects of Glucose or Lipid Infusion on Hemodynamics, Myocardial Blood Flow (MBF) and Directly Measured Myocardial Oxygen Consumption (MVO$_2$) |
| Heart rate | Systolic blood pressure | Rate-pressure product | MBF | MVO$_2$' |
| Glucose study (n = 8) |
| Baseline | 126 ± 47 | 152 ± 20 | 19515 ± 7987 | 0.9 ± 0.4 | 4.4 ± 1.6 |
| Glucose infusion | 112 ± 39 | 177 ± 22 | 19928 ± 7351 | 1.2 ± 0.4 | 4.8 ± 1.5 |
| Intralipid study (n = 4) |
| Baseline | 122 ± 69 | 167 ± 10 | 18525 ± 9479 | 0.6 ± 0.2 | 3.7 ± 1.4 |
| Intralipid infusion | 132 ± 32 | 135 ± 58 | 16723 ± 6782 | 0.7 ± 0.2 | 4.3 ± 1.3 |

' Minor decreases in heart rate and increases in systolic blood pressure, rate pressure product, MBF, and MVO$_2$ were seen during glucose infusion, presumably because of osmotic effects. Minor increases in heart rate and decreases in systolic blood pressure and rate pressure product were observed during Intralipid infusion. No difference was statistically significant.

† MVO$_2$ was not able to be measured directly in two dogs in the glucose group because the coronary sinus catheter slipped out prior to the study.
TABLE 2
Effects of Glucose-Insulin-Potassium or Intralipid Infusion on Arterial Plasma Substrate Concentration (Art conc.), Myocardial Extraction Fraction and Arterial-Coronary Sinus Difference (A-V Δ)

<table>
<thead>
<tr>
<th></th>
<th>Glucose study (n = 6)</th>
<th>Intralipid study (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Art conc.</td>
<td>Extraction fraction (%)</td>
</tr>
<tr>
<td>Glucose (mM)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>5.4 ± 0.8</td>
<td>9.6 ± 10.4</td>
</tr>
<tr>
<td>Infusion</td>
<td>25.9 ± 6.7</td>
<td>3.1 ± 8.3</td>
</tr>
<tr>
<td>Fatty acid (μM)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>445 ± 182</td>
<td>42.0 ± 6.4</td>
</tr>
<tr>
<td>Infusion</td>
<td>202 ± 61†</td>
<td>17.4 ± 12.7†</td>
</tr>
<tr>
<td>Lactate (mM)</td>
<td></td>
<td></td>
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<tr>
<td>Baseline</td>
<td>1.9 ± 0.4</td>
<td>37.6 ± 19.7</td>
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<tr>
<td>Infusion</td>
<td>2.3 ± 0.7</td>
<td>44.0 ± 14.2</td>
</tr>
<tr>
<td>Acetate (μM)</td>
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<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>115 ± 25</td>
<td>18.0 ± 13.9</td>
</tr>
<tr>
<td>Infusion</td>
<td>124 ± 30</td>
<td>27.3 ± 20.5</td>
</tr>
</tbody>
</table>

† p < 0.05 compared with baseline.

consumption before and after glucose administration showed no significant difference (Figure 3).

Similar rate constants were found before and after Intralipid infusion (0.15 ± 0.06 compared to 0.14 ± 0.04 min⁻¹, p = N.S.). No statistical differences were observed after normalization for rate-pressure product or oxygen consumption. Comparison of the regression of clearance rate on myocardial oxygen consumption showed no significant difference between baseline and post lipid studies (Fig. 3).

As depicted by the relationship shown in Figure 3, the myocardial turnover rate constant correlated closely with directly measured myocardial oxygen consumption and with the rate-pressure product, an index of total cardiac work that reflects the energy demands of the heart.

DISCUSSION

The major factor effecting the clearance of 11C radioactivity after administration of [11C]acetate is independent of variations in the pattern of substrate utilization by the heart, and reflects overall myocardial oxygen consumption.

These findings contrast with those of [11C]palmitate (9,10). After a similar dose of glucose, the major rapid phase of myocardial clearance normally observed after [11C]palmitate administration under fasting conditions and thought to reflect beta-oxidation was either attenuated or was not detectable (9). Similar findings were reported in patients after oral glucose (10). This change in fatty acid metabolism has been considered to be due to inhibition of beta oxidation with preferential shunting of [11C]palmitate into neutral or phospholipid pools. Slow clearance of tracer subsequently occurs due to slow turnover of these pools. In addition, although rates of beta-oxidation can be estimated during normoxia with [11C]palmitate, during ischemia, backdiffusion of unmetabolized tracer results in overestimates of beta-oxidation (21). Nonetheless, shunting of tracer into neutral and phospholipids diminishes the turnover rate constant and permits identification of ischemic myocardium (21,22).

Estimates of glucose utilization with [18F]fluorodeoxyglucose are also profoundly affected by changes in substrate concentration (11-13). In fact, if subjects are not given glucose prior to administration of [18F]fluorodeoxyglucose, myocardial uptake of tracer is small (11).
As we recently demonstrated, because of the rapid changes in the pattern of substrate utilization during ischemia and reperfusion, neither [1^1C]palmitate nor [1^8F]fluorodeoxyglucose alone is likely to provide an estimate of overall oxygen utilization (14). In contrast, as demonstrated by results from this study, [1^1C]acetate oxidation appears to be relatively insensitive to the pattern of utilization of myocardial substrate. As previously reported, valid estimates of MVO_2 can be made with [1^1C]acetate during ischemia and reperfusion and are unaffected by infusion of unlabeled acetate at physiological concentrations (1).

Metabolism of Acetate
Acetate is readily utilized by the heart, with extraction fractions of 18–27% observed in this study, with a mean extraction fraction of ~40% reported in human volunteers (23). Metabolism is predominantly by oxidation in the tricarboxylic acid cycle after activation to acetyl CoA (3,4). Alternative metabolic routes appear to be minor. Nuclear magnetic resonance spectroscopy has demonstrated the production of 1^3C-labeled amino acids from [1^3C]acetate (24). Minor incorporation of [1^4C]acetate into lipid occurs with hypoxia in rat hearts (7,8), and production of C-14 labeled ketone bodies occurs with ischemia in rabbit hearts (1).

The initial activation of acetate to acetyl CoA appears to be poorly regulated in contrast to the intermediary metabolism of other substrates, as suggested by the tenfold increase in acetyl CoA concentration found in isolated perfused rat hearts when perfused with 5 mM acetate (7) (supraphysiological compared with normal plasma levels, 0.03–0.06 mM (24,25)). The simplified metabolism and apparent poor regulation may explain the insensitivity of acetate metabolism in the heart to changes in substrate condition found in this study. Since the size of the acetyl-CoA pool is small compared with its rapid turnover (5–8), changes in pool size do not seem to alter the validity of measurements of MVO_2 with [1^1C]acetate under conditions studied.

The clearance of 11^C radioactivity from the heart after [1^1C]acetate administration is biphasic in canine studies and in isolated perfused rabbit hearts, consisting of a major rapidly clearing phase and a minor phase. In this study, the size of the major rapid phase was not altered by glucose or lipid infusion. Although the intracellular distribution of the 11^C label after [1^1C]acetate extraction by the heart is unclear, biexponential clearance suggests that the label is incorporated into at least

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**FIGURE 1**
An example of a composite [1^5O]H_2O (left) and [1^1C]acetate (right) midventricular tomogram in a control study showing the concordant, homogeneous distribution of flow and metabolism.

**FIGURE 2**
Examples of myocardial residue time activity curves from a control and post glucose study. Clearance is biexponential, consisting of a major rapidly clearing phase and a minor phase. In this study, rate pressure product was 18,850 and 20,700 mmHg × bpm, respectively, and measured oxygen consumption 5.0 and 4.8 μmol O_2/g/min, respectively. No difference in k was observed.
two distinct intracellular pools. Based on the metabolism of acetate discussed above, it is likely that the dominant phase represents oxidation of acetyl CoA, acetylcarnitine and tricarboxylic acid cycle intermediates. The minor phase may represent incorporation of the $^{13}$C label into lipids and amino acids. In preliminary studies in humans, only a single monoexponential clearance has been observed (26).

**SUMMARY**

Alterations in myocardial substrate utilization or plasma substrate concentrations do not appear to influence validity of estimates of MVO$_2$ with $[^{13}]$acetate independent of changes in myocardial oxygen consumption or work load induced by the interventions. Although changes in substrate utilization will alter the relationship between tricarboxylic acid cycle flux and oxygen consumption, this effect is theoretically small (<4%). Assessment of MVO$_2$ with $[^{13}]$acetate and PET thus offers a promising noninvasive approach for assessment of regional oxygen utilization which should be useful in studying the effect of cardiac disease on energy utilization, and for evaluation of the effects of therapeutic interventions.

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