Regulation of cardiac malonyl-CoA content and fatty acid oxidation during increased cardiac power

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LONG-CHAIN FATTY ACID OXIDATION in the heart is controlled at the level of the mitochondrial membrane by the activity of carnitine palmitoyltransferase I (CPT I). The endogenous inhibitor of CPT I is malonyl-CoA, which has a rapid turnover in the heart and is produced from acetyl-CoA by acetyl-CoA carboxylase (ACC) and degraded by malonyl-CoA decarboxylase (MCD); thus a fall in malonyl-CoA could be due to activation of MCD, inhibition of ACC, or both. This study assessed the effects of increased cardiac power on malonyl-CoA content and ACC and MCD activities. Anesthetized pigs were studied under control conditions and during increased cardiac power in response to dobutamine infusion and aortic constriction alone, under hyperglycemic conditions, or with the CPT I inhibitor oxofenicine. An increase in cardiac power was accompanied by increased myocardial oxygen consumption, decreased malonyl-CoA concentration, and increased fatty acid oxidation. There were no differences among groups in activity of ACC or AMP-activated protein kinase (AMPK), which physiologically inhibits ACC. There also were no differences in Vmax or Km of MCD. Previous studies have demonstrated that AMPK can be inhibited by protein kinase B (PKB); however, PKB was activated by dobutamine and the elevated insulin that accompanied hyperglycemia, but there was no effect on AMPK activity. In conclusion, the fall in malonyl-CoA and increase in fatty acid oxidation that occur with increased cardiac work were not due to inhibition of ACC or activation of MCD, suggesting alternative regulatory mechanisms for the work-induced decrease in malonyl-CoA concentration.

acetyl coenzyme A carboxylase; heart; malonyl coenzyme A decarboxylase; mitochondria; protein kinase B

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cultured mouse cardiac muscle cells show that the addition of palmitate to the medium partially prevents the insulin-induced increase in PKB activity (34). In addition, palmitate is able to prevent the inhibition of AMPK by insulin in isolated, perfused rat hearts (5).

The mechanism for palmitate inhibition of insulin activation of PKB is unclear; however, it does not appear to be due to alterations in the mitochondrial oxidation of fatty acids, as evidenced by the lack of effect on CPT I inhibition or pyruvate dehydrogenase activity and citric acid cycle intermediates from these experiments have been reported separately (34).

In the present study we investigated the effects of an acute increase in left ventricular contractile power on key regulators of myocardial malonyl-CoA content. We hypothesized that 1) increased cardiac power results in activation of MCD (i.e., greater \( V_{\text{max}} \) and/or lower \( K_m \)); 2) hyperglycemia plus hyperinsulinemia during increased cardiac power elevates malonyl-CoA content; 3) suppression of fatty acid oxidation by CPT I inhibition reduces malonyl-CoA content; and 4) PKB activity increases during increased cardiac power and the elevated insulin that accompanies hyperglycemia and corresponds with a decrease in AMPK activity. Experiments were performed in anesthetized pigs under unstimulated conditions and with acute aortic constriction plus an infusion of dobutamine to increase cardiac power and \( MV_\text{O}_2 \). PKB activity was increased by hyperinsulinemia induced by an infusion of glucose, and fatty acid oxidation was blocked by CPT I inhibition with oxfenicine (2, 38). It is important to note that data on the regulation of pyruvate dehydrogenase activity and citric acid cycle intermediates from these experiments have been reported separately (33).

**METHODS**

Studies were performed in anesthetized open-chest domestic swine (35–45 kg) in accordance with the Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23). The protocol was approved by the Institutional Animal Care and Use Committee at Case Western Reserve University.

**Surgical preparation.** The surgical preparation was described in detail previously (33). Briefly, pigs fasted overnight were sedated with Telazol (6 mg/kg im), anesthetized with isoflurane by mask (5%), intubated by tracheotomy, ventilated with 100% \( O_2 \), and maintained on isoflurane (0.75–1.5%) and ketamine (4 mg·kg\(^{-1}\)·h\(^{-1}\) iv). Ventilation was adjusted to maintain \( P_\text{CO}_2 \) and pH in the normal range (35–45 mmHg and 7.35–7.45, respectively) (36). A midline sternotomy was performed, a femoral artery and vein were catheterized, and the pig was heparinized (200 U/kg iv). A 24-mm vascular hydraulic occluder (Harvard Apparatus) was placed around the ascending aorta, and the anterior interventricular vein was catheterized for coronary venous blood sampling. The left atrial appendage was cannulated for injection of microspheres for measurement of blood flow (4, 33).

**Experimental protocol.** Four experimental groups were studied: control (CON; \( n = 7 \)) with sham instrumentation; dobutamine infusion (DOB; \( n = 6 \), 25 \( \mu \text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \)); dobutamine and glucose infusion (DOB+GLU; \( n = 9 \)); and dobutamine, glucose, and oxfenicine (DOB+GLU+OXF). \( MV_\text{O}_2 \), myocardial oxygen consumption. \(* P < 0.01\) compared with CON. There were no differences among the DOB, DOB+GLU, and DOB+GLU+OXF groups.

**Table 1. Rate-pressure product and \( MV_\text{O}_2 \) during treatment period**

<table>
<thead>
<tr>
<th></th>
<th>CON</th>
<th>DOB</th>
<th>DOB+GLU</th>
<th>DOB+GLU+OXF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rate pressure product ( \text{mmHg} \cdot \text{beats} \cdot \text{min}^{-1} )</td>
<td>7,770±510</td>
<td>31,510±3,080*</td>
<td>27,820±1,780</td>
<td>26,950±2,900</td>
</tr>
<tr>
<td>( MV_\text{O}_2 ), ( \mu \text{mol} \cdot \text{g}^{-1} \cdot \text{min}^{-1} )</td>
<td>2.90±0.53</td>
<td>10.59±3.14*</td>
<td>10.16±2.63</td>
<td>10.19±1.35</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>CON</th>
<th>DOB</th>
<th>DOB+GLU</th>
<th>DOB+GLU+OXF</th>
</tr>
</thead>
<tbody>
<tr>
<td>( VOL \cdot \text{H} \cdot \text{min}^{-1} )</td>
<td>3.14±10.16</td>
<td>3.08±1.96</td>
<td>3.08±1.96</td>
<td>3.08±1.96</td>
</tr>
<tr>
<td>( 1\text{7,770} \cdot 31,510 )</td>
<td>3,080±27,820</td>
<td>5,103±31,510</td>
<td>5,103±31,510</td>
<td>5,103±31,510</td>
</tr>
</tbody>
</table>

Values are means ± SE for control (CON) group and groups infused with dobutamine (DOB), dobutamine and glucose (DOB+GLU), and dobutamine, glucose, and oxfenicine (DOB+GLU+OXF). \( MV_\text{O}_2 \), myocardial oxygen consumption. \(* P < 0.01\) compared with CON. There were no differences among the DOB, DOB+GLU, and DOB+GLU+OXF groups.

Hemoglobin concentration and saturation were measured spectrophotometrically with a hemoximeter (AVOximeter 1000; A-VOX Systems, San Antonio, TX), and \( P_\text{O}_2 \), \( P_\text{CO}_2 \), and pH were measured (2). Fig. 1. Myocardial malonyl-CoA content at the end of the protocol. Four experimental groups were studied: control (CON; \( n = 7 \)) with sham instrumentation; dobutamine infusion (DOB; \( n = 6 \)); dobutamine and glucose infusion (DOB+GLU; \( n = 9 \)); and dobutamine, glucose, and oxfenicine infusion (DOB+GLU+OXF; \( n = 9 \)).
Table 2. Activities of enzymes that regulate myocardial malonyl-CoA content

<table>
<thead>
<tr>
<th></th>
<th>CON</th>
<th>DOB</th>
<th>DOB + GLU</th>
<th>DOB + GLU + OXF</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCD Vmax, μmol·g wet wt⁻¹·min⁻¹</td>
<td>0.68±0.098</td>
<td>0.58±0.06</td>
<td>0.68±0.08</td>
<td>0.75±0.06</td>
</tr>
<tr>
<td>MCD Km, μM</td>
<td>47±10</td>
<td>45±6</td>
<td>47±9</td>
<td>42±5</td>
</tr>
<tr>
<td>ACC activity, mmol·mg protein⁻¹·min⁻¹</td>
<td>5.27±0.97</td>
<td>4.13±0.22</td>
<td>4.30±0.28</td>
<td></td>
</tr>
<tr>
<td>AMPK activity, mmol·mg protein⁻¹·min⁻¹</td>
<td>0.61±0.03</td>
<td>0.66±0.05</td>
<td>0.64±0.02</td>
<td>0.63±0.03</td>
</tr>
</tbody>
</table>

Values are means ± SE. MCD, malonyl-CoA decarboxylase; ACC, acetyl-CoA carboxylase; AMPK, AMP-activated protein kinase. There were no differences among treatment groups.

were measured with a blood gas meter (Nova Biomedical, Waltham, MA). Plasma concentrations of free fatty acid and 3H2O and the 3H-fatty acid specific activity were measured as previously described (3, 24). Myocardial microsphere content was measured by neutron activation (25). Tissue malonyl-CoA concentration was determined by gas chromatography-mass spectroscopy as described by Reszko et al. (26).

RESULTS

Before treatment, there were no differences in rate-pressure product or MVO₂ among groups, and during the treatment period there was a significant increase in both parameters in all groups except CON (Table 1). Data on heart rate and blood pressure for these animal have been reported previously (33). MVO₂ was similar before treatment in all groups and increased significantly and to the same extent in the DOB, DOB + GLU, and DOB + GLU + OXF groups (P < 0.01). As previously reported, the infusion of glucose in the DOB + GLU and DOB + GLU + OXF groups resulted in a 3.5-fold increase in arterial glucose concentration (from 4 to 14 mM) and a 9.0-fold increase in insulin concentration (from 15 to 135 pM) (33).

Fatty acid oxidation. Arterial plasma free fatty acid concentration was twofold higher in the DOB group compared with the CON group but was not elevated in the DOB + GLU and DOB + GLU + OXF groups (see Ref. 33). The rate of free fatty acid oxidation was greater in the DOB group (0.357 ± 0.138 μmol·g⁻¹·min⁻¹) than in the CON group (0.137 ± 0.048 μmol·g⁻¹·min⁻¹), as previously reported (33). Free fatty acid oxidation was not significantly different in the DOB + GLU group (0.177 ± 0.067 μmol·g⁻¹·min⁻¹) compared with the DOB group; however, it was significantly suppressed in the DOB + GLU + OXF group (0.006 ± 0.016 μmol·g⁻¹·min⁻¹, P < 0.05 compared with DOB).

Malonyl-CoA content. There was a significant 35% decrease in malonyl-CoA content in the DOB group compared with the CON group (P < 0.006) (Fig. 1). The DOB + GLU group had significantly higher malonyl-CoA content than either the DOB (P < 0.004) or DOB + GLU + OXF groups (P < 0.001) (Fig. 1).

Enzyme activities. There were no significant differences in either the Km or Vmax value of MCD or in ACC or AMPK activities (see Table 2).

PKB phosphorylation. Because increased phosphorylation of PKB at Ser473 is indicative of PKB’s activation status (17), we also determined the level of PKB phosphorylation in our treatment groups. An increase in cardiac power with DOB resulted in a significant increase in the phosphorylation of PKB compared with the CON group (Fig. 2). There were no differences among the DOB, DOB + GLU, and DOB + GLU + OXF groups as determined by one-way ANOVA; however, when data from the DOB + GLU and DOB + GLU + OXF groups...
were pooled, they were different from the DOB group as shown using a t-test ($P < 0.05$).

**DISCUSSION**

The results of this study demonstrate that malonyl-CoA content falls with increased cardiac work and that this decrease in malonyl-CoA is highly dependent on the metabolic milieu and the rate of myocardial fatty acid oxidation. When cardiac work was increased under hyperglycemic and hyperinsulinemic conditions, the malonyl-CoA content was increased relative to stimulated conditions with normal glucose and insulin levels; however, when fatty acid oxidation was inhibited by oxfenicine, there was a sharp decrease in malonyl-CoA content. These responses were not associated with changes in MCD, ACC, AMPK, or PKB activities. These findings add further support to the concept that the elevated availability of acetyl-CoA from the oxidation of either carbohydrate or fatty acids plays a key role in the regulation of myocardial malonyl-CoA content (20, 28).

The results of the present investigation support the concept that the increase in fatty acid oxidation that occurs with an increase in cardiac energy expenditures is due to a fall in malonyl-CoA content and less inhibition of CPT I (10, 11, 28); however, the mechanism for the reduction in malonyl-CoA remains elusive. It is important to note that the inhibitory site for malonyl-CoA is on the cytosolic side of CPT I (12, 15, 22, 40) and that malonyl-CoA is found in both the cytosol and the mitochondria (12, 15, 22, 40). It is possible that adrenergic stimulation and/or high rates of cardiac energy expenditure cause a selective decrease in cytosolic acetyl-CoA or result in selective alterations in the activity or cellular location of cytosolic ACC or MCD, which lead to reduced production or greater clearance of cytosolic malonyl-CoA. However, we cannot draw conclusions regarding cytosolic levels from the whole tissue content. On the other hand, we can estimate the cytosolic concentration on the basis of assumed mitochondrial and cytosolic volumes and malonyl-CoA distribution between the two compartments. If we assume that $J$) the mitochondrial and the cytosolic compartments comprise 20 and 80% of the intracellular volume, 2) under normal cardiac work conditions malonyl-CoA concentration is the same in both compartments and 3) there is no change in malonyl-CoA concentration in the mitochondria during increased cardiac work, we can then estimate that the cytosolic malonyl-CoA content in the cytosol will decrease by 44% with dobutamine [compared with only a 35% decrease in whole tissue content (Fig. 1)]. Furthermore, if we assume that dobutamine stimulation results in a doubling of mitochondrial malonyl-CoA [secondary to an increase in acetyl-CoA concentration (13)], then we would predict a 69% decrease in cytosolic malonyl-CoA concentration. Thus, if the mitochondrial pool of malonyl-CoA does not change or if it increases when cardiac work is increased, there is a greater change in cytosolic malonyl-CoA. Future studies should assess the cellular distribution of malonyl-CoA by rapidly separating the cytosolic and mitochondrial compartments and measuring malonyl-CoA content in the two fractions.

The supply of acetyl-CoA in the cytosol is thought to be a major regulator of cytosolic malonyl-CoA concentration. Cytosolic malonyl-CoA is formed in the cytosol from acetyl-CoA by ACC (9–11, 18, 30, 31, 39); however, most of the acetyl-CoA in cardiomyocytes resides in the mitochondria (14). In the present experiment we did not observe any significant differences in total tissue acetyl-CoA content among groups (5.6 ± 0.4, 7.1 ± 1.2, 7.7 ± 0.8, and 8.5 ± 0.8 mmol/g for CON, DOB, DOB + GLU, and DOB + GLU + OXF groups, respectively). There are three sources of cytosolic acetyl-CoA: 1) cleavage of citrate via the ATP-citrate lyase reaction (23, 32), 2) acetyl carnitine that has been exported out of the mitochondrial matrix (21, 31), and 3) peroxisomal β-oxidation. Reszko et al. (27) recently provided convincing evidence that the latter process predominates in the heart. Clearly, additional work is needed before the role of cellular compartmentation in the regulation of malonyl-CoA kinetics and CPT I inhibition is understood.

There are several limitations in the present study that need to be addressed. First, our protocol did not include groups under normal workload conditions treated with hyperglycemia or hyperglycemia plus oxfenicine, because the goal of this study was to assess the effects of increased cardiac energy expenditure on malonyl-CoA content and the role of substrate supply on malonyl-CoA content during increased work. Ideally, one would also assess the effects of hyperglycemia and oxfenicine under normal workload conditions; however, this was not the primary goal of this study, and therefore we did not include these groups. A second limitation was the lack of measurement of the cytosolic concentration of malonyl-CoA. Future studies should rapidly separate the cytosol and mitochondria and measure malonyl-CoA content in the two compartments.

In conclusion, the results of the present study show that an acute increase in LV contractile power and $\dot{MV}_O_2$ reduces myocardial malonyl-CoA content independently of changes in the activities of MCD, ACC, and AMPK. These findings run counter to other situations, such as diabetes, ischemia-reperfusion, or maturation, where the activities of MCD, ACC, and AMPK clearly regulate malonyl-CoA content. On the other hand, the results of the present study show that the supply of substrate was a major determinant of malonyl-CoA levels under increased work conditions, with an increase in malonyl-CoA content with hyperglycemia plus hyperinsulinemia and a significant decrease in malonyl-CoA content with inhibition of fatty acid oxidation via CPT I inhibition. In addition, PKB activity was increased by an increase in cardiac power; however, this did not correspond with a decrease in AMPK activity, suggesting that there is no interaction between the two enzymes under these conditions.

**GRANTS**

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**REFERENCES**


MALONYL-COA REGULATION OF FATTY ACID OXIDATION

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