Positron Emission Tomography Analysis of $[1^{-11}\text{C}]$Acetate Kinetics in Short-term Hibernating Myocardium

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Background—Modeling of the time-$[1^{-11}\text{C}]$acetate activity curve assumes a constant concentration of labeled tricarboxylic acid cycle intermediates and associated metabolites, such as glutamate and aspartate, which may, however, decrease in short-term hibernating myocardium.

Methods and Results—In 12 anesthetized pigs, $[1^{-11}\text{C}]$acetate was injected as a bolus into the cannulated left anterior descending coronary artery during normoperfusion, inotropic stimulation, and early (5 to 45 minutes) and prolonged ischemia (60 to 90 minutes). Regional myocardial oxygen consumption ($\text{MVO}_2$, microliters per minute per gram) was measured, and the absence of necrosis was verified by triphenyl tetrazolium chloride staining. Inotropic stimulation increased $\text{MVO}_2$ from 52.5±7.4 to 195.4±36.2 (mean±SD) and the rate constant ($k_{\text{mono}}$, minutes$^{-1}$) of $[1^{-11}\text{C}]$acetate clearance from 0.094±0.018 to 0.322±0.076. During early ischemia, $\text{MVO}_2$ and $k_{\text{mono}}$ were decreased to 24.3±8.5 and 0.061±0.011, respectively. $k_{\text{mono}}$ closely correlated to $\text{MVO}_2$ during normoperfusion, inotropic stimulation, and early ischemia. In short-term hibernating myocardium, however, at an unchanged $\text{MVO}_2$, $k_{\text{mono}}$ increased toward control values (0.080±0.014). Myocardial glutamate and aspartate concentrations (biopsies) decreased to 47±6 and 77±18%; the peak count rate decreased to 66±22% of its respective control value. After correction for the decreases in glutamate and aspartate or in peak count rate, $k_{\text{mono}}$ was again decreased (0.050±0.016 or 0.052±0.014, respectively), and a close relationship to $\text{MVO}_2$ was restored.

Conclusions—$k_{\text{mono}}$ correlates to $\text{MVO}_2$ in short-term hibernating myocardium when the decreases in aspartate and glutamate or in peak count rate are considered. (Circulation. 1998;97:1009-1016.)

Key Words: tomography ■ ischemia, myocardial ■ hibernation, myocardial ■ metabolism
Methods

The experimental protocols used in this study were approved by the bioethical committee of the district of Düsseldorf, and they adhere to the guiding principles of the American Physiological Society.

Experimental Model

The experimental model has been described extensively elsewhere\textsuperscript{29,30}; in brief, in 12 enflurane-anesthetized Göttinger miniswine (20 to 40 kg) of either sex, both common carotid arteries were cannulated for measurement of arterial pressure and to supply blood to an extracorporeal circuit. A left lateral thoracotomy was performed, and a micromanometer was placed in the left ventricle through the apex. Ultrasound dimension gauges were implanted in the LV myocardium in brief, in 12 enflurane-anesthetized Göttinger miniswine (20 to 40 kg) of either sex, both common carotid arteries were cannulated for measurement of arterial pressure and to supply blood to an extracorporeal circuit. A left lateral thoracotomy was performed, and a micromanometer was placed in the left ventricle through the apex. Ultrasound dimension gauges were implanted in the LV myocardium. The systemic arterial pressure was recorded and analyzed using a guiding principle of the American Physiological Society. The experimental protocols used in this study were approved by the bioethical committee of the district of Düsseldorf, and they adhere to the guiding principles of the American Physiological Society.

Regional Myocardial Dimensions

End diastole was defined as the point when the first derivative of LV pressure (LV dp/dt) started its rapid upstroke after crossing the zero line. Global LV end systole was defined as the time point of peak systolic wall thickness within 20 ms before peak negative LV dp/dt. \textsuperscript{31} Anterior systolic wall thickening and regional myocardial work index are reported.

Regional Myocardial Blood Flow

Radiolabeled microspheres were injected into the coronary perfusion circuit to determine regional myocardial blood flow. Blood flow to the tissue at the site of the ultrasound crystals is reported, and this piece of tissue was divided into transmural thirds of approximately equal thickness. Transmural blood flows in different pieces (average weight, 1.08±0.51 g) within and along different heart slices revealed a coefficient of variation of 2.6±1.7%.

Regional Myocardial Oxygen and Lactate Consumption

Oxygen content was measured with anaerobically sampled blood drawn simultaneously from the coronary vein and an artery (Cavitron/LexOx-CON-k, Dr B.G. Schlag, Bergisch Gladbach). Lactate was measured in simultaneously drawn coronary venous and arterial blood samples by use of enzymatic dehydrogenation and subsequent photometry of NADH. Myocardial oxygen (MV\textsubscript{O}2) and lactate consumption were calculated by multiplying the arterial-coronary venous difference by the transmural blood flow at the crystal site.

Myocardial Aspartate and Glutamate Concentrations

Transmural myocardial biopsies (≈10 µg each) were taken in six swine of group 1 under control conditions and at the end of the 90-minute ischemic period (see below) with a modified dental drill. Samples requiring more than 1 to 2 seconds for acquisition were not used for this analysis. Samples were homogenized in 0.33 mol/L perchloric acid (Mikro–Disembrator, B. Braun Melsungen), and the pH of the supernatant was adjusted to 7.2 with 1.1 mol/L K\textsubscript{2}PO\textsubscript{4}. Aspartate was determined by transamination with \textalpha-ketoglutarate and glutamic-oxidase transaminase, followed by hydrogenation of the formed oxaloacetate with NADH and malic dehydrogenase. The decrease in NADH extinction was measured photometrically at 340 nm (model 8452, Hewlett-Packard Co). \textsuperscript{32} Glutamate was determined by the glutamic dehydrogenase reaction. In this reaction, NADH is formed stoichiometrically when the equilibrium is shifted by the addition of hydrazine sulfate at pH 9. \textsuperscript{33} The NADH formation was measured at 340 nm.

Radiochemistry

[1-\textsuperscript{11}C]acetate was prepared via carboxylation of methylmagnesium bromide. \textsuperscript{34} The radiochemical yield, defined as the ratio of \textsuperscript{11}C activity determined in [1-\textsuperscript{11}C]acetate to the \textsuperscript{11}C activity at the beginning of the acetate synthesis, averaged 68±7%. The specific activity was in the range of 10 to 20 GBq \textmu mol\textsuperscript{-1}. Radiochemical purity was determined by high-performance liquid chromatography to be >97.5% by use of a LiChrospher 100 RP-18 column (250×4 mm, 10 µm; Merck) with 0.0005N H\textsubscript{2}SO\textsubscript{4} as eluent (1.0 mL⋅min\textsuperscript{-1}). For each intracoronary injection, activities of about 1 to 3 mCi 1-\textsuperscript{11}C]acetate were administered within 1 minute.

Coronary Venous \textsuperscript{11}CO\textsubscript{2}

The vein that paralleled the LAD was cannulated and drained into an unpressurized reservoir to avoid recirculation of labeled substance. If [1-\textsuperscript{11}C]acetate is completely metabolized to \textsuperscript{11}CO\textsubscript{2}, within the TCA cycle, its clearance from the myocardium reflects myocardial oxygen consumption. The coronary venous blood was sampled during and at 2, 3, 5, 7, 10, 15, 20, 25, 30, 35, and 40 minutes after the injection of [1-\textsuperscript{11}C]acetate. The total activity of the coronary venous blood was counted, and thereafter \textsuperscript{11}CO\textsubscript{2} was eliminated from the coronary venous blood by acidification (pH 1.0) and bubbling with N\textsubscript{2} for 5 minutes. The remaining activity within the coronary venous blood was once again assessed, corrected for the activity decay over time, and related to total activity.

Morphology

At the end of each study, the heart was removed and sectioned from base to apex into five transverse slices in a plane parallel to the AV groove. The tissue slices were immersed in a 0.09 mol/L sodium phosphate buffer, pH 7.4, containing 1.0% triphenyl tetrazolium chloride and 8% dextran to verify the absence of infarcted tissue.

Experimental Protocol

Two groups of swine were studied. Under control conditions, the perfusion pump was adjusted so that the minimum coronary arterial pressure was not <70 mm Hg to avoid any initial hypoperfusion. Therefore, mean coronary arterial pressure exceeded peak LV pressure. In both groups of swine, each observation period began with the simultaneous withdrawal of a pair of arterial and coronary venous blood samples. During the blood sampling, microspheres and [1-\textsuperscript{11}C]acetate were injected into the LAD perfusion system. Systemic hemodynamic and regional myocardial dimension data were recorded throughout the microspheres injection.

Group 1 (n=6)

After control measurements, epinephrine was infused intravenously (0.5 to 1.0 µg ⋅ kg\textsuperscript{-1} ⋅ min\textsuperscript{-1}) to increase heart rate by ≈30 bpm. During inotropic stimulation, the perfusion pump was set to maintain mean coronary arterial pressure equal to LV peak pressure to avoid any hypoperfusion. The dose of epinephrine was then maintained, and regional myocardial blood flow, function, and metabolism were measured while [1-\textsuperscript{11}C]acetate was once again injected intracoronarily. Thereafter, heart rate was further increased by ≈30 bpm with a further increased dose of intravenous epinephrine (1.0 to 4.0 µg ⋅ kg\textsuperscript{-1} ⋅ min\textsuperscript{-1}) and additional left atrial pacing, and measurements were once again repeated. Thereafter, the infusion of epinephrine and left atrial pacing were maintained until the end of the study period.
pacing were stopped, and restoration of systemic hemodynamics and regional myocardial function was ensured. The perfusion pump was then adjusted to reduce coronary inflow by ~50%, a level previously shown to be compatible with the development of short-term hibernating myocardium over 90 minutes.29-30 Flow, function, and myocardial oxygen consumption were measured at 5 minutes of hypoperfusion, whereas the PET data for early ischemia were acquired between 5 and 45 minutes of hyperperfusion. Flow, function, and myocardial oxygen consumption were once more measured at 90 minutes of hypoperfusion, and biopsies for the determination of glutamate and aspartate concentration were taken, whereas the PET data for prolonged ischemia were acquired between 60 and 90 minutes of hypoperfusion.

**Group 2 (n=6)**

To ensure that prior inotropic stimulation had no impact on the results obtained during ischemia, in a second group of swine, blood flow to the LAD was reduced to 50% immediately after control measurements. Measurements were taken during early and prolonged ischemia, as in group 1. After 90 minutes of ischemia, the myocardium was reperfused for 2 hours.

**Data Acquisition**

PETs were acquired with an ECAT 953/15 scanner (Siemens/CTI) with an axial field of view of 54 mm. Plane separation was 3.4 mm. The data were reconstructed with a Hanning filter and a frequency cutoff of 0.5 to 128 matrix. The emission data were corrected for radioactive decay and for attenuation with a transmission scan. A dynamic acquisition protocol with 30 frames of 1 second, 10 frames of 10 seconds, 8 frames of 60 seconds, and 6 frames of 300 seconds was used. The emission data were evaluated with a monoeponential model. Transaxial scans were analyzed. The axial and in-plane spatial resolution of the images was about 9 mm. Because labeled acetate was injected into the cannulated LAD, the perfusion territory was clearly delineated from the left circumflex and right coronary artery perfusion territory. Within the LAD perfusion territory, to avoid influences of mixed perfusion territory (border zone), only the central area with a distance of 2 to 3 mm from the left and right borders was used for analysis. Therefore, the size of the region of interest ranged from 17 to 28 mm in diameter, depending on heart size (different swine) and the intervention used (control versus inotropic stimulation versus ischemia). During ischemia, however, the size of the region of interest remained unchanged within a given animal. Because recirculation of labeled substance was avoided and acetate taken up by the myocardium was metabolized to CO2, the peak-to-noise ratio exceeded 50:1 during normoperfusion and 30:1 during ischemia. The parameters a and kmono of the monoeponential model \( y(t) = a \cdot e^{-k_{\text{mono}} \cdot t} \) were determined pixelwise by linear least-squares fitting. The linear fit was performed over the time interval when the washout of tracer was dominated by \(^1\text{C} \text{O}_2\) (>95% of baseline) and the logarithmic plot of the data was obviously linear. The kmono values for a given region of interest were averaged. Values for kmono thus are mean values of six to eight heart slices, depending on the size of the perfusion territory. The coefficient of variation of measured activity within the region of interest throughout the slices averaged 11±5%, 10±4%, 12±4%, 20±10%, and 24±11% during control conditions, the two degrees of inotropic stimulation, and early and late ischemia, respectively. Within the same region of interest, the peak count rate was determined during control conditions, inotropic stimulation, and early and prolonged ischemia. To account for the different amounts of injected activity, the true count rates are expressed per millicurie of injected activity.

**Data Analysis**

Hemodynamic data were recorded on an eight-channel recorder and stored directly to the hard disk of an AT-type computer. Hemodynamic and dimension parameters were digitized and recorded over the time period of microspheres injection with CORDAT II software.29 The calculation of all hemodynamic parameters was done on a beat-to-beat basis, and data were then averaged over at least 33 cardiac cycles. kmono was corrected for the observed decreases in the amino acid pool sizes with the following assumptions: If acetate is metabolized completely to \(^1\text{C} \text{O}_2\), the flux rate through the TCA cycle closely correlates to myocardial oxygen consumption.3 The flux rate through the TCA cycle is given by the product of the TCA cycle intermediates and metabolites in equilibrium with the TCA cycle times kmono. Therefore, as assumed in most instances, at constant pool sizes of TCA cycle intermediates and metabolites such as aspartate and glutamate, kmono indeed correlates to myocardial oxygen consumption. The total concentration of aspartate and glutamate is 8 to 10 times higher than the sum of the TCA cycle metabolites in isolated rat hearts4,5,29 and in the myocardium of anesthetized dogs.3 Therefore, the flux rate can be simplified as the sum of the aspartate and glutamate concentrations times kmono. Alterations in the aspartate and glutamate concentrations, at constant regional myocardial oxygen consumption and thus flux rate through the TCA cycle, will then inversely affect kmono. In conclusion, given the observed changes in the glutamate and aspartate concentrations, kmono was corrected by multiplying kmono with the fraction of the glutamate (Glu) and aspartate (Asp) concentration relative to their control values: kmono,corr = kmono × \((\text{Asp},\text{ischemia}/\text{(Asp),control})^2 \cdot (\text{Asp},\text{control}/\text{(Asp),ischemia})^2\). Similarly, kmono was corrected for the decrease in the peak count rate within the region of interest. The peak count rate during early and more prolonged ischemia was expressed as a fraction of the peak count rate under control conditions and then multiplied with kmono: kmono,corr = kmono × \((\text{countsROI},\text{ischemia}/\text{countsROI},\text{control})^2\).

**Statistics**

Statistical analysis was performed by use of SYSTAT software. Because comparison of systemic hemodynamics, regional myocardial blood flow, function, and metabolism during normoperfusion and ischemia revealed no statistical significant differences between groups 1 and 2 by use of a two-way ANOVA, data at these time points were pooled and compared by use of a one-way ANOVA for repeated measures. When significant differences were detected, individual mean values were compared by use of least-significant-difference post hoc tests. All data are reported as mean±SD, and a value of \(P<.05\) was accepted as indicating a significant difference in mean values. Linear regression analyses between regional myocardial oxygen consumption in the LV area at risk and kmono and corrected kmono were performed.

**Results**

**Systemic Hemodynamics and Regional Myocardial Dimensions**

With infusion of epinephrine, heart rate (\(P<.05\)), LV end-diastolic pressure (\(P=\text{NS}\)), peak pressure (\(P<.05\)), LV dP/dt\text{max} (\(P<.05\)), LV dP/dt\text{min} (\(P<.05\)), and coronary blood flow (\(P<.05\)) increased (Table 1). End-diastolic wall thickness of the anterior myocardium (\(P=\text{NS}\)) increased, whereas systolic wall thickening, in the presence of increased heart rate and LV peak pressure, remained unaltered. The anterior myocardial work index (\(P<.05\)) increased. Further increases in the epinephrine dose in combination with left atrial pacing resulted in a further increase in LV peak pressure (\(P=\text{NS}\)), LV dP/dt\text{max} and LV dP/dt\text{min} (both \(P<.05\)) and coronary blood flow (\(P=\text{NS}\)). The end-diastolic wall thickness of the anterior myocardium tended to increase (\(P=\text{NS}\)), and systolic wall thickening tended to decrease (\(P=\text{NS}\)); the anterior myocardial work index remained unchanged. With ischemia, the LV end-diastolic pressure was increased (\(P=\text{NS}\)), and LV peak pressure (\(P=\text{NS}\)), LV dP/dt\text{max} (\(P=\text{NS}\)), and LV dP/dt\text{min} (\(P<.05\)) were reduced. In the presence of significantly reduced coronary blood flow and coronary arterial pressure (both \(P<.05\)), end-diastolic wall thickness decreased (\(P=\text{NS}\), and both systolic wall thickening and the work index of the anterior myocardium were reduced by ~70% (both \(P<.05\)). Prolongation of ischemia resulted in no further changes compared
with early ischemia, except that systolic wall thickening and the work index of the anterior myocardium tended to be further depressed.

**Coronary Venous Blood Activity**

During normoperfusion, >95% of total activity was related to $^{11}$CO$_2$ at 5 minutes and longer after the [1-11C]acetate injection (Fig 1A). During inotropic stimulation, >95% of total activity was related to $^{11}$CO$_2$ already at 3 minutes after the [1-11C]acetate injection, whereas it took almost 7 minutes during early and more prolonged myocardial ischemia (Fig 1B) until >95% of total activity was related to $^{11}$CO$_2$.

**Regional Myocardial Blood Flow, Metabolism, and [1-11C]Acetate Kinetics**

Infusion of low-dose epinephrine increased transmural myocardial blood flow ($P<.05$) and oxygen consumption of the anterior myocardium to twofold ($P<.05$) (Table 2). Myocardial lactate consumption increased ($P<.05$). The rate constant of the monoeponential flt of the time [1-11C]acetate activity curve ($k_{mon}$) also increased to twofold ($P<.05$). Further increases in the epinephrine dose in combination with left atrial pacing resulted in an additional increase in transmural myocardial blood flow to about fourfold the baseline level ($P<.05$ versus preceding value) and a similar increase in regional myocardial oxygen consumption ($P<.05$ versus preceding value). Myocardial lactate consumption returned toward control values. The time constant $k_{mon}$ increased further, however, only to 3.5-fold the baseline level ($P<.05$ versus preceding value). With ischemia, transmural myocardial blood flow and regional myocardial oxygen consumption were reduced by ~60% (both $P<.05$), and lactate consumption under control conditions was reversed to net lactate production ($P<.05$). The time constant $k_{mon}$ was significantly reduced. With prolonged ischemia, transmural myocardial blood flow and regional myocardial oxygen consumption were not changed, whereas lactate production was attenuated. In the absence of changes in regional myocardial oxygen consumption, $k_{mon}$ returned toward baseline values. Myocardial glutamate and aspartate concentrations averaged 2.19±0.54 and 1.35±0.17 μmol·g$^{-1}$ wet weight under control conditions, respectively, and were reduced to 47±26% and 77±18% of their control levels (both $P<.05$ versus control conditions) after 90 minutes of ischemia. The true count rates averaged

**TABLE 1. Systemic Hemodynamics and Regional Myocardial Dimensions During Control Conditions, Inotropic Stimulation, and Low-Flow Ischemia**

<table>
<thead>
<tr>
<th></th>
<th>Control (n=12)</th>
<th>Inotropic Stimulation (n=6)</th>
<th>Early Ischemia (n=12)</th>
<th>Prolonged Ischemia (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR, bpm</td>
<td>90±7</td>
<td>125±10*</td>
<td>152±6*</td>
<td>93±6†</td>
</tr>
<tr>
<td>LVEDP, mm Hg</td>
<td>8±4</td>
<td>12±5</td>
<td>12±2</td>
<td>13±5</td>
</tr>
<tr>
<td>LVPP, mm Hg</td>
<td>89±9</td>
<td>135±7*</td>
<td>152±22*</td>
<td>84±7†</td>
</tr>
<tr>
<td>LV dP/dt$_{max}$, mm Hg · s$^{-1}$</td>
<td>1645±277</td>
<td>4846±715*</td>
<td>6180±1682†</td>
<td>1408±314†</td>
</tr>
<tr>
<td>LV dP/dt$_{min}$, mm Hg · s$^{-1}$</td>
<td>1299±239</td>
<td>2384±278</td>
<td>2898±665†</td>
<td>812±238†</td>
</tr>
<tr>
<td>CAP, mm Hg</td>
<td>119±10</td>
<td>137±9*</td>
<td>154±14*</td>
<td>44±5†</td>
</tr>
<tr>
<td>CBF, ml · min$^{-1}$</td>
<td>28.5±7.8</td>
<td>53.3±15.8*</td>
<td>94.8±27.0*</td>
<td>11.9±3.1†</td>
</tr>
<tr>
<td>AWTED, mm</td>
<td>10.52±1.80</td>
<td>11.38±1.51</td>
<td>11.74±2.10</td>
<td>9.23±1.91</td>
</tr>
<tr>
<td>AWT, %</td>
<td>37.0±6.9</td>
<td>37.7±8.6</td>
<td>31.4±9.8</td>
<td>10.4±6.9†</td>
</tr>
<tr>
<td>AWI, mm Hg · mm Hg</td>
<td>277±49</td>
<td>497±128*</td>
<td>493±161*</td>
<td>80±40†</td>
</tr>
</tbody>
</table>

$HR$ indicates heart rate; LVEDP, LV end-diastolic pressure; LVPP, LV peak pressure; LV dP/dt$_{max}$, maximum of the first derivative of LV pressure; LV dP/dt$_{min}$, minimum of the first derivative of LV pressure; CAP, mean coronary arterial pressure; CBF, mean coronary blood flow; AWTed, end-diastolic wall thickness of the anterior wall; AWT, systolic wall thickening of the anterior wall; and AWI, anterior work index.

*P<.05 vs control; †P<.05 vs preceding value.

**Figure 1.** Original data from one animal on total and $^{11}$CO$_2$-related activity in coronary venous blood. During control conditions, >95% of total activity was related to $^{11}$CO$_2$ at 5 minutes and longer after the [1-11C]acetate injection (A). During acute and prolonged myocardial ischemia (B), it took almost 7 minutes until >95% of total activity was related to $^{11}$CO$_2$. 
35 044±6540 cps/mCi under control conditions, 30 329±7658 cps/mCi during inotropic stimulation, 27 077±6858 cps/mCi during early ischemia, and 22 121±6358 cps/mCi during late ischemia. In the six swine in which decreases in both the peak count rate and glutamate and aspartate concentrations were measured, a close correlation between these two variables existed (percent change in peak count rate = 0.99 times the percent change in the glutamate and aspartate concentrations = 1.76, r = 0.84). Correction of kmono for the attenuation in peak count rate during early ischemia decreased kmono from 0.061±0.011 to 0.046±0.021 min⁻¹. After correction for the decreases in glutamate and aspartate or in peak count rate during prolonged ischemia, kmono was again decreased from 0.080±0.014 to 0.050±0.016 or 0.052±0.014 minutes⁻¹, respectively.

Relationships Between kmono and MVO₂

A close correlation between kmono and MVO₂ was observed when data obtained during control conditions, inotropic stimulation, and early ischemia were combined (Fig 2). With prolonged ischemia, kmono was increased, so that in short-term hibernating myocardium, all data points relating kmono to regional myocardial oxygen consumption were located above the regression line determined during normoperfusion, inotropic stimulation, and early ischemia (Fig 3A). Correction for the significant decrease in labeled pool sizes of aspartate and glutamate in group 1 restored a close relationship between kmono and regional myocardial oxygen consumption in short-term hibernating myocardium (Fig 3B).

Correction for the decreases in peak count rate based on all data points obtained throughout the experiment (y = 1.11×10⁻³×x + 2.96×10⁻²; r = 0.91) only slightly increased the y-axis intercept of the relationship between myocardial oxygen consumption and kmono compared with the uncorrected relationship (Fig 4A). However, correction for the decreases in peak count rate during prolonged ischemia once more restored a close relationship between kmono and regional myocardial oxygen consumption in short-term hibernating myocardium (Fig 4B).

Discussion

In short-term hibernating myocardium, the monoexponential rate constant per se does not correctly reflect regional myo-

![Figure 2](image)

**Figure 2.** A close correlation between kmono and regional myocardial oxygen consumption existed during normoperfusion, inotropic stimulation, and early myocardial ischemia.

![Figure 3](image)

**Figure 3.** Values for kmono in short-term hibernating myocardium were located above the regression line obtained during normoperfusion, inotropic stimulation, and early acute myocardial ischemia (A). Correction for the significant decrease in labeled pool sizes of aspartate and glutamate restored a close relationship between kmono and MVO₂ also in short-term hibernating myocardium (B).
cardiac oxygen consumption. The lack of decrease in the rate constant, in the presence of a significantly reduced regional myocardial oxygen consumption, is related to alterations in the amino acid pool sizes; correction for such a decrease in the pool sizes of glutamate and aspartate or in peak count rate within the region of interest restores a close relationship between the rate constant and regional myocardial oxygen consumption.

Critique of Methods

In the present study, an established model of short-term hibernation was used. A limitation of all controlled experimental studies, including the present one, on myocardial hibernation, however, is the limited observation period.

With systemic administration of the tracer, its delivery will be flow dependent, vary between the region of interest and the reference region, and therefore require normalization to myocardial blood flow. In the present study, however, labeled acetate was injected only into the cannulated LAD, and such normalization to myocardial blood flow was not necessary.

The variation in the measured $^{13}$C activity within the region of interest could represent the physiological variation of either regional myocardial blood flow or metabolism. With myocardial tissue with an average weight $<100$ mg, substantial variations in regional blood flow have indeed been demonstrated. Thus, the variation of activity from pixel to pixel might reflect the differences in blood flow distribution, because the pixel volume averaged $2.4 \times 3.4 \times 3$ mm$^3$, which, when a density of 1 is assumed, results in an average sample weight of only 25 mg.

The time constants $k_1$ and $k_2$ from a two-compartment model, as first introduced by Armbrecht et al., were not calculated. Because of the limited acquisition period, especially during ischemia, a reasonable determination of $k_2$ in the two-compartment model was not possible because of the slow kinetics of the second component. It therefore remains unclear whether the limitations outlined for the monoexponential model with respect to altered amino acid pool sizes are also valid for other compartment models. Because the exchange rate between the TCA cycle and the glutamate pool is known to be rapid, it is unlikely that the second, slow component adequately reflects such rapid exchange. The second, slow component of the two-compartment model might, however, reflect the slow conversion of labeled glutamate to glutamine or the further metabolism of glutamine that has already been formed. In the present study, lactate consumption increased during the first degree of inotropic stimulation but once again decreased toward control values when inotropic stimulation was further increased. Using $^{14}$C-labeled glucose, Massie et al demonstrated an increased lactate uptake but also lactate production during dobutamine stimulation in the anesthetized pig in situ, resulting in an almost unchanged net lactate uptake. Similar findings were also obtained in anesthetized dogs. The increased lactate production during inotropic stimulation indicates some myocardium with an oxygen supply-demand imbalance. Thus, during inotropic stimulation, areas with increased oxygen demand and increased oxygen supply coexist with areas with increased oxygen demand but insufficient oxygen supply.

Myocardial alanine, which can be produced from either aspartate or glutamate during ischemia, was not measured in the present study. The increase in the alanine concentration by transamination of glutamate would not trap activity within the myocardium because only NH$_3$ is transferred to pyruvate. The myocardial aspartate concentration decreased by 23%.

Amino Acid Metabolism and $k_{mono}$

Under aerobic conditions, the oxidative metabolism of fatty acids, lactate, and glucose provides most of the cardiac energy requirements. Glutamate participates in the transport of electrons from the cytosol into the mitochondria during oxidation of glucose or lactate (malate-aspartate shuttle). This process is strictly dependent on a high concentration of glutamate because of the low affinity of the mitochondrial carrier. High rates of $\beta$-oxidation delay and, vice versa, predominant use of glucose accelerate the incorporation of the $^{13}$C label into glutamate in isolated rabbit hearts. The somewhat smaller increase in $k_{mono}$ than in regional myocardial oxygen consumption during maximal inotropic stimulation (3.5-fold versus
4-fold) observed in the present study could relate to such altered substrate use.

During ischemia, anaerobic glycolysis is the major energy source, and lactate accumulates. In the presence of high concentrations of tissue lactate, the conversion of pyruvate to lactate is retarded owing to the mass action effect of lactate accumulation with subsequent augmentation of glutamate–pyruvate transamination.24 This might result in a net reduction in lactate because alanine is produced from pyruvate; the predominant amino acid for transamination of pyruvate is glutamate.25 Indeed, also in the present study, lactate production was attenuated during prolonged ischemia, and the glutamate concentration significantly decreased. Glutamate is further involved in the clearance of ammonia from the heart. During anoxia and ischemia, ammonia is produced within the myocardium by breakdown of adenosine to inosine.26,46 Alamine, which is formed through transamination of pyruvate (thereby accepting ammonia), acts as a nontoxic carrier of ammonia.77 Therefore, despite an increased glutamate uptake,44,48 the myocardial glutamate concentration is significantly decreased during ischemia.24,26

The decrease in amino acid concentrations during prolonged ischemia—at an equivalent reduction of regional myocardial oxygen consumption during early and late ischemia—was associated with a relative increase in \( k_{\text{mono}} \) back toward control values. Correction of \( k_{\text{mono}} \) was performed under the assumption that at an unchanged regional myocardial oxygen consumption, the flux rate through the TCA cycle also remained unchanged. Understandably, then, correction for the decrease in the aspartate and glutamate concentrations restored the close correlation between regional myocardial oxygen consumption and \( k_{\text{mono}} \) observed during normoperfusion, isotropic stimulation, and early ischemia (Fig 3B). Similarly, correction of \( k_{\text{mono}} \) for the decrease in the peak count rate within the region of interest also restored the close correlation between regional myocardial oxygen consumption and \( k_{\text{mono}} \) (Fig 4B). These findings indicate that the peak count rate and the decay of activity from the myocardium after \([1–11C]\)acetate injection are closely related to the myocardial glutamate and aspartate concentrations.

**Clinical Implications**

In patients with coronary artery disease, dynamic PET imaging with \([1–11C]\)acetate facilitates the accurate identification of dysfunctional but still viable myocardium, which is capable of recovering systolic function after coronary revascularization.11,13,14,49 \([1–11C]\)acetate even exhibits positive and negative predictive values superior to fluorine-18–gluoroethylglucose.49

While in clinical practice the correction of \( k_{\text{mono}} \) might be less important for the assessment of myocardial viability, such correction offers the potential to better identify the underlying mechanism of contractile dysfunction. It is currently unclear whether hibernation as observed in the clinical setting is a manifestation of and adaptation to a persistent reduction in blood flow,50 or the result of repetitive stunning.51 The correct measurement of myocardial oxygen consumption is a prerequisite to quantitatively correlate the observed reduction in function and oxygen demand to that in oxygen supply, and the matching between demand and supply is a hallmark of the classic concept of hibernation.52,53 Whether or not in a clinical setting with a single-spot PET measurement, comparing the region of interest to a reference region, the correction proposed by the present study using serial PET measurements in the region of interest will be useful remains to be determined.

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