Mitochondrial Preference for Short Chain Fatty Acid Oxidation During Coronary Artery Constriction

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Background—Reduced fatty acid oxidation in hypoperfused myocardium is believed to result from impaired oxidation in mitochondria. This study suggests another mechanism, that oxidative capacity exceeds regulated entry of long chain fatty acid (LCFA). The ability of myocardium to oxidize fatty acids and metabolize glucose during stenosis was examined in open chest, anesthetized pigs.

Methods and Results—The left anterior descending (LAD) coronary artery was infused for 40 minutes (5 mL/min LAD) with [2-13C] butyrate (4 mmol/L), a short chain fatty acid (SCFA), plus [2-13C] glucose (10 mmol/L) in either nonischemic controls (n = 4) or at the end of 5 hours of LAD flow reduction (40%, n = 7). With LAD constriction, left ventricular wall thickening fell 45±8% (P<0.01). Despite glycolytic production of lactate and alanine, hypoperfused myocardium preferentially oxidized SCFA over endogenous LCFA. SCFA accounted for 63±4% (mean±SEM) of carbon units entering oxidation in both ischemic epicardium and endocardium versus only 38±4% and 40±6% in respective samples from normal myocardium (P<0.002). Unexpectedly, SCFA contributions were elevated in both endocardium and epicardium despite preserved epicardial blood flow versus a 58±9% drop in endocardial flow (P<0.05). No significant oxidation of glucose was evident, indicating that unlabeled fuels were primarily LCFA.

Conclusions—Because SCFA bypass LCFA transport into mitochondria, during LAD constriction, mitochondrial capacity to oxidize fatty acid exceeds LCFA entry for oxidation. Importantly, metabolic changes were disassociated from transmural tissue perfusion. These findings suggest that signals other than oxygen availability regulate fatty acid use during hypoperfusion. (Circulation. 2002;105:367-372.)

Key Words: ischemia ■ spectroscopy ■ hibernation

Hypoperfused myocardium has been related to reduced oxidative metabolism, increased anaerobic energy production, and depressed contractility.1 In vivo animal heart preparations of regional blood flow deprivation have demonstrated reduced oxidation of fatty acids, the primary fuel for energy production in the heart,1 and this reduction presumably occurs as the result of limited oxygen availability. Reduced long chain fatty acid (LCFA) oxidation in ischemic but viable human hearts has also been reported.2 3 However, oxidation of fuels within the mitochondria of the hypoperfused myocardium has not been examined with substrates that bypass the highly regulated transfer of LCFA into the mitochondrial matrix. The current protocol tests the oxidative capability of mitochondria in the in vivo heart during restricted blood flow.

To date, reduced oxidation of both glucose and LCFA during low-flow ischemia has been generally considered to be the result of negative feedback inhibition from accumulating metabolic intermediates that are not oxidized and sequester coenzyme A.4-7 However, evidence exists that translocation-transesterification limits fatty acid oxidation in mitochondrial preparations that are isolated from severely ischemic canine hearts.8 Thus, the role of energy substrate entry into the mitochondrial matrix must be further considered as a central factor that limits LCFA oxidation in the hibernating myocardium,9 10 aside from the reduced availability of oxygen.

This study was aimed at determining regional substrate utilization for oxidative energy production in the in vivo porcine heart subjected to coronary artery constriction during infusion of a short chain fatty acid to fuel β-oxidation while bypassing the highly regulated transport of LCFA. The results offer surprising, new considerations in understanding the metabolic mechanisms that limit fatty acid oxidation in an in vivo model of coronary artery constriction that is consistent with short-term, hibernating myocardium.
Methods

Animal Preparations
Domestic swine (weight, 37 ± 1 kg) were sedated with 5 mg/kg IM telazol and 0.05 mg/kg IM atropine and intubated. General anesthesia was maintained with isoflurane (0.5 to 1.5 vol%). The open-chest animals were instrumented and hemodynamics recorded as previously described. The left main coronary artery was dissected proximal to its bifurcation, and a catheter was placed as previously described. A Silastic catheter was placed retrograde into the coronary vein for myocardial venous sampling. Arterial blood gases and electrolytes were monitored and maintained throughout the study. A heating pad, drapes, and infusion of warmed 0.9% NaCl solution were used to maintain rectal temperature between 36°C and 37°C. Animals used in this study were maintained in accordance with the “Guide for the Care and Use of Laboratory Animals” (National Research Council, revised 1996).

Experimental Protocols
The open chest heart preparation was subjected to either a 5-hour left anterior descending (LAD) coronary artery constriction protocol (n = 7) or a 5-hour protocol without constriction as control (n = 5). An LAD constriction was induced in experimental groups with a hydraulic occluder, reducing flow by ~40%. The degree of coronary blood flow reduction was continuously monitored and sustained for 5 hours. In the nonischemic, control group, a 5-hour protocol controlled for potential effects of the duration of the experiment. For both groups, infusion of labeled substrates was initiated at 4 hours and 20 minutes into the protocol.

Labeled substrate, [2,4-13C] glucose (10 mmol/L), and [2,4,5,13C4] butyrate, a short chain fatty acid (4 mmol/L) in saline were infused into the coronary artery catheter proximal to the occluder during the final 40 minutes of each protocol. Labeled butyrate was chosen as a metabolic probe of fatty acid oxidation to bypass LCFA transport into mitochondria. Although 13C labeling studies have used acetate, butyrate is a fatty acid that enters mitochondria. Although 13C labeling studies have used acetate, butyrate is a fatty acid that enters mitochondria. An infusion pump was used to deliver the labeled substrates at 5 mL/min per 25 mL/min of measured flow through the LAD, so that the infusion was always 20% of blood flow for 40 minutes. This time period was conservative in the intent to establish steady-state isotope enrichment. Myocardial oxygen consumption was determined with a blood gas machine (ABL 725, Radiometer Copenhagen) and calculated as oxygen uptake (volume percent from A-V difference) multiplied by mean coronary blood flow (mL/min). Regional myocardial blood flow measurement with radioactive microspheres was administered at baseline, twice during LAD constriction before substrate infusion (30 minutes and just before substrate infusion), and again during constriction with substrate infusion (20-minute infusion).

At 5 hours, during continued infusion, a section of the anterior left ventricular (LV) wall was excised, divided rapidly into endocardial and epicardial segments, and freeze-clamped in liquid nitrogen, all in less than 30 seconds. An additional experiment was performed in an open chest pig receiving a 40-minute infusion to determine the effects of transmural sectioning on the excised tissue before freezing. In unsectioned tissue, lactate was 1.9 ± 0.4 mmol/g dry wt versus values in sectioned myocardium of 1.7 in endocardium and 1.2 in epicardium. Thus, sectioning did not introduce any significant degree of incidental ischemia that may have influenced the measurements.

Additional experiments, both control (n = 3) and LAD constriction (n = 4), were performed over the 4-hour and 20-minute interval for tissue sampling and triglyceride assay. This sampling time provided information on the metabolic state of the myocardium at the time corresponding to the infusion protocol.

In Vitro Nuclear Magnetic Resonance Spectroscopy and Tissue Chemistry
Tissue metabolites were extracted from frozen ventricle with the use of 7% perchloric acid and assayed according to published methods. A Silastic catheter was placed retrograde into the coronary vein for myocardial venous sampling. Arterial blood gases and electrolytes were monitored and maintained throughout the study. A heating pad, drapes, and infusion of warmed 0.9% NaCl solution were used to maintain rectal temperature between 36°C and 37°C. Animals used in this study were maintained in accordance with the “Guide for the Care and Use of Laboratory Animals” (National Research Council, revised 1996).

Figure 1. Labeling scheme. Label from [2,4-13C] butyrate incorporates at the 2-, 3-, and 4- positions of the glutamate molecule. Label from [2-13C] glucose appears at the 5-carbon position of glutamate. At bottom, solid circles are glutamate sites labeled from butyrate; striped circle is label from glucose.

Figure 2. Regional blood flow. A. Values shown are for endocardium and epicardium of control animals (CON). Closed circle indicates epicardium; open circle, endocardium. B. Values shown are for endocardium and epicardium of heart subjected to 40% reduction of LAD flow (CS). Closed squares indicate epicardium; open squares, endocardium. Final data point corresponds to infusion of labeled substrates. Note normal epicardial flow during stenosis vs reduced endocardial flow. *P<0.05 vs control.
or more groups. Differences between mean values were considered statistically significant at a probability level of $P < 0.05$.

**Results**

**Physiological Function and Preparation Stability**

Neither the administration of substrates nor the infusion itself affected global hemodynamics, regional LV wall thickening, or blood flow. LAD constriction produced a sustained 40% drop from baseline blood flow. Figure 2 illustrates the effects of LAD constriction on regional blood flow. No drop from baseline flow occurred in the epicardial layer, but stenosis produced a sustained drop in endocardial blood flow. Figure 3 shows the stability of regional wall thickening in the nonischemic control group over 5 hours, whereas LAD constriction produced a sustained drop.

During constriction, regional oxygen use dropped from a preischemic baseline of $1.4 \pm 0.2 \text{ mL/min (\% volume)}$ to a value at 4 hours of hypoperfusion of $1.0 \pm 0.2 (P < 0.003)$. This 29% reduction in oxygen use remained unchanged at the midpoint of the 40-minute infusion with labeled substrates ($1.0 \pm 0.2$ at 4.3 hours).

**Oxidative and Nonoxidative Metabolism**

$^{13}$C NMR spectra from myocardial extracts displayed the expected increases in signals from glycolytic end products in the ischemic tissue (Figure 4), whereas signals from glutamate indicated active oxidation of the short chain fatty acid. Despite nonoxidative glycolytic metabolism in the ischemic myocardium, short chain fatty acid oxidation was retained, albeit at the expected reduction in oxidative rate, with a drop in oxygen consumption during the stenosis. Figure 5 displays an expanded region of a representative $^{13}$C NMR spectrum from an extract of ischemic epicardial tissue that displays the high-resolution, multiplet structures within the resonance peaks of the $^{13}$C-enriched glutamate.

Data demonstrating the $^{13}$C enrichment of glutamate caused by short chain fatty acid oxidation provided the primary results of this study: Short chain fatty acid accounted for

![Figure 3](link-to-image)

**Figure 3.** Regional wall thickening (% baseline) in control hearts (closed diamonds) vs reduced thickening with coronary stenosis (open diamonds). Note lack of change during infusion at final point. *$P<0.05$ vs control values.*

![Figure 4](link-to-image)

**Figure 4.** $^{13}$C NMR spectra from A, control myocardium, and B, hypoperfused myocardium. Glutamate labeled at 2-, 3-, and 4-carbons indicates oxidation of $[2,4,^{13}\text{C}]$ butyrate. Signals from lactate and alanine (2-carbons) indicate glycolysis of $[2,^{13}\text{C}]$ glucose. No evidence of glucose oxidation appeared at the 5-carbon of glutamate (see text). GLU indicates glutamate; LAC, lactate; and ALA, alanine. Signal at 49 ppm is endogenous taurine.

![Figure 5](link-to-image)

**Figure 5.** Expanded region of $^{13}$C NMR spectrum from LV epicardium during 40% reduction of LAD flow. Multiplet structures of resonances result from J-coupling to adjacent labeled nucleus. Peak at 32 ppm is 4-carbon of glutamine (GLU) and peak at 35 ppm is 2- and 3-carbons of succinate. Multiplet with predominately central signal at 27.3 ppm is 3-carbon of glutamate, and resolution was sufficient to factor out small symmetrical glutamine C-3 multiplets from the larger Glu C-3 signal.
63±4% of acetyl-CoA produced in the mitochondria in both ischemic epicardium and endocardium versus only 46±5% and 49±5% in respective controls (P<0.002). These data (Figure 6) indicate that the hypoperfused myocardium preferentially oxidized short chain fatty acid over endogenous LCFA, in contrast to normal myocardium.

Even more unexpected was the finding that the epicardium and endocardium used similar contributions from short chain fatty acid despite preservation of essentially normal blood flow rates in the epicardial layer and a 58±9% reduction in endocardial blood flow. These data, shown in Figure 7, indicate that the inhibition of fatty acid entry into the mitochondria during coronary stenosis was independent of regional, transmural blood flow.

Increased nonoxidative, glycolytic metabolism in the ischemic hearts is confirmed by lactate and alanine content (Table). As expected, lactate was increased in ischemic tissue (Table). In control tissue, the alanine and lactate were approximately equal, whereas in ischemic tissue, lactate represented slightly more of this pool of glycolytic end products (59% in endocardium and 57% in epicardium). This distribution reflects the increased ratio of cytosolic NADH/NAD⁺ during ischemia.¹⁷,¹⁸

Glutamate content was similar at the end of both the control and ischemia protocols, indicating that the low-flow ischemia did not reduce total tissue glutamate levels that remained at steady state to enable comparisons of isotope enrichment (Table). With stenosis, substrate infusion did not affect glutamate content, as seen in comparison to saline infusion: epicardial glutamate=18 µmol/g dry weight and endocardial glutamate=13 µmol/g dry weight. A low level of glucose oxidation was demonstrated in both layers by the absence of ¹³C NMR signal at 182 ppm (lack of signal not shown) from the glutamate 5-carbon. As a percentage of Krebs cycle flux, anaerolysis remained similarly low in all groups (controls: epicardium=9±2%, endocardium=9±2% versus hypoperfused: epicardium=11±3%, endocardium=13±2%, NS).

Hypoperfused myocardium showed the expected increase in triglyceride content before the time of infusion of labeled substrate. Total glycerol, as triglyceride content after saponification, increased from a control value of 16.2±1.7 mmol/g dry wt to 27.8±4.3 mmol/g dry wt during ischemia (mean±SEM, P<0.04). These data prove that the relative reduction in LCFA oxidation to that of short chain fatty acid during ischemia was not due to any unexpected reduction in lipid content during hypoperfusion.

Discussion

We report, for the first time, the finding that exogenous, short chain fatty acid outcompeted unlabeled, endogenous substrates for oxidative metabolism in the myocardium during coronary artery constriction in the in vivo pig heart. The actual increase in the contribution from the administered butyrate, in competition with endogenous fuels during 5 hours of hypoperfusion, indicates that the change occurred at the point of LCFA entry into oxidative metabolism in the mitochondria. In either case, the data demonstrate a paradoxical increase in the proportion of short chain fatty acid that is contributing as an oxidative fuel in the hypoperfused myocardium.

Competition between the short chain fatty acid and LCFA is notable because the established literature shows that fatty acid levels increase in ischemic myocardium.²³–²⁵ Consistent with these reports, endogenous triglyceride increased as a result of hypoperfusion. The importance of this approach, using exogenous short chain fatty acids to bypass LCFA utilization, is that if a direct measure of LCFA were performed, we would only demonstrate the known reduction in LCFA oxidation.¹–³

The novelty of these findings is that by using a short chain fatty acid, we have elucidated a continued oxidation of fatty acids that would otherwise not be detected with labeled

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**Figure 6.** Fraction of acetyl-CoA formation in the Krebs cycle that is attributed to the exogenous short chain fatty acid (SCFA). CON, 5-hour control with 40-minute infusion (dark bar); CS, 5-hour 40% reduction of LAD flow with 40-minutes infusion (open bar). Note elevated contribution of short chain fatty acid in CS. *P<0.05 from CON.

**Figure 7.** Discrepancy between blood flow and short chain fatty acid oxidation during LAD occlusion. A, Normal blood flow to epicardium (dark bar, EPI) and reduced flow to endocardium (light bar, ENDO). *P<0.05. B, Contribution of exogenous ¹³C butyrate to the Krebs cycle in epicardium (dark bar, EPI) and endocardium (light bar, ENDO). The fraction of oxidized butyrate is similar albeit increased over control (Figure 5), but blood flow is markedly different between endocardium and epicardium.

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**Metabolite Content: Lactate, Alanine, and Glutamate Content in Normal and Ischemic Myocardium**

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>Tissue Lactate</th>
<th>Tissue Alanine</th>
<th>Tissue Glutamate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ischemic epicardium</td>
<td>21±6*</td>
<td>15±3*</td>
<td>13±1</td>
</tr>
<tr>
<td>Ischemic endocardium</td>
<td>27±8*</td>
<td>21±6*</td>
<td>12±1</td>
</tr>
<tr>
<td>Control epicardium</td>
<td>5±2</td>
<td>4±1</td>
<td>14±1</td>
</tr>
<tr>
<td>Control endocardium</td>
<td>5±2</td>
<td>5±1</td>
<td>15±1</td>
</tr>
</tbody>
</table>

Values shown as µmol/g dry weight (mean±SEM). *P<0.05 between control and ischemic.
LCFA. The indication that butyrate contributions increased during ischemia oxidation in this study may be the result of inhibition of LCFA oxidation through CPT1, as suggested by previous work on the regulation of LCFA oxidation through malonyl CoA sensitivity in mitochondria from ischemic dog hearts. Alternatively, differences in the relative sensitivities of the long, medium, and short chain acyl-CoA dehydrogenases to ischemia may be reflected in these results, with the short chain acyl-CoA dehydrogenase accommodating butyrate oxidation beyond the capacity of the other dehydrogenases to support LCFA oxidation. Nonetheless, the argument remains that short chain fatty acid oxidation is preferred over the oxidation of LCFA in the hyperperfused myocardium. Two additional factors clearly implicate the entry of fatty acid into the mitochondria and subsequently β-oxidation as the reason for the increased role of short chain fatty acids in oxidative metabolism during hypoperfusion: (1) scaling the infusion rates to account for reductions in baseline blood flow and (2) the choice of exogenous substrates in the infusion, 13C-enriched butyrate and 13C-enriched glucose.

Despite the demonstrated glycolytic metabolism, 13C NMR data show no evidence of significant oxidation of glycolytic end products in either group. This finding is not surprising, based on previous reports. The low level of glucose oxidation does establish that carbohydrate metabolism was not a confounding variable. It is possible that a change in glycolgen breakdown occurred, but the proportion of butyrate use relative to other fuels went up, and not down. Nor are the findings obscured by potential changes in ketone levels, because this would have reduced, not increased, the contributions of the labeled short chain fatty acid.

Interestingly, both the epicardium and endocardium displayed the same elevated contributions to acetyl CoA formation from short chain fatty acid, despite preservation of normal blood flow in the epicardial layer versus a 58±9% drop in endocardial flow. These data (Figure 6) indicate that the metabolic changes during stenosis were independent of transmural blood flow. This finding suggests the intriguing concept that signals other than oxygen delivery determine fatty acid oxidation in the hyperperfused myocardium. The LAD constriction produced a 29% reduction in oxygen use by the myocardium. However, a relative increase in butyrate utilization, in comparison to the other circulating substrates, suggests a non–oxygen-mediated change in substrate selection for mitochondrial metabolism. Therefore, a potential mechanism for future consideration is the elevation of metabolic intermediates, such as malonyl-CoA, which may build up as the result of increased acetyl-CoA content in the heart as the rate of substrate oxidation slows. However, were availability and utilization of oxygen the sole mechanisms, then the reduced rates of oxidative metabolism would otherwise have limited short chain fatty acid oxidation in the same proportion to the other available substrates as during normal perfusion.

The findings indicate that regulation of fatty acid oxidation in a physiological preparation is more dependent on fatty acid transport than the oxidative capacity of the mitochondria. Recent work has shown that factors other than tissue oxygenation, such as nitric oxide, affect the oxidation of fatty acids.

The results shown here indicate that mechanisms invoked during regional hypoperfusion may become important in regulating the extent of substrate oxidation in the myocardium during limited flow, even though sufficient tissue oxygenation exists to support a higher level of oxidative metabolism. Even more surprising are the changes in butyrate use that appeared to be independent of transmural blood flow. Although fatty acid accumulation has been implicated in reperfusion damage, fatty acid oxidation does require more oxygen than that of carbohydrates and may be a countering limitation during hypoperfusion. This apparent drop in the oxidation of endogenous LCFA, despite the ability to support short chain fatty acid oxidation, may prove to be a protective mechanism against the potential deleterious effects of high oxygen demand by lipid oxidation during low-flow conditions.

Acknowledgments

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