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

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Myocardial fatty acid oxidation is regulated by carnitine palmitoyltransferase I (CPT I), which is inhibited by malonyl-CoA. Increased cardiac power causes a fall in malonyl-CoA content and accelerated fatty acid oxidation; however, the mechanism for the decrease in malonyl-CoA is unclear. Malonyl-CoA is formed 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 oxfenicine. An increase in cardiac power was accompanied by increased myocardial O2 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 AMPK activity. In conclusion, the fall in malonyl-CoA content with β-adrenergic stimulation coincides with a reduced malonyl-CoA turnover that occurs in perfused rat hearts (28). There are conflicting results on the effects of β-adrenergic stimulation on MCD activity; in the isolated rat heart, Goodwin and Taegmeyer (9) observed a decrease in the Kmb of MCD with no change in Vmax with isoproterenol stimulation, whereas investigators in our laboratory (28) found no change in MCD activity with epinephrine stimulation.

Myocardial malonyl-CoA content and turnover also are determined by the supply of acetyl-CoA. Both parameters increase despite no change in the activities of ACC or MCD if acetyl-CoA levels increase, such as when the heart is perfused with the medium-chain fatty acid octanoate instead of with oleate or palmitate (20, 28). Cardiac malonyl-CoA content also is increased when acetyl-CoA levels are elevated by activation of pyruvate dehydrogenase with dichloroacetate, and this correlates with a decrease in fatty acid oxidation (31, 36). These observations suggest that increased availability of acetyl-CoA from the oxidation of either carbohydrate or fatty acids would increase malonyl-CoA content. Conversely, one would hypothesize that a reduction in acetyl-CoA production from fatty acid oxidation would decrease malonyl-CoA content.

Recent studies suggest that there is cross talk among cardiac workload, fatty acid metabolism, and the activation of protein kinase B (PKB) and AMPK (1, 17, 34). PKB is activated by an increase in cardiac afterload (1), and insulin activation of PKB is associated with inhibition of AMPK activity (17). A decrease in AMPK activity activates ACC, increases malonyl-CoA content, and reduces fatty acid oxidation. Studies in controlling MCD activity are less clear. Inhibition of malonyl-CoA degradation by MCD decreases malonyl-CoA turnover, increases malonyl-CoA content, and inhibits fatty acid oxidation, demonstrating a critical role for MCD in the regulation of fatty acid oxidation in the heart (7, 28). An increase in cardiac workload and myocardial oxygen consumption (MV02), such as occurs with physical exercise or catecholamine stress, increases fatty acid uptake and oxidation (8, 19, 35). Dobutamine infusion in pigs increases MV02 and fatty acid uptake and decreases malonyl-CoA content (10, 11), suggesting that there is less malonyl-CoA inhibition of CPT I and accelerated fatty acid oxidation. This fall in malonyl-CoA content is not accompanied by activation of AMPK or reduced ACC activity (10). The fall in malonyl-CoA content with β-adrenergic stimulation coincides with a reduced malonyl-CoA turnover that occurs in perfused rat hearts (28). There are conflicting results on the effects of β-adrenergic stimulation on MCD activity; in the isolated rat heart, Goodwin and Taegmeyer (9) observed a decrease in the Kmb of MCD with no change in Vmax with isoproterenol stimulation, whereas investigators in our laboratory (28) found no change in MCD activity with epinephrine stimulation.

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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 activation (34). It is not known whether β-adrenergic stimulation activates PKB in the heart, and it is not clear whether there is a relationship between insulin activation of PKB and AMPK activity. In addition, the combined effects of β-adrenergic stimulation, insulin stimulation, and fatty acid oxidation on PKB activation have not been evaluated.

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 \( \text{MV}_{\text{O}_2} \). 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 \( \mu \)g·kg\(^{-1}\)·min\(^{-1} \)); dobutamine and glucose infusion (DOB+GLU; \( n = 9 \), glucose infused at 15 mg·kg\(^{-1}\)·h\(^{-1} \)); and dobutamine, glucose, and oxfenicine infusion (DOB+GLU+OXF; \( n = 9 \), oxfenicine infused at 30 mg·kg\(^{-1}\)·h\(^{-1} \)). Each group underwent a 50-min equilibrium period followed by a 15-min treatment period. An infusion of \( [9,10-^3\text{H}]\text{oleate} \) tracer (0.2 \( \mu \text{Ci}/\text{min} \)) was initiated at the beginning of the equilibrium period to determine the free fatty acid oxidation rate. After 50 min of equilibration, animals received either no treatment (CON group) or were subject to a 15-min period of increased cardiac power induced by 1) a bolus injection of atropine (2 mg) to increase heart rate, 2) an infusion of dobutamine to increase cardiac contractility and further increase heart rate, and 3) inflation of the aortic cuff to maintain peak left ventricular (LV) systolic pressure at 170–180 mmHg. Arterial and coronary venous blood samples were taken immediately before treatment (at 42 and 47 min into the equilibration period) and during treatment (60 and 65 min) and were analyzed for blood oxygen content and plasma concentrations of free fatty acids, \([^3\text{H}]\text{oleate},\) and \( \text{H}_2\text{O}. \) Cardiac function was recorded on a commercial online data acquisition system. Microspheres for flow measurement were injected into the left atrial appendage at 40 and 58 min, and a reference withdrawal sample was taken from the femoral artery (4 ml/min). After 15 min of treatment, a large punch biopsy (4–5 g) was rapidly obtained from the anterior free wall, freeze clamped, placed in liquid nitrogen, and stored at −80°C until analyzed.

Analytical methods. 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

![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; \( n = 9 \)).](http://ajpheart.physiology.org/ By 10.220.33.1 on July 6, 2017 http://ajpheart.physiology.org/ Downloaded from http://ajpheart.physiology.org/ By 10.220.33.1 on July 6, 2017

Table 1. Rate-pressure product and \( \text{MV}_{\text{O}_2} \) 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·beats}·\min^{-1} )</td>
<td>7.770±510</td>
<td>3.150±3.080*</td>
<td>2.7820±1.780</td>
<td>2.6950±2.900</td>
</tr>
<tr>
<td>( \text{MV}_{\text{O}_2} ) ( \mu \text{mol}·\text{g}^{-1}·\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>

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). \( \text{MV}_{\text{O}_2} \), myocardial oxygen consumption. *\( P < 0.01 \) compared with CON. There were no differences among the DOB, DOB+GLU, and DOB+GLU+OXF groups.
Table 2. Activities of enzymes that regulate myocardial malonyl-CoA content

<table>
<thead>
<tr>
<th>Activity</th>
<th>CON</th>
<th>DOB</th>
<th>DOB + GLU</th>
<th>DOB + GLU + OXF</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCD V_max, μmol·g·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 K_max, μM</td>
<td>47±10</td>
<td>45±6</td>
<td>47±9</td>
<td>42±5</td>
</tr>
<tr>
<td>ACC activity, nmol·mg·protein⁻¹·min⁻¹</td>
<td>4.86±0.43</td>
<td>5.27±0.97</td>
<td>4.13±0.22</td>
<td>4.30±0.28</td>
</tr>
<tr>
<td>AMPK activity, nmol·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). MCD activity was measured in triplicate at 37°C by using the radiochemical assay of Kerner et al. (16) at malonyl-CoA concentrations of 10, 30, 100, and 300 μM and a run time of 10 min. ACC activity was measured in the absence of citrate as previously described (18). AMPK activities were measured as previously described (18) with substitution of AMARA as the peptide substrate in the AMPK assay.

*Immunoblot analysis.* Myocardial PKB phosphorylation was determined in homogenates of myocardium that were subjected to SDS-PAGE and transferred to nitrocellulose as previously described (17). Membranes were blocked in 5% milk-Tris-buffered saline (TBS)-0.1% Tween 20 and then immunoblotted at 1:1,000 dilution with rabbit anti-phospho-PKB (Ser473) antibody or rabbit anti-PKB (Cell Signaling Technology) overnight at 4°C. After being washed, the membranes were incubated with peroxidase-conjugated goat anti-rabbit secondary antibodies in 5% milk-TBS-0.1% Tween 20. After being further washed, the antibodies were visualized using the Pharmacia enhanced chemiluminescence Western blotting detection system.

*Calculations.* MVroscope, blood flow, and fatty acid oxidation were calculated as previously described (37). Rate-pressure product was calculated as heart rate × peak LV pressure. K_max and V_max values for MCD were calculated for each animal from Lineweaver-Burke plots.

*Statistical analysis.* Data are presented as means ± SE. Data for the CON and DOB groups were compared using a nonparametric t-test, and differences among data for the DOB, DOB + GLU, and DOB + GLU + OXF groups were determined using a Kruskal-Wallis one-way analysis of variance (ANOVA) on ranks with a Dunn’s pairwise multiple comparison. A P value < 0.05 was considered significant.

**RESULTS**

Before treatment, there were no differences in rate-pressure product or MVroscope 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). MVroscope 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 K_max or V_max 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 analyzed separately, we found that the phosphorylation of PKB was significantly greater in the DOB + GLU and DOB + GLU + OXF groups compared with the CON group (P < 0.05).

![Fig. 2. Myocardial protein kinase B (PKB) phosphorylation at the end of the protocol. Whole heart homogenates from the different treatment groups were subjected to immunoblot analysis using anti-phospho-PKB (Ser473) and anti-total-PKB antibodies. Blots were scanned, and data were quantified, expressed as phospho-PKB/PKB ratios, and presented as a fraction of the mean of the CON group. *P < 0.05, PKB in the DOB group was significantly greater than in the CON group.](http://ajpheart.physiology.org/)

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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 oxenifine, there was a sharp decrease in malonyl-CoA content. These responses were not associated with changes in 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 contents. 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 1) 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 nmol/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 oxenifine, 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 oxenifine 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 MVO2 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.

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