Impact of High Glucose/High Insulin and Dichloroacetate Treatment on Carbohydrate Oxidation and Functional Recovery After Low-Flow Ischemia and Reperfusion in the Isolated Perfused Rat Heart

Peipei Wang, MD, PhD; Steven G. Lloyd, MD, PhD; John C. Chatham, DPhil

Background—It is believed that increasing cardiac glucose metabolism in the setting of ischemia and reperfusion is protective because of the resulting decrease in fatty acid oxidation, which improves cardiac efficiency and increases glucose oxidation relative to glycolysis; however, these conclusions are based primarily on studies in which glucose is the only carbohydrate provided. The goal of this study was to examine the effect of stimulating myocardial carbohydrate use either by increasing glucose and insulin levels or by using dichloroacetate on the response to ischemia and reperfusion in hearts perfused with physiological concentrations of lactate and pyruvate plus glucose and fatty acids.

Methods and Results—Metabolic fluxes were determined in hearts from male Sprague-Dawley rats perfused with 13C-labeled substrates using 13C/1H-NMR isotopomer analysis after 30 minutes of low-flow ischemia (0.3 mL/min) and 60 minutes of reperfusion. Measurements were made under control conditions: 5 mmol/L glucose, 1 mmol/L lactate, 0.1 mmol/L pyruvate, 0.3 mmol/L palmitate, and 50 μU/mL insulin plus dichloroacetate 5 mmol/L or glucose and insulin increased to 30 mmol/L and 1000 μU/mL, respectively. Dichloroacetate increased carbohydrate oxidation and the ratio of glucose oxidation to glycolysis but did not improve functional recovery or cardiac efficiency; however, elevated glucose and insulin levels improved functional recovery and cardiac efficiency but did not increase carbohydrate oxidation or the ratio of glucose oxidation to glycolysis.

Conclusions—These data support the notion that increasing myocardial glucose use is beneficial in the setting of ischemia and reperfusion; however, the protective effect appears not to be mediated by shifting the balance between carbohydrate and fatty acid oxidation. (Circulation. 2005;111:2066-2072.)

Key Words: fatty acids • glucose • ischemia • metabolism • reperfusion

Modulation of cardiac metabolism as a therapeutic approach for treating ischemic heart disease has been the subject of investigation for >40 years and generally is predicated on increasing cardiac glucose use while decreasing fatty acid use. In this context, glucose-insulin-potassium (GIK) therapy is one of the most widely investigated approaches used in a clinical setting; it was first reported in 1962,1 with results from new trials continuing to be published.2,3 Although in general GIK therapy is found to improve outcome after acute myocardial infarction,4,5 this is not a uniform observation.5 The discrepancies could be due in part to the relatively large fluid volumes administered with this protocol, which may adversely affect some patient groups more than others.2 An alternative approach that avoids these complications is direct pharmacological modulation of cardiac fatty acid and carbohydrate metabolism.6 This has been the focus of many studies, leading to the development of putative fatty acid oxidation inhibitors such as trimetazidine and ranolazine for the treatment of ischemic disease.7,8 Their protective effects are typically attributed to increased glucose oxidation and decreased fatty acid oxidation.6–8

Despite the continuing interest in GIK therapy and the apparent efficacy of trimetazidine and ranolazine, there is no consensus about the mechanisms underlying their beneficial effects. One of the most frequently proposed mechanisms is that decreasing fatty acid oxidation and increasing glucose oxidation improve efficiency by decreasing the amount of oxygen required for ATP synthesis.6 Theoretically, there is an ≈12% decrease in oxygen required for ATP synthesis in shifting from 100% palmitate oxidation to 100% glucose oxidation9; however, such extreme metabolic shifts are unlikely to occur under physiological conditions. Another explanation for the beneficial consequences of increasing glucose oxidation is that it improves the “coupling” between...
glucose oxidation and glycolysis, decreasing proton production and thus reducing intracellular acidosis. It is noteworthy that both of these mechanisms are based on the premise that glucose and palmitate are the primary substrates for cardiac energy production despite the fact that lactate is more readily oxidized than glucose in the heart. We found that when lactate and pyruvate, as well as glucose and palmitate, are available for oxidation, decreasing fatty acid oxidation from ~60% to ~5% of total energy production did not alter the efficiency of ATP production.

Given the broad acceptance of the notion that increasing glucose use is beneficial, relatively few studies have examined the impact of these interventions on the regulation of carbohydrate and fatty acid metabolism after ischemia and reperfusion, particularly in the presence of substrates other than glucose and fatty acids. Therefore, the aim of this study was to compare the effect of stimulating cardiac glucose use by increasing glucose and insulin concentrations with direct activation of carbohydrate oxidation using dichloroacetate (DCA) on the metabolic and functional responses to ischemia and reperfusion in hearts perfused with physiological concentrations of lactate and pyruvate, as well as glucose and palmitate. The results demonstrated that increasing glucose and insulin improved functional recovery compared with DCA treatment, whereas DCA had a greater impact on increasing carbohydrate oxidation. We also found that the protection associated with increased glucose and insulin levels was not related to changes in cardiac efficiency or to the coupling of glucose oxidation to glycolysis.

Methods

Animals

Animal experiments were approved by the Institutional Animal Care and Use Committee at the University of Alabama at Birmingham and followed the Guidelines for the Care and Use of Laboratory Animals (National Academy of Sciences, 1996). Male Sprague-Dawley rats (Charles Rivers Laboratories) weighing ~300 to 325 g were used throughout.

Isolated Heart Preparation

Hearts were isolated and perfused as previously described with a Krebs-Henseleit buffer containing 3% BSA (essentially fatty acid free, Intergen Corp), 0.32 mmol/L sodium palmitate, 1.0 mmol/L sodium lactate, 0.1 mmol/L sodium pyruvate, 5 mmol/L glucose, and 50 μU/mL insulin unless stated otherwise. Cardiac function was monitored via a fluid-filled balloon placed in the left ventricle and connected to a pressure transducer. End-diastolic pressure was set to a constant perfusion pressure of 75 mm Hg and paced at 320 bpm throughout the experiments.

Experimental Protocol

Hearts were assigned to 1 of 3 groups: (1) control, 5 mmol/L glucose and 50 μU/mL insulin; (2) high glucose and high insulin (HG/HI), 30 mmol/L glucose and 1000 μU/mL insulin; or (3) DCA (5 mmol/L), 5 mmol/L glucose and 50 μU/mL insulin. Hearts from all groups were equilibrated for 30 minutes under normal-flow perfusion and subjected to low-flow ischemia (LFI; 0.3 mL/min) for 30 minutes followed by 60 minutes of reperfusion, during which time flow was restored to achieve a pressure perfusion of 75 mm Hg as described previously. Hearts from the control and HG/HI groups were also perfused under normal-flow conditions for 60 minutes. In some experiments, at the onset of LFI, unlabeled lactate was replaced with [3-13C]lactate to permit the rates of exogenous lactate uptake and glycolytic lactate efflux to be determined as previously described. During the last 30 minutes of each protocol, hearts were perfused with [U-13C]palmitate, [2-13C]pyruvate, [3-13C]lactate, and unlabeled glucose at the same concentrations used during preceding perfusion period.

Coronary effluent samples were collected immediately before ischemia, every 5 minutes during ischemia, every 1 minute during the first 5 minutes of reperfusion, and at the end of reperfusion in the LFI protocol and at the end of the experiments in the normal-flow experiments. Oxygen consumption was determined as previously described. At the end of each protocol, hearts were freeze-clamped.

As described previously, tissue and coronary effluent samples were acid extracted, freeze-dried, and redissolved in a potassium phosphate buffer (pH 7.5, 50 mmol/L) with 2H2O solvent (99.9%; Cambridge Isotope Laboratories) before 13C- and 1H-nuclear magnetic resonance (NMR) analysis.

13C-NMR Glutamate Isotopomer Analysis and Determination of Substrate Oxidation Rates

13C-NMR spectra of heart extracts were collected and 13C-glutamate isotopomer analysis was performed to determine the fraction of total acetyl-CoA entering the tricarboxylic acid cycle originating from unlabeled, [1,2-13C]-, [2-13C]-, and [1-13C]-acetyl-CoA resulting from oxidation of unlabeled glucose, [U-13C]-palmitate, [3-13C]-lactate, and [2-13C]-pyruvate, respectively. Combined with measurements of tissue oxygen consumption, the absolute oxidation rates of each substrate were determined. Theoretical oxidative ATP production was calculated from the oxidation rates of each substrate multiplied by the oxidative component of the calculated ATP yield for each substrate as described by Opie using the corrections for loss of energy during oxidative phosphorylation as reported by Hinkle et al.

Determination of Lactate Efflux and Uptake Rates

1H-NMR spectroscopy was used to determine the fraction of unlabeled lactate formed by metabolism of exogenous glucose or glycogen and [3-13C] lactate added to the perfusate. These data, multiplied by the total lactate concentration in the effluent determined by enzymatic colorimetric methods and the coronary flow rates, were used to determine the rates of exogenous [3-13C] lactate uptake and unlabeled glycolytic lactate efflux.

Statistical Analysis

All data are presented as mean ± SEM. One-way ANOVA and repeated-measures ANOVA were used when appropriate, combined with Scheffé post hoc test (Statview, Abacus Concepts, Inc). A value of P < 0.05 was considered significant.

Results

Baseline cardiac function was not significantly different between the 3 groups (Figure 1A). The onset of LFI led to a rapid cessation of contractile function and a gradual increase in end-diastolic pressure characteristic of the development of contracture (Figure 1B); this increase in contracture was completely abolished in the HG/HI group. The time to maximum contracture was more rapid in the DCA group than the control group (15.0 ± 1.6 versus 22.3 ± 1.4 minutes, respectively; P < 0.05). During reperfusion, contractile function recovered more rapidly in the HG/HI group than in either the control or DCA group. For example, the rate of recovery of the rate-pressure product (RPP; maximum RPP in reperfusion divided by time to maximum RPP) in the HG/HI group was 2932 ± 656 mm Hg · min⁻¹ compared with 1206 ± 249 and 926 ± 235 mm Hg · min⁻¹ in the control and DCA groups, respectively (Figure 1C). In the HG/HI group, RPP
was 87% of baseline at the end of reperfusion, which was significantly higher than in the control group (74%; *P*<0.05). An important contributing factor to the improved function in the HG/HI group was a significantly lower end-diastolic pressure (Figure 1C).

As summarized in Figure 2A, DCA significantly increased total carbohydrate oxidation rate by >2-fold with an ≈5-fold decrease in palmitate oxidation. Surprisingly, there were no significant differences in carbohydrate and fatty acid oxidation rates between the control and HG/HI groups. As shown in Figure 2B, lactate represented the predominant source of total carbohydrate oxidation. Interestingly, although DCA increased total carbohydrate oxidation, it did not change the relative contributions of lactate, pyruvate, and glucose. Although total carbohydrate oxidation was not increased in the HG/HI group, the contribution of glucose to total carbohydrate oxidation was significantly increased and the contribution from lactate was decreased compared with the control group.

During LFI, glycolytic lactate efflux was increased ≈2-fold in the HG/HI group compared with the other 2 groups, whereas there was no significant difference in lactate uptake between groups (Figure 3A). During reperfusion, the rate of glycolytic lactate efflux remained higher in the HG/HI group; the rate of exogenous lactate uptake was also increased in the HG/HI group compared with the control group (Figure 3B).

From the lactate efflux rates and the substrate oxidation rates, we determined oxidative, glycolytic, and total ATP synthesis rates during reperfusion (Table 1). There were no differences in total ATP synthesis rates between the groups; however, in the HG/HI group, there was a significant decrease in oxidative ATP production and an increase in the contribution of glycolytic ATP production to total ATP production. The rates of oxidative ATP synthesis from the individual substrates are shown in Table 2. These data...
reinforce the results in Figure 2, demonstrating that DCA decreases the contributions of lactate, pyruvate, and glucose to oxidative ATP synthesis relative to both the control and HG/HI groups. In the HG/HI group, the contribution to oxidative ATP synthesis from pyruvate is decreased by $>$50% and that from palmitate is decreased by $\approx$30%.

To evaluate the impact of DCA and HG/HI treatment on the coupling of glucose oxidation to glycolysis, we calculated the ratio of the rate of glycolytically derived pyruvate oxidation to the total rate of glycolysis (defined as the sum of the rates of glycolytically derived pyruvate oxidation plus glycolytically derived lactate efflux) (Table 3). DCA increased this ratio, i.e., improved the coupling of glucose oxidation to glycolysis, compared with the HG/HI group, consistent with their effects on both lactate efflux (Figure 3) and glucose oxidation (Figure 2). To estimate the efficiency of converting oxygen consumption to contractile work, we calculated the ratio of RPP to oxygen consumption (MVO$_2$). This ratio was significantly higher in the HG/HI group compared with the control and DCA groups, demonstrating that the HG/HI group consumed less oxygen to perform the same level of contractile work. Thus, the HG/HI group showed improved recovery of function and cardiac efficiency compared with the DCA group, despite a decrease in the coupling of glucose oxidation to glycolysis and increased contribution of fatty acids to energy production.

The lack of effect of HG/HI on carbohydrate oxidation compared with control conditions during reperfusion was surprising. Therefore, we determined the rates of substrate oxidation and lactate uptake and efflux in these 2 groups during normal-flow conditions (Figure 4). In contrast to our findings during reperfusion, in normal flow, there was a significant increase in total carbohydrate oxidation in the HG/HI group compared with the control group. Interestingly, this increase was a result of increased lactate and glucose oxidation. Furthermore, whereas glycolytic lactate efflux was increased during reperfusion in the HG/HI group, there was no difference during normal perfusion. Nevertheless, lactate uptake was increased with HG/HI in accordance with the increase in lactate oxidation; this is consistent with previous reports of insulin stimulating lactate uptake. A larger increase in carbohydrate oxidation might have been anticipated with 30 mmol/L glucose and 1000 $\mu$U/mL insulin; however, these data are consistent with our earlier report in which we found that the greatest increase in carbohydrate oxidation was between 0 and 100 $\mu$U/mL insulin, whereas increasing insulin from 100 to 1000 $\mu$U/mL increased total carbohydrate oxidation by only $\approx$20%.

To gain further insight into the effects of HG/HI on glycolysis and lactate metabolism, we calculated the ratio of lactate oxidation to lactate uptake and the ratio of lactate efflux to lactate uptake (Table 3). Previous reports have shown that $\approx$70% of lactate extracted by the heart is subsequently oxidized. In contrast, during reperfusion in the control and DCA groups, only $\approx$30% of lactate taken up was subsequently oxidized; in the HG/HI group, this was reduced to $\approx$12%. Here, we show that, in both the control and DCA groups, the rate of lactate uptake exceeds lactate efflux rates (Table 4); in the HG/HI group, however, these rates are approximately equal.

### Discussion

The most frequently cited mechanisms underlying the protection associated with increased myocardial glucose use are that it improves efficiency by decreasing oxygen consumption and improves the coupling between glucose oxidation and glycolysis, thereby reducing intracellular acidosis. Surprisingly, however, given the considerable interest in this area, remarkably few studies have directly evaluated these mechanisms, particularly under conditions in which, in addition to glucose, other exogenous sources of pyruvate such as lactate and pyruvate were present at physiologically relevant concentrations. We report here, for the first time, a comparison between the effect of direct activation of carbohydrate oxidation using DCA with stimulation of cardiac glucose use by increasing glucose and insulin concentrations (HG/HI) on cardiac efficiency and the relationship between glucose oxidation and glycolysis after ischemia and reperfusion. The results demonstrated that HG/HI significantly improved the rate of functional recovery and markedly increased efficiency compared with the control and DCA groups; however, this

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**TABLE 1. Total, Oxidative, and Glycolytic ATP Production Rates at the End of Reperfusion**

<table>
<thead>
<tr>
<th>Group</th>
<th>Total, $\mu$mol · min$^{-1}$ · g$^{-1}$</th>
<th>Oxidative, $\mu$mol · min$^{-1}$ · g$^{-1}$</th>
<th>Glycolytic, $\mu$mol · min$^{-1}$ · g$^{-1}$</th>
<th>Glycolytic, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=6)</td>
<td>16.4±0.8</td>
<td>15.7±0.8</td>
<td>0.7±0.2</td>
<td>4±1</td>
</tr>
<tr>
<td>DCA (n=4)</td>
<td>16.8±1.8</td>
<td>15.7±1.8‡</td>
<td>1.1±0.3</td>
<td>7±2</td>
</tr>
<tr>
<td>HG/HI (n=7)</td>
<td>14.3±0.8</td>
<td>12.4±0.7*</td>
<td>1.9±0.3*</td>
<td>13±2‡</td>
</tr>
</tbody>
</table>

*P<0.05 vs control; †P<0.05 vs HG/HI; ‡P=0.065 vs HG/HI.

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**TABLE 2. Rates of Oxidative ATP Production From Individual Substrates During Reperfusion**

<table>
<thead>
<tr>
<th>Group</th>
<th>Palmitate, $\mu$mol · min$^{-1}$ · g$^{-1}$</th>
<th>Lactate, $\mu$mol · min$^{-1}$ · g$^{-1}$</th>
<th>Pyruvate, $\mu$mol · min$^{-1}$ · g$^{-1}$</th>
<th>Glucose, $\mu$mol · min$^{-1}$ · g$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=6)</td>
<td>9.5±1.0</td>
<td>3.9±0.4</td>
<td>1.1±0.1</td>
<td>1.2±0.3</td>
</tr>
<tr>
<td>DCA (n=4)</td>
<td>1.8±0.6¶</td>
<td>8.5±1.1¶</td>
<td>2.6±0.5†</td>
<td>2.9±0.2†</td>
</tr>
<tr>
<td>HG/HI (n=7)</td>
<td>6.9±0.6*</td>
<td>3.0±0.2</td>
<td>0.6±0.1*</td>
<td>1.9±0.1</td>
</tr>
</tbody>
</table>

*P<0.05 vs control; †P<0.05 vs HG/HI.
was not accompanied by a reduction in fatty acid oxidation or an increase in the ratio of glucose oxidation to glycolysis. Conversely, although DCA decreased fatty acid oxidation and increased the ratio of glucose oxidation to glycolysis, this result had no beneficial effect on the response to ischemia and reperfusion. Consequently, although these data clearly support the notion that increasing myocardial glucose use is beneficial in the setting of ischemia and reperfusion, this protective effect does not appear to be mediated by the mechanisms typically associated with this phenomenon.

The relationship between improved efficiency and decreased fatty acid oxidation is usually attributed to the fact that the amount of ATP produced per unit of oxygen consumed is \(\approx 12\%\) greater when glucose compared with palmitate is the sole energy source; however, the amount of ATP produced per oxygen consumed by lactate or pyruvate oxidation falls midway between that for glucose and palmitate. Because lactate and pyruvate contribute appreciably to oxidative energy metabolism both under normal conditions and during reperfusion (Figure 2), it seems unlikely that physiologically relevant shifts in substrate oxidation would greatly affect myocardial oxygen consumption. Furthermore, even though DCA decreased fatty acid oxidation by 5-fold after reperfusion, it did not reduce oxygen consumption. Although increased levels of exogenous fatty acids significantly increase basal myocardial oxygen requirements, the increase in oxygen consumption is too large to be accounted for by differences in efficiency of ATP production. This suggests that if exogenous fatty acid concentrations are constant, as was the case here, shifts in the contribution of fatty acids to oxidative energy metabolism do not lead to measurable changes in oxygen consumed per unit work, whereas elevated exogenous fatty acids could impair cardiac efficiency via mechanisms independent of changes in substrate oxidation. Thus, strategies designed to reduced circulating levels of fatty acids may well be of greater benefit in the treatment of myocardial ischemia than those targeting the regulation of cardiac substrate oxidation.

The detrimental effects of increased fatty acid oxidation in a range of pathological conditions, including cardiac ischemia, have also been attributed to the “uncoupling” of glucose oxidation from glycolysis, protecting against ischemic injury; however, this proposed mechanism is based almost exclusively on studies in which glucose is considered to be the primary, if not the only, source for pyruvate oxidation. Clearly, this is not the case in vivo where resting lactate levels are \(\approx 1\) mmol/L and can easily reach \(\approx 5\) mmol/L in response to exercise, trauma, or stress. As shown in Table 2, both lactate and pyruvate contribute significantly to oxidative ATP production. It is noteworthy that even though lactate is present at a 5-fold-lower concentration than glucose in the control group, its contribution to ATP production is 3-fold higher. Furthermore, despite the fact that pyruvate is present at a 50-fold-lower concentration than glucose, its contribution to ATP production is similar to that from glucose. This is consistent with other reports that examine glucose, lactate, and pyruvate oxidation. Here, we found that although DCA increased the ratio of glucose oxidation to glycolysis by \(\approx 5\)-fold compared with the HG/HI group, functional recovery was not improved; indeed, we found that an increase in glycolytic flux relative to glucose oxidation was associated with improved functional recovery. Thus, at least in this setting, increasing glucose oxidation rates relative to glycolysis rates was not protective. It is possible that this mechanism is applicable when glucose is the only exogenous source of pyruvate.

Contrary to the concept of decreasing glycolytic flux relative to glucose oxidation, the primary metabolic difference between the HG/HI group and the other 2 groups was an increase in glycolytic lactate efflux during both ischemia and reperfusion. The latter resulted in a significant increase in total ATP production coming from glycolysis during reperfusion. The increase in glycolytic lactate efflux during LFI in the HG/HI group could be a result of increased glucose entry into the cell or increased mobilization of glycogen, possibly resulting from higher glycogen levels in this group before ischemia. This could lead to

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**Table 3. Ratio of Glucose Oxidation to Glycolysis, \(V_{O_2\text{max}}\), and Cardiac Efficiency at the End of Reperfusion**

<table>
<thead>
<tr>
<th></th>
<th>(\text{GOx/Gly} )</th>
<th>(\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{g}^{-1} )</th>
<th>(\text{RPP} / V_{O_2\text{max}})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=6)</td>
<td>0.2 ± 0.1</td>
<td>3.1 ± 0.2</td>
<td>7800 ± 900</td>
</tr>
<tr>
<td>DCA (n=4)</td>
<td>0.4 ± 0.2†</td>
<td>3.3 ± 0.4</td>
<td>7600 ± 600†</td>
</tr>
<tr>
<td>HG/HI (n=7)</td>
<td>0.08 ± 0.01</td>
<td>2.8 ± 0.1</td>
<td>11300 ± 300*</td>
</tr>
</tbody>
</table>

GOx/Gly indicates ratio of glucose oxidation to glycolysis; RPP/\(V_{O_2\text{max}}\), cardiac efficiency.

*P < 0.05 vs control and DCA; †P < 0.05 vs HG/HI.

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**Table 4. Ratios of Lactate Oxidation to Lactate Uptake and Lactate Efflux to Lactate Uptake During Reperfusion**

<table>
<thead>
<tr>
<th></th>
<th>Oxidation/Uptake</th>
<th>Efflux/Uptake</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.30 ± 0.09</td>
<td>0.8 ± 0.3</td>
</tr>
<tr>
<td>DCA</td>
<td>0.31 ± 0.03</td>
<td>0.59 ± 0.04</td>
</tr>
<tr>
<td>HG/HI</td>
<td>0.12 ± 0.02†</td>
<td>1.04 ± 0.04†</td>
</tr>
</tbody>
</table>

*P < 0.05 vs control; †P < 0.05 vs DCA.

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**Figure 4. Substrate oxidation rates (A) and lactate efflux and uptake rates (B) under baseline perfusion conditions in control (Cont; open bars) and HG/HI (solid bars) groups.**

\(*)P < 0.05\) vs control. CHO indicates total carbohydrate oxidation.
higher ATP levels at the end of ischemia and on reperfusion, which may be an important contributing factor to the improved cardiac efficiency and functional recovery in the HG/HI group; however, the relationship between bioenergetic status and cardiac efficiency remains to be determined.

The importance of glycolytic ATP production in improving recovery from ischemia has been demonstrated by a number of investigators. Cross et al. showed that, in the presence of residual flow, increasing glycolysis during ischemia was protective. Others have shown that inhibition of glycolysis during reperfusion markedly impaired recovery, supporting the concept that glycolytic ATP was critical in maintaining cardiomyocyte function. Furthermore, several studies have demonstrated a direct link between glycolytic ATP production and membrane ion transport, which may reflect compartmentation of glycolytic ATP production in the vicinity of sarcolemmal or sarcoplasmic reticulum ion pumping ATPases. Thus, improved glycolytic ATP production could account for both the improved ischemic and postischemic diastolic function observed in the HG/HI group.

Ischemic contracture, typically associated with increased severity of ischemic injury, can be attenuated by increasing glycolysis. The reduction in contracture in the HG/HI group associated with increased glycolytic lactate efflux and improved recovery of function is certainly consistent with this concept. Surprisingly, in the DCA group, the development of contracture was accelerated relative to the control group, although functional recovery ultimately was not significantly different. A dissociation between the severity of ischemic contracture and functional recovery has been previously reported by Kolocassides et al., who found that the onset of contracture was associated with ATP depletion whereas the improved recovery of function was associated with less severe acidosis. It is worth noting that although DCA is a well-established activator of pyruvate oxidation, it has been reported to inhibit glycolysis, which could account for the accelerated development of contracture. Further studies are needed to better understand the impact of DCA on ATP hydrolysis, intracellular acidosis, and development of ischemic contracture.

It is also possible that insulin may exert a direct protective effect independent of its effects on metabolism; however, the development of acidosis during no-flow ischemia has been shown to inhibit insulin receptor tyrosine kinase signaling, thereby preventing activation of downstream targets. Thus, it is possible that the insulin effects are mediated primarily at the time of reperfusion. For example, Jonassen et al. reported that increased levels of insulin administered at the time of reperfusion afforded significant protection that was mediated independently of effects of glucose metabolism and was associated with activation of cell survival signaling pathways. This could also account, at least in part, for the improved recovery of function seen here in the HG/HI group. Interestingly, we found that under normal perfusion conditions, there was a significant increase in carbohydrate oxidation in the HG/HI group that was absent during reperfusion. This suggests that, after ischemia and reperfusion, the effect of insulin on stimulating carbohydrate oxidation was blunted and could account for the increased contribution of fatty acids to oxidative energy metabolism after ischemia and reperfusion compared with normoxic perfusion. It was also intriguing that HG/HI treatment also significantly affected the ratio of lactate oxidation to uptake and the ratio of lactate efflux to uptake (Table 3). The mechanisms underlying these changes and the relationship to the improved recovery of function remain to be determined; however, hyperinsulinemia has been reported to increase lactate extraction in vivo, and it has been proposed that changes in the ratio of lactate efflux to uptake may reflect alterations in cytosolic redox state. At the very least, these data demonstrate that the impact of HG/HI treatment on cardiac metabolism go beyond simply altering the relationship between glucose and fatty acid oxidation.

An important distinction between this report and many of those that have examined the impact of altered substrate use in mediating ischemic injury is that we used a model of LFI rather than zero-flow ischemia. Cross et al. reported that increased glycolytic flux resulting from glycogen loading was beneficial during LFI but was detrimental in a zero-flow ischemia model. Furthermore, whereas oxidative energy production is minimal during zero-flow ischemia, we have recently shown that oxidative energy metabolism contributes 30% to 50% of ATP production during the LFI protocol used here. Interestingly, King et al. showed that the provision of additional oxidizable substrates such as long-chain fatty acids improved functional recovery after a similar LFI protocol compared with situations when glucose was the only available substrate. We believe that using an LFI protocol is relevant because, in both large animal studies and patients, there is considerable residual flow in the infarct zone. Furthermore, areas of reduced flow represent regions that could be accessible to therapeutic interventions before reperfusion. Although, much of the focus on development of agents designed to improve recovery from ischemic injury has emphasized treatment at the time of reperfusion, it is noteworthy that treatment was started before revascularization began in many GIK trials. Furthermore, interventions before the onset of ischemia are possible in the context of perioperative myocardial infarction, which affects 3% to 6% of patients undergoing coronary artery bypass and is considered one of the most important potentially reversible risk factors for mortality after noncardiac surgery.

In conclusion, we compared the effect of stimulating cardiac glucose use by increasing glucose and insulin concentrations with direct activation of carbohydrate oxidation (with DCA) on the metabolic and functional response to ischemia and reperfusion under conditions in which physiological concentrations of lactate, pyruvate, glucose, and palmitate were provided. We found that the combination of increased glucose and insulin improved functional recovery compared with DCA treatment despite the fact that DCA treatment had a greater impact on increasing carbohydrate oxidation. Furthermore, the primary metabolic difference between the HG/HI group and the other 2 groups was an increase in glycolytic lactate efflux during both ischemia and reperfusion, which is contrary to the concept that decreasing glycolytic flux relative to glucose oxidation is beneficial. This dissociation between functional recovery and increased carbohydrate oxidation suggests that the mechanisms underlying the protection associated with increased myocardial glucose use are not related to changes in cardiac efficiency or the coupling of glucose oxidation to glycolysis.
Acknowledgments
This work was supported in part by grants from the National Heart, Lung and Blood Institute (HL-67464 and HL-48789 to Dr Chatham). All NMR studies were performed at the UAB NMR Core Facility funded by NCI Cancer Center support grant CA-13148. We thank Charlye Brooks and Clarence Forrest for technical assistance.

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_Circulation._ 2005;111:2066-2072; originally published online April 11, 2005;
doi: 10.1161/01.CIR.0000162466.06150.D4
_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2005 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

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