Absence of Malonyl Coenzyme A Decarboxylase in Mice Increases Cardiac Glucose Oxidation and Protects the Heart From Ischemic Injury

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Background—Acute pharmacological inhibition of cardiac malonyl coenzyme A decarboxylase (MCD) protects the heart from ischemic damage by inhibiting fatty acid oxidation and stimulating glucose oxidation. However, it is unknown whether chronic inhibition of MCD results in altered cardiac function, energy metabolism, or ischemic cardioprotection.

Methods and Results—Mcd-deficient mice were produced and assessed for in vivo cardiac function as well as ex vivo cardiac function, energy metabolism, and ischemic tolerance. In vivo and ex vivo cardiac function was similar in wild-type and mcd−/− mice. Ex vivo working hearts from mcd−/− and wild-type mice displayed no significant differences in rates of fatty acid oxidation, glucose oxidation, or glycolysis. However, cardiac deletion of mcd resulted in an increased expression of genes regulating fatty acid utilization that may compensate for the loss of MCD protein and likely contributes to the absence of changes in energy metabolism in the aerobic heart. Despite the lack of changes in fatty acid utilization, hearts from mcd−/− mice displayed a marked preference for glucose utilization after ischemia, which correlated with a significant cardioprotection of ischemic hearts from mcd−/− mice compared with wild-type mice.

Conclusions—Deletion of MCD markedly increases glucose oxidation and improves functional recovery of the heart after ischemia. As a result, chronic pharmacological inhibition of MCD may be a viable approach to treat myocardial ischemia. (Circulation. 2006;114:1721-1728.)

Key Words: drugs ■ fatty acids ■ glucose ■ ischemia ■ malonyl coenzyme A ■ metabolism ■ reperfusion

Malonyl coenzyme A (CoA) has emerged as a central mediator of many important cellular processes, including both fatty acid biosynthesis and fatty acid oxidation.1 Alterations in intracellular malonyl CoA levels have also been implicated in the regulation of a number of physiological and pathological processes, which include the following: (1) feeding behavior;2 (2) hepatocyte-mediated whole-body insulin resistance;3 (3) whole-body energy expenditure and subsequent storage of fat in adipose tissue;4 (4) pancreatic beta cell insulin secretion;5 (5) skeletal muscle–mediated insulin resistance and type 2 diabetes;6 and (6) cardiac ischemia/reperfusion injury.7

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In the heart, malonyl CoA is produced by acetyl CoA carboxylase and decarboxylated back to acetyl CoA by malonyl CoA decarboxylase (MCD). Cardiac MCD is now recognized as a central regulator of fatty acid oxidation via the control of intracellular malonyl CoA levels.8 The key enzyme involved in mitochondrial long-chain fatty acid uptake is carnitine palmitoyltransferase 1 (CPT1),1,9 and it is this enzyme that is inhibited by endogenous malonyl CoA.9–12 Therefore, by regulating malonyl CoA levels in the heart, MCD can control fatty acid oxidation through modifying malonyl CoA–mediated inhibition of CPT1.

Using recently developed MCD inhibitors, we confirmed that MCD is a major regulator of intracellular malonyl CoA levels in the heart.7 Pharmacological MCD inhibition increases intracellular malonyl CoA levels, decreases fatty acid oxidation, and accelerates glucose oxidation in both ex vivo rat hearts and in vivo pig hearts.7 In addition, this switch in energy substrate preference improves cardiac function during and after ischemia, suggesting that pharmacological inhibition of MCD is a novel approach to treating ischemic heart disease.7
Although the short-term regulation of MCD with the use of MCD inhibitors has shown promise for switching energy substrate preference and preventing ischemia/reperfusion injury, the effects of long-term inhibition of cardiac MCD on energy metabolism and ischemia/reperfusion injury are unknown. Similarly, the effects of long-term systemic in vivo inhibition of MCD are also unknown. Because MCD deficiency in humans is associated with a metabolic disorder known as malonic aciduria, with symptoms that include cardiomyopathy, it is possible that long-term systemic inhibition of MCD may be associated with undesirable side effects. To address these issues, mcd knockout mice were generated to study the effect of long-term MCD deficiency on mouse phenotype and cardiac metabolism. Using mice lacking MCD, we also determined whether long-term inhibition of MCD alters cardiac function and protects the heart from ischemic injury.

Methods

Targeted Mutation of the mcd Locus and Breeding of mcd Knockout Mice
Selected stem cells containing the linearized targeting vector with exon 1 of the mcd gene replaced with a neomycin cassette were injected into C57BL6J (CLEA Japan, Tokyo, Japan) blastocysts to generate chimeras. To obtain mcd<sup>+/-</sup> (homozygous) mutants, male chimeras were mated with C57BL6 females. mcd<sup>+/-</sup> (homozygous) mutant mice (B6;129S7-mcd<sup>tm1Jish</sup>) were produced from mcd<sup>+/-</sup> crosses (detailed methods are provided in the online-only Data Supplement).

In Vivo Assessment of Cardiac Function and Exercise Tolerance Test in Wild-type and mcd<sup>+/-</sup> Mice
Pulmonary artery acceleration time (PAAT), a validated measure of mean pulmonary artery (PA) pressure in rodent models, and left ventricular wall thickness were assessed serially in nonanesthetized mice with the use of Doppler and 2-dimensional/M-mode echocardiography with a 12-MHz transducer (Phillips, Andover, Mass), as we have previously described. Exercise tolerance tests were performed with incremental increases in treadmill belt speed until the mouse exhibited signs of exhaustion (detailed methods are provided in the online-only Data Supplement).

Isolated Working Mouse Heart Perfusions and Measurement of Ex Vivo Cardiac Function
Ex vivo perfused working mouse hearts from wild-type and mcd<sup>+/-</sup> mice were aerobically perfused essentially as we have previously described (detailed methods are provided in the online-only Data Supplement). Heart rates, pressures, and flows were also determined as previously described (detailed methods are provided in the online-only Data Supplement).

Isolation of Tissue From Wild-type and mcd<sup>+/-</sup> Mice
Control and mcd knockout mouse (male mice; aged 12 to 14 weeks) hearts were excised and immediately frozen in liquid nitrogen.

MCD Activity and Malonyl Coenzyme A Measurements
MCD activities were measured from homogenized frozen mouse hearts with the use of a radiometric assay as previously described. Extraction of CoA esters and the measurement of malonyl CoA levels were performed as previously described.

Quantitative Reverse Transcriptase–Polymerase Chain Reaction Analysis
RNA extraction and quantitative reverse transcriptase–polymerase chain reaction (RT-PCR) of samples were performed by previously described methods. Specific quantitative assays were designed from mouse sequences available in GenBank. Taqman assays used in this study are presented in the Table in the online-only Data Supplement or have been reported previously. All gene expression data are normalized to the housekeeping gene cyclophilin, which did not differ between the groups investigated (data not shown).

Statistical Analysis
Statistical analysis of the data was performed with the use of either a 2-tailed Student t test or a 2-way ANOVA when appropriate. A probability value of <0.05 is considered significant.

Animal Care
The University of Alberta adheres to the principles for biomedical research involving mice developed by the Council for International Organizations of Medical Sciences and complies with the Canadian Council on Animal Care guidelines. The University of Alberta Animal Policy and Welfare Committee approved all experimental procedures involving animals.

The authors had full access to the data and take responsibility for its integrity. All authors have read and agree to the manuscript as written.

Results

Although we have previously shown that short-term pharmacological inhibition of MCD results in a switch in energy substrate preference from fatty acid oxidation to glucose oxidation and ischemic cardioprotection, it is not clear whether long-term inhibition of MCD results in a similar switch in energy metabolism and ischemic cardioprotection. To investigate this further, we produced mice in which the MCD gene was deleted (mcd<sup>-/-</sup>). mcd<sup>-/-</sup> mice were generated by replacing exon 1 of the MCD gene with a neomycin cassette. Offspring heterozygous for the mutated mcd allele were interbred, and the offspring were genotyped by Southern blot analysis (Figure 1a). Heterozygous and mcd<sup>-/-</sup> mice were produced with a near-mendelian frequency, and neither the heterozygous nor the mcd<sup>-/-</sup> mice displayed any overt phenotypes. Northern blot analysis of liver and heart total RNA demonstrated complete deletion of the mcd transcript (Figure 1b), a deletion which led to the absence of MCD protein in the heart (Figure 1c) and in all other tissues measured (not shown). Cardiac MCD activity was also completely abolished in the mcd<sup>−/−</sup> mouse hearts (Figure 1d). In accordance with the loss of MCD activity, cardiac malonyl CoA levels were significantly elevated by ~3-fold (Figure 1c).

To assess in vivo heart function, wild-type and mcd<sup>−/−</sup> mice (12 to 18 weeks of age) were evaluated by Doppler and 2-dimensional/M-mode echocardiography. The heart rates in wild-type and mcd<sup>−/−</sup> mice were not significantly different (481±25 versus 433±23 bpm, respectively). Pulsed Doppler interrogation in the pulmonary arteries (short-axis parasternal view) allowed for measurement of the PAAT, ie, the time from the beginning to the peak of the Doppler signal. PAAT correlates very well with mean PA pressure and is used routinely in clinical echocardiography. We found no difference in the PAAT between wild-type and mcd<sup>−/−</sup> mice (Figure 2a). Because the mean PA pressure increases as the
left ventricular end-diastolic pressure and left atrial pressure increase (as commonly occurs in secondary pulmonary hypopertension because of left ventricular failure), these data indirectly suggest that the filling pressures in the left ventricle between wild-type and mcd⁻/⁻ mice are not different.

Further evidence that left ventricular function (and thus cardiac output) is similar between wild-type and mcd⁻/⁻ mice comes from our observation that the mice had similar maximal exercise performance in the rodent treadmill, where wild-type and mcd⁻/⁻ mice ran a similar distance and for a similar time (Figure 2b and 2c). The maximal distance covered in a 6-minute period (ie, the 6-minute walk test) is commonly used as an end point in heart failure clinical trials (the longer the 6-minute walk, the better is the prognosis). The mice treadmill test is similar to the 6-minute walk used in humans. M-mode echocardiography (Figure 2d) and gross morphology (Figure 2e) also revealed no differences in left ventricular thickness between the wild-type and mcd⁻/⁻ mice. Taken together, these noninvasive in vivo data provide strong evidence that the left ventricular function between the 2 groups of mice is similar under normal (ie, nonischemic) conditions.

To assess the functional and metabolic consequences of long-term MCD deletion and increased malonyl CoA levels in the heart, we subjected wild-type and mcd⁻/⁻ mouse hearts to ex vivo aerobic perfusions to measure cardiac function and rates of fatty acid oxidation, glucose oxidation, and glycolysis. As with observations in the in vivo mice hearts, ex vivo perfused working hearts from mcd⁻/⁻ mice displayed no significant differences in mechanical function compared with wild-type hearts (Table 1). Surprisingly, hearts from mcd⁻/⁻

<table>
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<th>TABLE 1. Cardiac Function in the Aerobically Perfused Ex Vivo Working Mouse Heart</th>
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<tr>
<td><strong>Wild-Type</strong> (n=13)</td>
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<tr>
<td>Heart rate, bpm</td>
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<tr>
<td>Rate pressure product, beat·min⁻¹·mm Hg·10⁻³</td>
</tr>
<tr>
<td>Cardiac work, mL·min⁻¹·mm Hg·10⁻²</td>
</tr>
<tr>
<td>Cardiac output, mL·min⁻¹</td>
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<tr>
<td>Aortic flow, mL·min⁻¹</td>
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<td>Cardiac power, mJ/min</td>
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Statistical analysis of the data was performed with a 2-tailed Student t test.
mice also did not exhibit significant changes in cardiac energy metabolism and had rates of palmitate oxidation, glucose oxidation, and glycolysis that were similar to those of hearts from wild-type mice (Figure 3a to 3c). This finding was inconsistent with our previous results showing that short-term MCD inhibition has profound effects on fatty acid oxidation and glucose oxidation. Therefore, we hypothesized that long-term MCD inhibition in the mcd⁻⁄⁻ mouse heart may lead to compensatory alterations in the expression of metabolic genes involved in fatty acid oxidation and glucose oxidation. This includes CD36, which is involved in fatty acid transport; uncoupling protein 3 (UCP3), mitochondrial thioesterase 1 (MTE1), and CPT1, which are associated with increased fatty acid oxidation; and pyruvate dehydrogenase kinase-4 (PDK-4), which is one isoform of the family of kinases responsible for phosphorylating and inhibiting the pyruvate dehydrogenase complex, thus decreasing glucose oxidation.

We therefore measured expression of these various metabolic genes and found that expressions of cd36, ucp3, mte1, and cpt1 were increased in mcd⁻⁄⁻ mouse hearts compared with wild-types (Figure 4a to 4d). In addition, pdk4 expression was significantly increased, providing a mechanism by which glucose oxidation rates could be reduced (Figure 4e). In contrast, expressions of medium-chain acyl CoA dehydrogenase (mcad), which is involved in fatty acid β-oxidation, and long-chain acyl CoA synthetase 1 (acsl1), which converts intracellular long-chain fatty acids to long-chain fatty acyl-CoAs, were not significantly different between wild-type and mcd⁻⁄⁻ hearts (not shown), suggesting that not all peroxisome proliferator-activated receptor-α (PPARα)–responsive genes involved in fatty acid utilization have been upregulated.

Our data suggest that the absence of any metabolic changes in aerobically perfused mcd⁻⁄⁻ mouse hearts could be accounted for by compensatory alterations in the expression of fatty acid metabolic genes. We also wanted to understand the effect of mcd deletion on energy metabolism in the postischemic setting. After a severe reversible period of ischemia, fatty acid oxidation rapidly recovers and dominates as a source of overall cardiac energy production. These high rates of fatty acid oxidation are detrimental to postischemic functional recovery primarily because of a decrease in glucose oxidation. We therefore investigated the effect of mcd deletion on fatty acid oxidation after an acute ischemic stress. Wild-type and mcd⁻⁄⁻ mouse hearts were subjected to 30 minutes of aerobic perfusion, followed by 20 minutes of no-flow ischemia and 40 minutes of aerobic reperfusion. Although plasma free fatty acids in wild-type and mcd⁻⁄⁻ mice were of normal value (~0.8 mmol/L) and were not different from one another (0.78 ± 0.06 versus 0.80 ± 0.10 mmol/L, respectively), hearts were perfused with 1.2 mmol/L palmitate to more closely mimic the clinical situation of ischemia and reperfusion (ie, after myocardial infarction or after surgery), in which case the heart is normally exposed to high levels of fatty acid primarily
because of activation of lipolysis of adipose tissue resulting from stress-induced increases in plasma catecholamines.\(^{25}\)

During reperfusion, hearts from mcd\(^{-/-}\) mice displayed no significant change in palmitate oxidation or glycolytic rates but did demonstrate a dramatic increase in glucose oxidation rates compared with wild-type hearts (Figure 5a to 5c). This resulted in an \(\approx50\%\) increase in the contribution of glucose oxidation to adenosine triphosphate (ATP) production in the mcd\(^{-/-}\) mice compared with wild-type mice (Figure 5d). Thus, after acute ischemic stress, hearts lacking MCD displayed a dramatic switch in energy substrate preference, in which glucose oxidation became the predominant source of energy for the heart. This change in energy substrate utilization represents a metabolic phenotype that can protect the heart against ischemic injury during reperfusion.\(^{7}\) Indeed, during aerobic reperfusion, functional recovery of cardiac energy metabolism and functional performance, whether measured in vivo (Figure 2) or ex vivo (Table 1). This finding is not consistent with what may have been predicted on the basis of MCD deficiency in humans and/or in patients with cardiomyopathies actually possess mutations in MCD.\(^{13,33–36}\) It is interesting to note that in patients who completely lack MCD, no cardiomyopathies were observed, and a number of the MCD-deficient patients with cardiomyopathies actually possess mutations in mcd that result in subcellular protein mistargeting and not the complete absence of MCD protein.\(^{35}\)

**Discussion**

High rates of fatty acid oxidation can contribute to myocardial ischemic injury by inhibiting glucose oxidation.\(^{26–28}\) Because glycolysis is accelerated during and after ischemia, fatty acid inhibition of glucose oxidation causes an imbalance between glycolysis and glucose oxidation, resulting in the accumulation of deleterious by-products (lactate and H\(^+\)) that contribute to a decrease in cardiac function and efficiency.\(^{29,30}\) As a result, therapeutic strategies that inhibit fatty acid oxidation and stimulate glucose oxidation have been shown to be cardioprotective in the ischemic/reperfused heart.\(^{31}\)

Inhibition of MCD is one approach to decreasing fatty acid oxidation, stimulating glucose oxidation, and decreasing ischemic injury. Indeed, we have previously demonstrated that short-term pharmacological inhibition of MCD is effective in switching energy substrate preference from fatty acid oxidation to glucose oxidation, and this switch in energy substrate preference is beneficial to the ischemic/reperfused heart.\(^{7}\)

In addition to examining the effects of acute MCD inhibition on cardiac energy metabolism and ischemic cardioprotection, we were also interested in determining the effect of chronic MCD inhibition on these parameters. Toward this end, we developed mice in which MCD was deleted. These mice did not display any overt phenotype compared with wild-type mice. Indeed, hearts from mcd\(^{-/-}\) mice appeared normal and did not display any alterations in mechanical performance, whether measured in vivo (Figure 2) or ex vivo (Table 1). This finding is not consistent with what may have been predicted on the basis of MCD deficiency in humans being associated, in some instances, with cardiomyopathies.\(^{13}\) However, only 18 patients with MCD deficiency have been reported in the literature,\(^{32}\) and of these, it appears that only 5 developed cardiomyopathy.\(^{13,33–36}\) It is interesting to note that in patients who completely lack MCD, no cardiomyopathies were observed, and a number of the MCD-deficient patients with cardiomyopathies actually possess mutations in mcd that result in subcellular protein mistargeting and not the complete absence of MCD protein.\(^{35}\) Together, these findings

**TABLE 2. Cardiac Function in the Aerobically Reperfused Ex Vivo Working Mouse Heart After Ischemia**

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<th>Wild-Type ((n=13))</th>
<th>MCD Knockout ((n=15))</th>
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<tbody>
<tr>
<td>Rate pressure product, b/min (\cdot) mm Hg (\cdot) 1 (\times) 10(^{-3})</td>
<td>10 ± 2.1</td>
<td>16 ± 1.3*</td>
</tr>
<tr>
<td>Cardiac work, mL (\cdot) min (^{-1}) \cdot) mm Hg (\cdot) 10(^{-2})</td>
<td>2.3 ± 0.8</td>
<td>5.7 ± 0.6*</td>
</tr>
<tr>
<td>Cardiac output, mL (\cdot) min (^{-1})</td>
<td>3.4 ± 1.2</td>
<td>9.4 ± 0.9*</td>
</tr>
<tr>
<td>Aortic flow, mL (\cdot) min (^{-1})</td>
<td>1.5 ± 0.6</td>
<td>5.5 ± 0.9*</td>
</tr>
<tr>
<td>Cardiac power, mJ/min</td>
<td>25.2 ± 9.1</td>
<td>61.4 ± 6.9*</td>
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Statistical analysis of the data was performed with a 2-tailed Student t test. *\(P<0.05\), significantly different from wild-type.
suggest that either MCD deficiency, by itself, does not directly cause cardiomyopathy or that MCD protein mistargeting is responsible for the cardiomyopathy. However, the low number of cases that possess this rare autosomal recessive disorder makes it difficult to establish a correlation of MCD deficiency with cardiomyopathy. Despite this, because fewer than half of the MCD-deficient patients develop cardiomyopathies, it is likely that MCD deficiency itself is not sufficient to cause cardiomyopathy, and other factors may be involved. Given these findings in humans and the observations that mcd−/− mice appear normal and that hearts from these mice are not functionally compromised, it is likely that long-term pharmacological inhibition of MCD may not produce any untoward cardiac side effects directly via MCD inhibition or by promoting MCD protein mistargeting. Clearly, however, these concepts need to be tested empirically in a variety of animal species before MCD inhibitors can be tested in the clinical setting.

One remarkable finding in our mcd−/− mice is that the cardiac energy substrate profile in the ex vivo perfused working heart is not different from that in wild-type mice (Figure 2a to 2c). Compensatory increases in PPARα-regulated genes controlling fatty acid utilization and glucose oxidation appear to be one explanation for maintained fatty acid oxidation rates despite high levels of malonyl CoA and a proposed inhibition of CPT1 activity. This is consistent with the findings of Lionetti et al,37 who reported that CPT1 inhibition increases expression of PPARα target genes. In the present study we examined a number of PPARα-regulated genes that control fatty acid utilization and glucose oxidation. For example, fatty acids transported into the heart by the fatty acid transport protein cd36 have been shown to account for ≅50% of the overall fatty acids utilized by the heart.15 Expression of cd36 is significantly increased in hearts from mcd−/− mice, suggesting that fatty acid transport is increased in these mice (Figure 4a). Furthermore, 2 other PPARα-regulated genes that are associated with accelerated rates of fatty acid oxidation are also significantly increased in hearts from the mcd−/− mice. These include ucp3 and mte1, which are increased 2-fold and 1.8-fold, respectively (Figure 4b and 4c). Because of the relative importance of CPT1 in the control of fatty acid oxidation in the mcd−/− mouse hearts, we also measured cpt1 mRNA expression in addition to CPT1 protein expression. Expression of cpt1 mRNA was significantly increased (1.6-fold) in hearts from mcd−/− mice compared with its expression in wild-type hearts (Figure 4d). Despite this increase in CPT1 mRNA expression, we did not observe a significant increase in CPT1 protein expression (Data Supplement Figure, a). This apparent disconnect may be explained by the lower sensitivity of immunoblots compared with quantitative RT-PCR and thus their inability to detect subtle changes in protein expression. Indeed, CPT1 protein increased 1.4-fold in hearts from mcd−/− mice compared with wild-type hearts, although this did not reach significance. Finally, pdk4 expression was significantly increased in hearts from the mcd−/− mice, providing a mechanism by which glucose oxidation rates could be reduced in a further attempt to promote fatty acid oxidation. That is, increased PDK4 would phosphorylate and inhibit the pyruvate dehydrogenase complex, thus decreasing glucose oxidation as a compensatory attempt to accelerate fatty acid oxidation via the Randle cycle. Although we suggest that the observed changes in gene expression are mediated by PPARα, other PPARα-responsive genes such as mcad and acs1 are not altered (not shown). However, because of high levels of expression of these genes in the adult heart, it is often difficult to further increase their expression through PPARα activation. Alternatively, PPARδ may be responsible for some of these changes in gene expression because PPARδ has also been shown to regulate several genes involved in fatty acid transport and oxidation along with pdk4 expression.38,39 On the basis of our data, we propose that initial inhibition of fatty acid oxidation, through loss of MCD, results in increased intracellular PPARα or PPARδ ligands (ie, fatty acyl derivatives). The lack of increases in expression of either PPARα or PPARδ (Data Supplement Figure, b and c) provides added support to this hypothesis. Interestingly, triacylglycerol levels (an indirect marker of the levels of fatty acyl derivatives) were not increased in mcd−/− mouse hearts compared with wild-type hearts (44.1±3.3 versus 39.5±3.1 μmol/g dry wt, respectively). However, it cannot be ruled out that total intracellular lipids may not accurately reflect the amount of nuclear lipids that act as PPARα or PPARδ ligands in mcd−/− mouse hearts. In addition, because increases in these PPARα- and/or PPARδ-responsive genes may be a mechanism by which the mcd−/− mouse hearts would prevent overt lipotoxicity, significant increases in intracellular lipid content in mcd−/− mouse hearts at the age at which these mice were studied may not be readily observed.

An interesting finding in our mcd−/− mice is that cardiac energy metabolism is altered after acute ischemic stress (Figure 5) despite the fact that energy metabolism is not altered in the aerobically perfused, nonischemic hearts (Figure 3). During reperfusion, hearts from mcd−/− mice displayed no significant change in palmitate oxidation or glycolytic rates relative to wild-type hearts but did demonstrate a significant and dramatic increase in glucose oxidation rates (Figure 5). This finding is not consistent with the compensatory increase in PDK4 expression in the hearts from mcd−/− mice. However, flux through the pyruvate dehydrogenase enzyme complex (PDC) is dependent on both direct feedback inhibition of PDC by-products of fatty acid oxidation and PDK phosphorylation of PDC. Indeed, inhibition of pyruvate oxidation in the heart can readily occur independently of PDK phosphorylation of PDC.40,41 We have also previously shown that glucose oxidation rates in the mouse heart can be dramatically altered independently of PDK phosphorylation and inhibition of PDC and that flux of pyruvate through PDC is regulated by fatty acid oxidation reaction products.42 In the case of hearts from the mcd−/− mice, these fatty acid oxidation reaction products would be dramatically reduced after ischemia relative to wild-type hearts, likely relieving the inhibition of PDC. In the normal heart, it is clear42 that during reperfusion of ischemic hearts, fatty acid oxidation quickly recovers and dominates as a source of ATP production.24 In accordance with our observations in short-term inhibition of MCD in rat hearts,7 postischemic mcd−/− mouse hearts exhibit lower fatty acid oxidation contribution to overall ATP production.
production compared with wild-type hearts (Figure 5d), as well as a shift toward glucose oxidation. This switch in substrate utilization profile was associated with a significant improvement in cardiac function during reperfusion after ischemia (Figure 5e) and is in agreement with experimental observations in a number of ex vivo and in vivo animal models. The increase in glucose oxidation seen during reperfusion may have occurred because of an inability of the heart to increase fatty acid oxidation as a result of the lack of MCD. From this, we propose that the ability of the mcd−/− mouse heart to use fatty acids for β-oxidation during acute stress is likely inhibited, fatty acid oxidation reaction products are subsequently reduced, and flux of pyruvate through PDC is increased independent of PDK activity. Together, these changes result in the mcd−/− mouse heart relying more on glucose oxidation for the ATP necessary for increased energy demand. Indeed, this is exactly what we observed in our study (Figure 5). Therefore, despite the compensatory adaptations in cardiac substrate utilization that occur in the mcd−/− mice, acute stresses such as ischemia/reperfusion may alter cardiac metabolism such that the metabolic phenotype of hearts from mcd−/− mice becomes more evident. As a result, this switch in cardiac substrate preference away from fatty acids and toward glucose suggests that chronic pharmacological inhibition of MCD may be a viable approach to the treatment of myocardial ischemia.

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Disclosures
A portion of this work was performed in collaboration with Chugai Pharma Japan, which assisted in the creation of the MCD knockout mice. Metabolic Modulators Research Ltd, a University of Alberta spinoff company, was also involved in the study. Both Chugai Pharma Japan and Metabolic Modulators Research Ltd have commercial interests in the development of MCD inhibitors. The authors and their respective affiliations have been disclosed in the byline and affiliations paragraph. The other authors report no conflicts.

References


**CLINICAL PERSPECTIVE**

Ischemic heart disease is a major cause of death and disability in Western society. A major problem associated with myocardial ischemia is that it results in an energetic deficit in the heart, thereby compromising contractile function and muscle viability. Ischemia also results in alterations in the subcellular control of energy metabolism, with the result that the heart does not optimally produce and use energy. This inefficient energy use includes the ischemic heart’s excessive reliance on fatty acid oxidation as a source of energy. Despite the contribution of alterations in energy metabolism to the severity of ischemic injury, current pharmacological treatment of ischemic heart disease does not routinely target myocardial energy metabolism. Recent clinical and experimental evidence has shown that switching fuel utilization in the heart away from fatty acids and towards glucose is an effective approach to relieving the symptoms associated with ischemic heart disease. In this article, we provide evidence that long-term inhibition of malonyl Coenzyme A decarboxylase (MCD), an important enzyme controlling fatty acid oxidation, will decrease cardiac fatty acid oxidation and can benefit the ischemic heart. We also show that dramatic inhibition of MCD is well tolerated, likely because of compensatory alternative mechanisms that restore fatty acid oxidation rates to normal levels. During the acute stresses of ischemia and reperfusion, MCD inhibition can switch cardiac substrate preference away from fatty acids and towards glucose, and can improve cardiac function. Our study suggests that long-term pharmacological inhibition of MCD may be a viable approach to optimizing cardiac energy metabolism and clinically treating myocardial ischemia.
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