Stimulation of glucose oxidation protects against acute myocardial infarction and reperfusion injury

John R. Ussher†, Wei Wang†, Manoj Gandhi†, Wendy Keung†, Victor Samokhvalov†, Tatsujiro Oka†, Cory S. Wagg†, Jagdip S. Jaswal†, Robert A. Harris‡, Alexander S. Clanachan†, Jason R.B. Dyck†, and Gary D. Lopaschuk†*

1Cardiovascular Research Centre, Mazankowski Alberta Heart Institute, University of Alberta, 423 Heritage Medical Research Centre, Edmonton, Canada T6G 2S2; and 2Department of Biochemistry and Molecular Biology, Indiana University School of Medicine, Indianapolis, IA, USA

Received 23 November 2011; revised 17 February 2012; accepted 16 March 2012; online publish-ahead-of-print 21 March 2012

Aims

During reperfusion of the ischaemic myocardium, fatty acid oxidation rates quickly recover, while glucose oxidation rates remain depressed. Direct stimulation of glucose oxidation via activation of pyruvate dehydrogenase (PDH), or secondary to an inhibition of malonyl CoA decarboxylase (MCD), improves cardiac functional recovery during reperfusion following ischaemia. However, the effects of such interventions on the evolution of myocardial infarction are unknown. The purpose of this study was to determine whether infarct size is decreased in response to increased glucose oxidation.

Methods and results

In vivo, direct stimulation of PDH in mice with the PDH kinase (PDHK) inhibitor, dichloroacetate, significantly decreased infarct size following temporary ligation of the left anterior descending coronary artery. These results were recapitulated in PDHK 4-deficient (PDHK4−/−) mice, which have enhanced myocardial PDH activity. These interventions also protected against ischaemia/reperfusion injury in the working heart, and dichloroacetate failed to protect in PDHK4−/− mice. In addition, there was a dramatic reduction in the infarct size in malonyl CoA decarboxylase-deficient (MCD−/−) mice, in which glucose oxidation rates are enhanced (secondary to an inhibition of fatty acid oxidation) relative to their wild-type littermates (10.8 ± 3.8 vs. 39.5 ± 4.7%). This cardioprotective effect in MCD−/− mice was associated with increased PDH activity in the ischaemic area at risk (1.89 ± 0.18 vs. 1.52 ± 0.05 μmol/g wet weight/min).

Conclusion

These findings demonstrate that stimulating glucose oxidation via targeting either PDH or MCD decreases the infarct size, validating the concept that optimizing myocardial metabolism is a novel therapy for ischaemic heart disease.

Keywords

Acute myocardial infarction • Ischaemia/reperfusion • Glucose oxidation • Pyruvate dehydrogenase • Malonyl CoA decarboxylase

1. Introduction

Ischaemic heart disease is a major health problem worldwide, affecting North Americans more adversely than any other pathological condition. Although numerous mechanisms contribute to ischaemic injury, there is clear evidence that cardiac dysfunction during and following myocardial ischaemia is mediated, at least in part, by the type of energy substrate utilized by the heart. For example, following ischaemia and during reperfusion, an excessive reliance on fatty acids contributes to cardiac dysfunction. Elevated fatty acid oxidation rates result in the subsequent inhibition of glucose oxidation rates in the heart. Furthermore, glycolytic rates remain high and are thus uncoupled from glucose oxidation, thereby increasing proton production and decreasing cardiac efficiency. We and others have demonstrated that overcoming fatty acid oxidation-induced inhibition of glucose oxidation in the heart (either by directly stimulating glucose oxidation, or by inhibiting fatty acid oxidation), improves the recovery of post-ischaemic cardiac function.

Pyruvate dehydrogenase (PDH) is the rate-limiting enzyme for glucose oxidation, controlling the flux of pyruvate into the mitochondria for oxidative metabolism. With respect to fatty acid utilization,
malonyl CoA decarboxylase (MCD) is a key enzyme in the regulation of fatty acid oxidation, via degrading malonyl CoA, a potent endogenous inhibitor of carnitine palmitoyltransferase 1 (CPT-1), the rate-limiting enzyme for mitochondrial fatty acid uptake. In previous reports, we demonstrated that pharmacological activation of PDH, pharmacological inhibition of MCD, or genetic deficiency of MCD in mice (MCD−/−), increases myocardial glucose oxidation rates and provides protection against ischaemia/reperfusion injury in isolated working hearts. Whether such findings are transferable to a more clinically relevant in vivo model of acute myocardial infarction (AMI) is unknown. Our objective was to test the hypothesis that PDH activation or MCD inhibition decreases the infarct size following the induction of acute myocardial infarction (AMI) via temporary ligation of the LAD coronary artery.

2. Methods

2.1 Animal care and in vivo ischaemia/reperfusion

The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85–23, revised 1996). The University of Alberta adheres to the principles for biomedical research involving animals developed by the Council for International Organizations of Medical Sciences and complies with the Canadian Council on Animal Care guidelines. All animal procedures were approved by the University of Alberta Health Sciences Animal Welfare Committee. MCD−/− and pyruvate dehydrogenase kinase 4 (PDHK4−/−) mice were generated as previously described. Twelve-week-old MCD−/− mice, PDHK4−/− mice, and their wild-type (WT) littermate controls were anaesthetized with an ip injection of sodium pentobarbital (60 mg/kg). Once surgical anaesthesia is achieved (determined via absence of toe-pin reflex and regular respiration), a left thoracotomy is performed and the ribs are spread apart to expose the left ventricle. Ischaemia is induced by ligating the LAD coronary artery at ~2 mm below its bifurcation from the left main coronary artery with a 7–0 polypropylene suture against a 2–0 silk suture. After 30 min of ischaemia, the 7–0 suture is released to induce reperfusion. Once the skin is sutured back together, an intramuscular injection of buprenorphine (0.01 mg/kg) was provided for analgesia and the animal allowed to recover. After 24 h of reperfusion, animals were euthanized by ip injection of sodium pentobarbital (320 mg/kg), and hearts were subsequently excised and immersed in ice-cold Krebs–Henseleit bicarbonate solution, following which the aorta was cannulated and perfused aerobically for 45 min, followed by 15 min of global no-flow ischaemia, and 30 min of reperfusion. At the end of reperfusion, hearts were immediately frozen in liquid N₂ with Wollenberger tongs, and stored at −80°C.

2.2 Infarct size assessment

Hearts were excised from euthanized animals and immediately perfused as Langendorff preparations. The LAD coronary artery is recoupled and perfused retrogradely through the aorta with Evans blue dye to delineate the area at risk (AAR). The heart is frozen at −20°C for 30 min and subsequently cut into ~10 μM slices that are incubated for 10 min in 1% triphenyltetrazoliumchloride (TTC) at 37°C. Slices are left in 10% formaldehyde overnight to enhance colour contrast and photographed. Necrotic cells lack viable mitochondria and are unable to reduce TTC, and therefore stain white. The viable cells in the AAR stain red/pink, whereas the non-ischaemic viable myocardium stains an intense blue as a result of the Evans blue. Photographs of heart slices were analysed by planimetry with ImageJ to quantify the infarct size.

2.3 Area at risk isolation

In another set of experiments, 12-week-old MCD−/− mice and their WT littermate controls underwent our in vivo ischaemia/reperfusion protocol, but were euthanized at 30 min of reperfusion. Hearts were immediately excised, perfused as Langendorff preparations, and their LAD coronary artery was recoupled to determine the AAR with retrograde perfusion of the aorta with Evans blue dye. The heart was frozen in liquid nitrogen-cooled isopentane for 1 min and subsequently sectioned. The AAR is isolated and dissected from the viable myocardium and both fractions were stored at −80°C.

2.4 Isolated working mouse heart

Mice were anaesthetized with sodium pentobarbital (60 mg/kg ip), and the hearts were subsequently excised and immersed in ice-cold Krebs–Henseleit bicarbonate solution, following which the aorta was cannulated and equilibrated in the Langendorff mode. Hearts were switched to and perfused in the working mode as previously described. Oxygenated Krebs–Henseleit solution consisting of 1.2 mM palmitate bound to 3% fatty acid-free bovine serum albumin and 11 mM glucose, in the presence of 100 μU/mL insulin was delivered to the left atrium at a preload pressure of 15 mmHg. The perfusate was ejected from hearts paced at 380 b.p.m. into a compliance chamber and into the aortic outflow line against a hydrostatic afterload pressure of 80 mmHg. Hearts were perfused aerobically for 45 min, followed by 15 min of global no-flow ischaemia, and 30 min of reperfusion. At the end of reperfusion, hearts were immediately frozen in liquid N₂ with Wollenberger tongs, and stored at −80°C.

2.5 Mechanical function measurements in isolated working mouse hearts

Heart rate and aortic pressure (mmHg) were measured with a Gould P21 pressure transducer (Harvard Apparatus) connected to the aortic outflow line. Cardiac output and aortic flow (mL/min) were measured with Transonic T206 ultrasonic flow probes in the preload and afterload lines, respectively. Coronary flow (mL/min) was calculated as the difference between the cardiac output and the aortic flow. Cardiac work was calculated as the product of the cardiac output times the left ventricular (LV) developed pressure (aortic systolic pressure minus preload pressure). Data were collected utilizing an MP100 system from AcqKnowledge (BIOPAC Systems, Inc., USA).

2.6 Measurement of glycolysis, glucose, and palmitate oxidation

Glycolysis, glucose, lactate, and palmitate oxidation were measured by perfusing hearts with [5-3H/U-14C]glucose and [9,10-3H]palmitate, respectively, and measuring 3H₂O production and 14CO₂ production as previously described.

2.7 Calculation of proton production from glucose utilization

If glucose passes through glycolysis to lactate and the ATP formed is hydrolysed, a net production of 2 H⁺ per molecule of glucose occurs. In contrast, if glycolysis is coupled to glucose oxidation, the net production of H⁺ is zero. Therefore, subtracting the rate of glucose oxidation from the rate of glycolysis and multiplying by two determines the overall rate of H⁺ production derived from glucose utilization. This calculation of H⁺ production has been validated, whereby in a previous study in isolated working rat hearts utilizing a similar metabolic approach, the pH₄ determination using 31P NMR correlated well with their calculated values.
2.8 Culture of cardiac myocytes

Cardiac myocytes were isolated from the hearts of 2-day-old neonatal rat pups and plated on Primaria dishes (Falcon) at a density of 2.0 × 10^6 cells/plate, as previously described. After 18 h of culture, neonatal rat cardiac myocytes were rinsed twice with serum-free DMEM/F-12 containing 50 μg/mL gentamicin and cultured in serum-free DMEM/F-12 containing 50 μg/mL gentamicin supplemented with 1 × 1 ITS liquid media supplement and 10 μM cytosine β-D-arabinofuranoside to prevent the growth of fibroblasts.

9H2 ventricular myoblasts (American Type Culture Collection [ATCC], Rockville, MD, USA) were grown as myoblasts to confluence in 60-mm-diameter cell culture dishes in Dulbecco’s modified Eagle’s medium containing 10% (vol/vol) foetal bovine serum, 1% (vol/vol) Pen-Strep, and 0.25 mM L-carnitine. On reaching ~90% confluence, myoblasts were allowed to differentiate into myocytes following treatment with 10 nM retinoic acid for 1 week as previously described.

2.9 Treatment of neonatal cardiac myocytes with LPS

Neonatal cardiac myocytes were cultured for 12 h in DMEM (10 mL per 10-cm-diameter dish) in the presence or absence of the MCD inhibitor, CBM-301106 (10 μM). After pretreatment with the MCD inhibitor, the medium was replaced with either (i) fresh medium, (ii) 10 μM of CBM-301106, (iii) 1 μg/mL lipopolysaccharide (LPS), or (iv) 10 μM CBM-301106 + 1 μg/mL LPS. After 6 h of incubation, cardiac myocytes were harvested as previously described.

2.10 2-Deoxy-[3H]glucose uptake

Neonatal cardiac myocytes grown in 24-well plates were serum starved for 6 h and subsequently treated with or without insulin (100 nM, 20 min). 2-Deoxyglucose uptake was measured as previously described in Niu et al. in the presence of 1 mM glucose.

2.11 PDH activity

PDH activities were measured using a revised protocol based on the radiometric assay described by Constantin-Teodosiu et al. Briefly, for measurement of the active PDH complex, frozen myocardial tissue was homogenized in buffer containing 200 mM sucrose, 50 mM KCl, 5 mM EGTA, 50 mM Tris–HCl, 50 mM NaF, 50 mM sodium pyrophosphate (NaPpi), 5 mM dichloroacetate, and 0.1% Triton X-100 (pH 7.8). Samples were then incubated in assay buffer (150 mM Tris–HCl, 0.75 mM EDTA, 0.75 mM nicotineamide adenine dinucleotide, 1.5 mM thiamine pyrophosphate, 5 mM EGTA, 5 mM DCA, and 0.75 mM CoA). The reaction was initiated by the addition of pyruvate. The reaction was terminated by perchloric acid. Samples were neutralized and centrifuged, and the resulting supernatant was used for determination of acetyl-CoA content. Acetyl-CoA was converted to [14C]citrate and separated from unreacted radioactivity using Dowex resin (50W×8, 100–200 mesh). The amount of acetyl-CoA was determined by comparison of acetyl-CoA and analyte detection occurred at an absorbance of 254 nm on a Beckman System Gold model 168 diode array detector. The mobile phase consisted of a mixture of buffer A (0.2 M NaH2PO4, pH = 5.0) and buffer B (0.25 M NaH2PO4 and acetonitrile, pH 5.0). The gradient elution profile consisted of the following: 0–2.5 min, 97% A 3% B; 2.5–7.5 min, 97% A 3% B to 82% A 18% B; 7.5–15 min, 82% A 18% B; 15–18 min, 82% A 18% B to 63% A 37% B; 18–35 min, 63% A 37% B to 10% A 90% B; 35–42 min, 10% A 90% B. Peaks were integrated using the Beckman System Gold software package.

2.13 Lactate content

AAR and viable myocardium fractions were extracted in lactate assay buffer, and the resulting supernatant was utilized for quantification of lactate levels via a colorimetric method at a 450 nm wavelength with a 96-well micro-plate reader through a commercially available lactate assay kit (BioVision, Inc., CA, USA).

2.14 Determination of plasma DCA concentrations via HPLC

Levels of DCA in the plasma were determined via HPLC as outlined previously via Sarzanini et al.

2.15 Statistical analyses

All values are presented as mean ± SE (n observations). The significance of differences was determined by the use of an unpaired, two-tailed Student’s t-test, or a two-way analysis of variance followed by a Bonferroni post hoc analysis where appropriate. Differences were considered significant when P < 0.05.

3. Results

3.1 Direct stimulation of PDH with DCA decreases infarct size following ischaemia/reperfusion in vivo

C57BL/6 mice were subjected to in vivo ischaemia/reperfusion via temporary ligation of the LAD coronary artery. Upon reperfusion mice were injected each hour with saline or DCA (100 mg/kg at the first hour followed by 50 mg/kg each subsequent hour) for the first 8 h. DCA was then supplemented into the animal’s drinking water at a concentration of 110 mM, for 16 h, before infarct size assessment. TTC (1%) staining revealed that DCA treatment significantly reduced infarct size, despite similar AAR (Figure 1A). Plasma DCA concentrations at the end of reperfusion were also determined via HPLC, to ensure that treatment resulted in plasma DCA concentration >0.5 mM, which is required for the inhibition of myocardial PDHK and subsequent stimulation of PDH activity.

The DCA treatment protocol yielded plasma concentrations >0.5 mM (Figure 1C). Because DCA treatment decreased the infarct size, we subjected PDHK4−/− mice (which have increased PDH activity) and their WT littermates to in vivo ischaemia/reperfusion. Similar to DCA treatment, there was a significant decrease in the infarct size in PDHK4−/− mice subjected to in vivo ischaemia/reperfusion (Figure 1D).

3.2 DCA and PDHK4 deficiency enhance glucose oxidation and decrease H+ production to attenuate myocardial ischaemia/reperfusion injury in isolated working hearts

We have shown previously that DCA treatment elevates glucose oxidation rates and reduces H+ production during reperfusion in...
the working rat heart. In parallel, treatment of the mouse heart with 1.5 mM DCA (added upon reperfusion) resulted in an improvement in the recovery of cardiac function in isolated working hearts subjected to 15 min of global ischaemia (Figure 2A). Improved post-ischaemic recovery was accompanied by a stimulation of glucose oxidation rates (Figure 2C), while glycolysis rates were not altered during reperfusion (Figure 2B). As such, the rates of proton production were significantly decreased in response to DCA treatment (Figure 2D). In addition, genetic deficiency of PDHK4 in mice also enhanced the recovery of cardiac function (Figure 3A) during reperfusion in isolated working hearts subjected to 15 min of global ischaemia, and was associated with an identical metabolic profile to that observed with DCA treatment (Figure 3B–D). This improvement in cardiac function was due to an increase in both the peak systolic pressure and the cardiac output, resulting in an increase in both aortic and coronary flow (Table 1). The coronary flow was normal during the aerobic perfusion (Supplementary material online, Table S1), indicating that an increase in myocardial oxygenation before the ischaemic insult was not responsible for the improved function during reperfusion. In contrast, DCA failed to increase glucose oxidation rates and improve functional recovery during reperfusion in PDHK4−/− mice, validating that increased PDH activity and subsequent glucose oxidation rates are likely playing a key role in DCA’s protective effect against ischaemia/reperfusion injury (Figure 4). Interestingly, palmitate oxidation rates were not altered during reperfusion via DCA treatment, or in PDHK4−/− mice (Figure 5). These findings are in contrast to what we have previously reported in the rat heart, and suggest that the enhancement of glucose oxidation is more important than the inhibition of fatty acid oxidation for metabolic modulation-induced cardiac protection.

### 3.3 MCD deficiency decreases infarct size following in vivo ischaemia/reperfusion

Similar to what was observed in DCA-treated and PDHK4−/− mouse hearts, hearts from MCD−/− mice also performed better in an ex vivo model of ischaemia/reperfusion injury, an outcome associated with increased glucose oxidation rates and reduced proton

---

**Figure 1** DCA-treated mice and PDHK4−/− mice are protected against AMI. (A) Representative images showing infarcted myocardial tissue (white) in DCA-treated mice. (B) Quantification of the infarct size in vehicle control and DCA-treated mice (n = 5–7). (C) DCA plasma concentrations in vehicle control and DCA-treated mice (n = 4). (D) Quantification of the infarct size in WT and PDHK4−/− mice (n = 4). *P = 0.07. All values are presented as mean ± SE. The significance of differences was determined by the use of an unpaired, two-tailed Student’s t-test.
Glucose oxidation and myocardial infarction

To determine whether these alterations in energy metabolism decrease the infarct size, we subjected WT and MCD\(^{-/-}\) mice to temporary LAD coronary artery ligation. Similar to the findings observed following DCA treatment, and in PDHK4\(^{-/-}\) mice, the infarct size was markedly decreased, despite a similar AAR, in MCD\(^{-/-}\) mice following reperfusion after an acute ischaemic insult (Figure 6B). Furthermore, in vivo monitoring of cardiac function via ultrasound echocardiography demonstrated a significant improvement in LV function in MCD\(^{-/-}\) mice at 24 h of reperfusion (Figure 6C and D).
Table 1  Ex vivo haemodynamics during reperfusion in control and DCA-treated isolated mouse hearts, and WT and PDHK4−/− isolated mouse hearts

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>DCA (1.5 mM)</th>
<th>WT</th>
<th>PDHK4−/−</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (b.p.m.)</td>
<td>375 ± 6 (n = 12)</td>
<td>386 ± 4 (n = 11)</td>
<td>374 ± 4 (n = 15)</td>
<td>380 ± 1 (n = 10)</td>
</tr>
<tr>
<td>Peak systolic pressure (mmHg)</td>
<td>78.9 ± 7.4 (n = 12)</td>
<td>111.2 ± 2.4* (n = 11)</td>
<td>78.6 ± 6.4 (n = 15)</td>
<td>114. ± 3.69* (n = 10)</td>
</tr>
<tr>
<td>Developed pressure (mmHg)</td>
<td>58.5 ± 5.3 (n = 12)</td>
<td>69.6 ± 2.1 (n = 11)</td>
<td>55.8 ± 4.2 (n = 15)</td>
<td>63.7 ± 2.02 (n = 10)</td>
</tr>
<tr>
<td>Cardiac output (mL/min)</td>
<td>3.4 ± 0.7 (n = 12)</td>
<td>7.9 ± 0.8* (n = 11)</td>
<td>3.1 ± 0.4 (n = 15)</td>
<td>7.8 ± 0.7* (n = 10)</td>
</tr>
<tr>
<td>Aortic flow (mL/min)</td>
<td>1.1 ± 0.4 (n = 12)</td>
<td>3.7 ± 0.6* (n = 11)</td>
<td>0.6 ± 0.2 (n = 15)</td>
<td>2.8 ± 0.4* (n = 10)</td>
</tr>
<tr>
<td>Coronary flow (mL/min)</td>
<td>2.3 ± 0.4 (n = 12)</td>
<td>4.2 ± 0.3* (n = 11)</td>
<td>2.5 ± 0.3 (n = 15)</td>
<td>5.0 ± 0.7* (n = 10)</td>
</tr>
</tbody>
</table>

Values represent means ± SE (n = 10–15).
*P < 0.05, significantly different from control-treated counterpart or WT littermate.

3.4 Decreased infarct size in MCD−/− mice is associated with increased malonyl CoA levels and PDH activity in the AAR in vivo

Because the proposed metabolic changes that are harmful to recovery of post-ischaemic cardiac function take place during the initial stages of reperfusion, we subjected WT and MCD−/− mice to 30 min in vivo ischaemia and 30 min reperfusion. At the end of reperfusion, the AAR was delineated with Evans blue dye, and dissected from the viable myocardium. Because it is impossible during our in vivo model of ischaemia/reperfusion to measure myocardial metabolism, we assessed malonyl CoA levels, PDH activity, and lactate levels as surrogates for in vivo rates of fatty acid oxidation, glucose oxidation, and glycolysis, respectively. Interestingly, malonyl CoA levels (Figure 6E) and PDH activity were increased (Table 2), while lactate levels were unaltered in the AAR of MCD−/− mice (Table 2). Because PDH activity and subsequent glucose oxidation rates are likely higher in vivo in the AAR of MCD−/− mice, more pyruvate would enter the mitochondria for oxidative metabolism, limiting its conversion to lactate and subsequent accumulation in the AAR.

3.5 MCD inhibition reduces the inflammatory response in cardiac myocytes exposed to lipopolysaccharide and improves glucose utilization

As inflammation has been proposed to be a key component of ischaemia/reperfusion injury,9 we cultured neonatal rat cardiac myocytes and exposed them to LPS (1 μg/mL) to induce an inflammatory state, evinced by the increase in tumour necrosis factor α and monocyte chemoattractant protein-1 levels at 6 h post-LPS treatment (Figure 7A and B). However, neonatal rat cardiac myocytes pretreated with a novel MCD inhibitor (CBM-301106, 10 μM) had a dramatically reduced inflammatory response to LPS treatment (Figure 7A and B). Further support for a reduction in inflammation was evidenced by the marked decrease in nuclear factor kappa B DNA-binding activity in response to LPS in cardiac myocytes treated with the MCD inhibitor (Figure 7C). Of interest, the reduced inflammation following MCD inhibition in cardiac myocytes was associated with an improvement in insulin-stimulated glucose uptake following LPS treatment (Figure 7D).

4. Discussion

In summary, the beneficial effects of directly stimulating glucose oxidation with DCA,2,8 or inhibiting mitochondrial fatty acid uptake and oxidation via genetic deletion of MCD12 observed in isolated hearts, translate to a reduction in the infarct size following ischaemia/reperfusion in vivo. The decrease in the infarct size was associated with increased PDH activity in the AAR of MCD−/− hearts, suggesting that enhanced PDH activity and subsequent glucose oxidation secondary to the inhibition of fatty acid oxidation may be responsible for these beneficial effects. As such, mice deficient in PDHK4, the primary kinase responsible for inhibiting PDH in the heart, also exhibited a decrease in the infarct size following ischaemia/reperfusion in vivo.

Numerous studies from our laboratory2,8,11,13,22 and others32–35 have validated metabolic modulation as a novel therapy for angina and ischaemic heart disease. The objective of metabolic modulation in patients with ischaemic heart disease is to either stimulate glucose oxidation secondary to an inhibition of fatty acid oxidation,11,22,36 or to stimulate glucose oxidation directly.2,8 Indeed, we have shown that directly inhibiting fatty acid oxidation with trimetazidine improves glucose oxidation rates and the recovery of cardiac efficiency during reperfusion following ischaemia.13 and that direct stimulation of glucose oxidation with DCA also improves functional recovery of the heart following ex vivo ischaemia/reperfusion.2,8 Initial studies of metabolic modulation proposed that such a strategy is desirable in patients with ischaemic heart disease due to the fact that oxidizing one molecule of glucose consumes less oxygen than one molecule of a fatty acid such as palmitate.9 Because oxygen supply to the heart is already limited in patients with ischaemic heart disease, a large number of experimental and clinical studies were carried out to test the validity of this concept, from which many have reported positive findings.9,32–33 Moreover, the fact that metabolic modulation yields its effects independent of changes in cardiac haemodynamics makes it an attractive approach as an adjunct therapy for patients with various forms of ischaemic heart disease.

Aside from improving cardiac efficiency via decreasing oxygen consumption, stimulation of glucose oxidation may also improve cardiac efficiency during ischaemia/reperfusion by decreasing intracellular acidosis through improved coupling between glycolysis and glucose oxidation.2,8,9,36 During reperfusion, myocardial glycolysis rates quickly recover, whereas glucose oxidation rates are significantly
depressed, resulting in the accumulation of lactate and protons.\(^2,9\) Unfortunately, our in vivo experimental model is limited, as we cannot directly measure rates of flux through these metabolic pathways. Nonetheless, the observed increase in PDH activity in the AAR of MCD\(^2\)/\(^2\) mice following ischaemia/reperfusion is consistent with increased glucose oxidation rates and improved coupling between glycolysis and glucose oxidation. As ATP is required to deal with the proton load and subsequent intracellular acidosis observed during reperfusion, improved coupling of glucose metabolism will reduce the amount of ATP required to counteract this ionic imbalance during the critical stages of reperfusion, when the myocyte needs the available ATP to restore contractile function.\(^37\)

In addition, early glycolysis during reperfusion is essential for the recovery of cardiac function as glycolytic-derived ATP has been suggested to play a key role in cytosolic Ca\(^{2+}\) homoeostasis due to localization of glycolytic enzymes in close proximity to sarcolemmal ion channels.\(^38,39\) Indeed, elevated intracellular protons in reperfusion can be exchanged for extracellular Na\(^+\) via Na\(^+\)/H\(^+\) exchangers that induce reverse mode Ca\(^{2+}\) transport through the Na\(^+\)/Ca\(^{2+}\)-exchanger and induce Ca\(^{2+}\) overload and contractile dysfunction.\(^37\) Therefore, stimulating glucose oxidation decreases intracellular proton load and should limit cytosolic Ca\(^{2+}\) overload via reverse mode Ca\(^{2+}\) transport. At the same time, maintained glycolytic rates during reperfusion will provide sufficient ATP required to deal with any excess cytosolic Ca\(^{2+}\), while mitochondrial ATP generated via increased glucose oxidation can be used to restore contractile function in a more efficient manner. Further support for cytosolic Ca\(^{2+}\) overload being detrimental during reperfusion was demonstrated in Na\(^+\)/Ca\(^{2+}\)-exchanger-deficient mice, which exhibit a marked recovery of cardiac function in isolated Langendorff hearts subjected to 20 min of ischaemia and 2 h of reperfusion.\(^40\)

The fact that DCA treatment also decreased the infarct size validates that increased PDH activity contributes to the observed protection against ischaemia/reperfusion injury in MCD\(^2\)/\(^2\) mice. Additionally, we did not observe any changes in ex vivo myocardial fatty acid oxidation rates during reperfusion in PDHK4\(^2\)/\(^2\) or in DCA-treated mice. This supports our conclusion that decreased infarct size in MCD\(^2\)/\(^2\) mice is not a direct effect of reduced fatty

---

**Figure 4** Treatment with DCA does not increase glucose oxidation rates nor confer protection against ex vivo ischaemia/reperfusion injury in PDHK4\(^2\)/\(^2\) mice. (A) Recovery of cardiac function during reperfusion is similar in PDHK4\(^2\)/\(^2\) mouse hearts treated with either vehicle control or 1.5 mM DCA (n = 5). (B) Glucose oxidation rates are similar in PDHK4\(^2\)/\(^2\) mouse hearts treated with either vehicle control or 1.5 mM DCA (n = 5). All values are presented as mean ± SE. The significance of differences was determined by the use of an unpaired, two-tailed Student’s t-test.

**Figure 5** Palmitate oxidation rates are not altered via DCA treatment (during reperfusion), or in PDHK4\(^2\)/\(^2\) mice. (A) Reperfusion palmitate oxidation rates in vehicle control and DCA-treated (1.5 mM) hearts (n = 5). (B) Reperfusion palmitate oxidation rates in WT and PDHK4\(^2\)/\(^2\) mouse hearts (n = 7 WT, 5 PDHK4\(^2\)/\(^2\)). All values are presented as mean ± SE. The significance of differences was determined by the use of an unpaired, two-tailed Student’s t-test.
acid oxidation rates, but rather, it is secondary to the activation of PDH and subsequent stimulation of glucose oxidation. Furthermore, DCA failed to improve reperfusion injury in isolated working hearts from PDHK4<sup>2/2</sup> mice, illustrating that enhanced PDH activity likely plays a key role in the cardioprotection against ischaemic heart disease observed in PDHK4<sup>2/2</sup> mice <em>in vivo</em> and <em>ex vivo</em>. Because PDH activity is already enhanced in PDHK4<sup>1/2</sup> mice, DCA cannot increase glucose oxidation rates any further, resulting in the lack of protection. However, it should be noted that we cannot completely translate our <em>ex vivo</em> findings demonstrating a lack of effect of DCA in PDHK4<sup>1/2</sup> isolated mouse hearts to say that an increase in glucose oxidation will decrease the infarct size <em>in vivo</em>, as a limitation with our <em>in vivo</em> observations lies in their correlational nature, and not a true cause–effect relationship.

Of interest, the decrease in the infarct size <em>in vivo</em> appeared to be greater in MCD<sup>1/2</sup> mice, and may suggest that additional mechanisms aside from increased glucose oxidation and improved cardiac efficiency are responsible for the observed cardioprotection. As such, inhibition of fatty acid oxidation in MCD<sup>1/2</sup> mice secondary to the inhibition of mitochondrial fatty acid uptake may limit lipid-induced mitochondrial stress and also contribute to their reduction of the infarct size. In addition, we also showed in neonatal cardiac myocytes that MCD inhibition reduced LPS-induced inflammatory responses, which were associated with a restoration of insulin-stimulated glucose uptake. We propose that the increase in malonyl CoA following MCD inhibition increases cytosolic long-chain acyl-CoA and other fatty acid intermediates that can activate PPARγ, which has been shown in a number of studies to be anti-inflammatory. Indeed, we have shown previously that MCD<sup>1/2</sup> mice have increased mRNA expression of a number of PPARγ targets, and inhibition of CPT-1 in dogs with oxfenicine increases PPARγ target gene mRNA expression following pacing-induced cardiomyopathy. Furthermore, a multitude of studies in numerous cell types including the cardiac myocyte have shown that reducing inflammation inhibits apoptosis. However, our studies in H9c2 cardiac myocytes suggest that stimulation of glucose oxidation does not protect against inflammation-mediated apoptosis, as DCA-mediated prevention of PDH phosphorylation/inactivation did not improve markers of apoptotic signalling (caspase 3 cleavage, Bax and Bcl-2 expression) following treatment with TNFα (Supplementary material online, Figure S1). Because reperfusion injury is a phenomenon encompassing not only enhanced inflammation, but also oxidative stress, it is possible that stimulating glucose oxidation reduces oxidative stress-induced cardiac myocyte apoptosis, while also reducing inflammation, and that these dual mechanisms in combination contribute to alleviating ischaemia/reperfusion injury. Nonetheless, further work is necessary to determine precisely how stimulating glucose oxidation improves outcomes following AMI and subsequent reperfusion injury.

Any potential clinical ramifications for the use of therapeutic agents that inhibit fatty acid oxidation or stimulate glucose oxidation to treat ischaemic heart disease will require thorough investigation of the...
peripheral effects on other tissues such as the brain, muscle, and liver. Nevertheless, agents that increase malonyl CoA in the brain actually reduce appetite and subsequent body weight in obese rodents. Furthermore, despite the intriguing postulation that stimulating fatty acid oxidation can reverse obesity-induced insulin resistance by decreasing intramuscular fatty acid intermediates that impair insulin signalling, a number of recent studies have actually shown that inhibiting fatty acid oxidation in muscle can reverse obesity-induced skeletal muscle insulin resistance. Of interest, both the MCD and PDHK4 mice utilized in our studies are also protected against obesity-induced insulin resistance. As the patient population suffering from ischaemic heart disease also encompasses a large population of obese, insulin-resistant individuals, whole-body inhibition of fatty acid oxidation or stimulation of glucose oxidation may in general prove to be beneficial. However, inhibition of fatty acid oxidation may increase intrahepatic lipid accumulation and induce steatosis. Indeed, overexpression of MCD in the liver of rats increased hepatic fatty acid oxidation with subsequent potent effects on whole-body glucose disposal and insulin sensitivity. Because DCA treatment did not impact myocardial fatty acid oxidation rates, stimulation of glucose oxidation may be the better approach peripherally as it may not impact fatty acid oxidation and steatosis in the liver. Although the data clearly suggest the therapeutic potential for optimizing metabolism in the heart, more rigorous work in this area further delineating the effects on peripheral tissues is warranted.

In contrast to our findings, previous work from Haessler et al. demonstrated that DCA was able to increase PDH activity in the rabbit heart, but that this activation did not improve infarct size following in vivo ischaemia/reperfusion injury. It is important to note, however, that Haessler et al. only administered DCA at the onset of and 1 h into reperfusion, vs. our protocol administering DCA.

### Table 2: Myocardial PDH activity and lactate accumulation in WT and MCD−/− mice following 30 min ischaemia and 30 min reperfusion in vivo

<table>
<thead>
<tr>
<th></th>
<th>VM (WT)</th>
<th>VM (MCD−/−)</th>
<th>AAR (WT)</th>
<th>AAR (MCD−/−)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDH activity (µmol/g wet weight/min)</td>
<td>ND</td>
<td>ND</td>
<td>1.52 ± 0.05 (n = 4)</td>
<td>1.89 ± 0.18** (n = 4)</td>
</tr>
<tr>
<td>Lactate content (nmol/mg wet weight)</td>
<td>7.37 ± 1.35 (n = 3)</td>
<td>9.14 ± 2.50 (n = 3)</td>
<td>12.29 ± 1.39* (n = 3)</td>
<td>12.82 ± 2.76 (n = 3)</td>
</tr>
</tbody>
</table>

Values represent means ± SE (n = 3–4).
VM, viable myocardium; AAR, area at risk; ND, not determined.
*P < 0.05, significantly different from genotype-matched VM.
**P = 0.07, vs. AAR (WT).

Figure 7 MCD inhibition reduces the inflammatory response induced by LPS in neonatal cardiac myocytes. Neonatal cardiac myocytes were pre-treated with the MCD inhibitor, CBM-301106 (10 µM) for 12 h before being exposed to LPS (1 µg/mL) to induce an inflammatory state. MCD inhibition reduced plasma (A) MCP-1, and (B) tumour necrosis factor α (TNFα) levels 6 h after LPS exposure. (C) The reduced inflammatory state observed with MCD inhibition following LPS treatment was associated with a reduction in nuclear factor kappa B (NFkB) DNA-binding activity, as well as a (D) restoration of insulin-stimulated glucose uptake.
every hour for the first 8 h of reperfusion, and then administering DCA in the drinking water for the remaining 16 h of reperfusion. This difference is of significance, as DCA has a very short half-life, and it is likely that, in the Haessler et al. study, plasma concentrations of DCA were not >0.5 mM, which is required for inhibition of PDHK and subsequent activation of PDH during reperfusion. While the authors did note that DCA increased PDH activity acutely upon administration in rabbits, they did not confirm that the plasma levels of DCA were at the appropriate concentration as we did at the end of our 24 h reperfusion protocol. In addition, Gozel et al. also did not report any protective effect against the infarct size in rabbits treated with DCA. Differences between our observations may be due to differences in the reperfusion time (3 vs. 24 h in our study), or possibly species related.

Taken together, our findings validate that optimizing myocardial metabolism is a novel therapy for the treatment of ischaemic heart disease(s). However, the mechanism(s) by which stimulation of glucose oxidation actually prevents the death of cardiac myocytes during an AMI remains to be determined. Moreover, whether inhibition of fatty acid oxidation or stimulation of glucose oxidation confers benefit in the setting of chronic ischaemia, ischaemia-induced left ventricular remodelling, and heart failure needs to be determined. Despite these important questions, inhibition of MCD or stimulation of PDH may both represent promising new targets for the mono-therapy or adjunct therapies in patients with various forms of ischaemic heart disease.

Supplementary material

Supplementary material is available at Cardiovascular Research online.

Conflict of interest: G.D.L. and J.R.B.D. are shareholders and officers of Metabolic Modulators Research Ltd., a company with commercial interests in the development of MCD inhibitors.

Funding

This study was supported by a grant from the Canadian Institutes of Health Research to G.D.L. G.D.L. is an Alberta Heritage Foundation for Health Research to G.D.L. G.D.L. is an Alberta Heritage Foundation for Medical Research Medical Research Scientist. J.R.U. and W.W. are trainees of the Alberta Heritage Foundation for Medical Research and Tomorrow's Research Cardiovascular Health Professional.

References

22. Soltys CL, Kovacic S, Dyck JR. Activation of cardiac AMP-activated protein kinase by LKB1 expression or chemical hypoxia is blunted by increased Akt activity. Am J Physiol Heart Circ Physiol 2006;290:H2472–H2479.


