Differential modulation of glucose, lactate, and pyruvate oxidation by insulin and dichloroacetate in the rat heart

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Lloyd, Steven, Charlye Brocks, and John C. Chatham. Differential modulation of glucose, lactate, and pyruvate oxidation by insulin and dichloroacetate in the rat heart. Am J Physiol Heart Circ Physiol 285: H163–H172, 2003; 10.1152/ajpheart.01117.2002.—Despite the fact that lactate and pyruvate are potential substrates for energy production in the isolated, perfused rat heart over a range of insulin concentrations and after activation of pyruvate dehydrogenase with dichloroacetate (DCA), hearts were perfused with physiological concentrations of [1-13C]glucose, [U-13C]lactate, [2-13C]-dichloroacetate (DCA). Hearts were freeze clamped, and 13C NMR glutamate isotopomer analysis was performed on tissue extracts. Glucose, lactate, and pyruvate all contributed significantly to myocardial energy production; however, in the absence of insulin, glucose contributed only 25–30% of total pyruvate oxidation. Even under conditions where carbohydrates represented >95% of substrate entering the tricarboxylic acid (TCA) cycle, we found that glucose contributed at most 50–60% of total carbohydrate oxidation. Despite being present at only 0.1 mM, pyruvate contributed between ~10% and 30% of total acetyl-CoA entry into the TCA cycle. We also found that insulin and DCA not only increased glucose oxidation but also exogenous pyruvate oxidation; however, lactate oxidation was not increased. The differential effects of insulin and DCA on pyruvate and lactate oxidation provide further evidence for compartmentation of cardiac carbohydrate metabolism. These results may have important implications for understanding the mechanisms underlying the beneficial effects of increasing cardiac carbohydrate metabolism.

substrate metabolism; carbohydrates; fatty acids

THE PREVAILING VIEW of cardiac energy production is that long-chain fatty acids are the primary oxidative energy source, followed to a lesser extent by glucose use, with other substrates such as lactate playing a much smaller role. This is despite the fact that the myocardium is capable of utilizing a wide variety of fuels for oxidative energy production. At rest, circulating lactate concentrations are ~1 mM (Table 1) and during moderate exercise can easily reach up to 3–5 mM (37). More than 20 years ago, Drake et al. (20) showed that the uptake of lactate by the heart in vivo is directly proportional to its serum concentration. In addition, it has been shown that in the isolated perfused heart, lactate contributes significantly to acetyl-CoA formation, often contributing more than glucose (9, 10, 35).

In addition, serum pyruvate is typically in the range of 0.1–0.2 mM (Table 1), and, while this is relatively low, pyruvate is readily taken up and oxidized by the heart (36, 40, 45) and thus could contribute significantly to overall oxidative energy production in vivo.

There is a resurgence of interest in the regulation of cardiac metabolism, driven, at least in part, by the manipulation of cardiac metabolism as a potential therapeutic intervention (55). For example, drugs such as ranolazine and trimetazidine, which shift the balance from fatty acids to carbohydrate use, appear to be beneficial in the setting of myocardial ischemia (23, 52). There has also been renewed interest in the beneficial effects of glucose-insulin-potassium (GIK) infusions in the setting of acute myocardial infarction (19, 21, 46). It has been proposed that one benefit of decreasing fatty acid oxidation and increasing myocardial carbohydrate utilization is an increase in efficiency, because oxidation of fatty acids requires more oxygen per unit ATP produced than oxidation of glucose (1). It has also been hypothesized that increasing glucose oxidation improves the “coupling” between glycolysis and glucose oxidation, thereby reducing proton accumulation (44). However, much of the experimental data used to support these proposed mechanisms are based on studies where glucose and fatty acids are the only substrates considered. Consequently, it is unclear whether the conclusions from such studies are applicable to situations where other sources of pyruvate are available, as is the case in vivo.

There is also a growing body of literature indicating that increasing pyruvate use by the heart can be advantageous. For example, providing 1–5 mM pyruvate significantly improved cardiac function in the setting of ischemia and reperfusion in the isolated perfused heart.
(6, 58). Increasing flux through pyruvate dehydrogenase (PDH) by dichloroacetate (DCA) improved functional recovery after ischemia and reperfusion in isolated perfused hearts (41, 48) and in pig hearts in vivo (39). Pyruvate has also been shown to improve the contractile performance of cardiac muscle strip preparations from humans with end-stage heart failure (31). Furthermore, increased lactate use appeared to improve cardiac efficiency in an animal model for hemorrhagic shock (2, 38). Lactate has also been associated with increased recovery after ischemia and reperfusion (24, 28).

Thus there is accumulating evidence that modulation of carbohydrate utilization significantly affects contractile function in the setting of cardiac disease. However, our understanding of the control and regulation of carbohydrate metabolism is based principally on studies where glucose is the only available carbohydrate. Consequently, our knowledge regarding the regulation of glucose, lactate, and pyruvate to cardiac energy production at physiological concentrations is limited. Therefore, the purpose of this study was to determine the contributions of lactate, pyruvate, and glucose to energy production in the isolated, perfused rat heart over a range of insulin concentrations and in the presence of DCA.

METHODS

Heart perfusion. Animal experiments were approved by the Institutional Animal Care and Use Committee and conform with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Pub. No. 85-23, Revised 1996). Adult male Sprague-Dawley rats were anesthetized with ketamine (100 mg/kg ip) and decapitated as described previously (9, 11). Hearts were excised and perfused with ketamine (100 mg/kg ip) and decapitated as described previously (9, 11). The extract was freeze dried and redissolved in a potassium phosphate buffer (pH 7.5, 50 mM) with 2H2O solvent (99.9%, Cambridge Isotope Laboratories; Andover, MA).

NMR spectroscopy. 1H-decoupled, nuclear Overhauser enhancement-enhanced 13C NMR spectra were collected on a Bruker AVANCE 500 NMR spectrometer operating at 11.85 T using a TXI heteronuclear broadband probe. Spectra were collected at 300 K with a spectral width of 25 kHz until an adequate signal-to-noise ratio was achieved (typically 4,000–64,000 scans).

Spectra were analyzed using the Nuts software package (AcornNMR; Livermore, CA). 13C time-domain NMR spectra were processed with 1- or 2-Hz line broadening, zero filled, Fourier transformed, and referenced to the methyl carbon peak of lactate [at 21.1 parts per million (ppm)]. The glutamate, lactate, and alanine [13C]NMR multiplet relative areas were measured as described previously (11, 30).

13C-glutamate isotopomer analysis. As previously described (11, 30), 13C NMR glutamate isotopomer analysis was performed using tcaCALC (developed and provided by Dr. Mark Jeffrey, University of Texas Southwestern Medical Center, http://www2.swmed.edu/rogersmr/) to determine the fraction of total acetyl-CoA entering the tricarboxylic acid (TCA) cycle that originates from unlabeled, [1,2,13C]-, [2,13C]- and [1,13C]acetyl-CoA (Fig. 1). Fgl is the fraction of acetyl-CoA that originated from [1,13C]glucose. FIn and Fpy are the fractions of acetyl-CoA originating from [U,13C]lactate and [2,13C]pyruvate, respectively. Fb is the fraction of acetyl-CoA attributed to glucose was accounted for, the remainder was attributed to exogenous palmitate (i.e., Fb).

After 30 min of equilibration, the unlabeled glucose, lactate, and pyruvate in the buffer was switched to [1,13C]glucose, [U-13C]lactate, and [2,13C]pyruvate at the same concentrations as the unlabeled compounds, as described in more detail below. After 45 min of perfusion with labeled substrates, hearts were freeze clamped and extracted with 6% perchloric acid as described previously (9, 11). The extract was freeze dried and redissolved in a potassium phosphate buffer (pH 7.5, 50 mM) with 2H2O solvent (99.9%, Cambridge Isotope Laboratories; Andover, MA).

Values for the lactate and pyruvate concentrations are means ± SE.

<table>
<thead>
<tr>
<th>Lactate, mM</th>
<th>Pyruvate, mM</th>
<th>Lactate-to-Pyruvate Ratio</th>
<th>Species and Conditions</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.67 ± 0.06</td>
<td>0.046 ± 0.003</td>
<td>15</td>
<td>Human adult fasting</td>
<td>22</td>
</tr>
<tr>
<td>1.45 ± 0.12</td>
<td>0.119 ± 0.020</td>
<td>12</td>
<td>Human adult insulin clamp</td>
<td>22</td>
</tr>
<tr>
<td>1.6 ± 0.7</td>
<td>0.07 ± 0.03</td>
<td>23</td>
<td>Human neonatal (presurgery)</td>
<td>56</td>
</tr>
<tr>
<td>2.1 ± 0.8</td>
<td>0.07 ± 0.01</td>
<td>24</td>
<td>Human neonatal (posturgery)</td>
<td>56</td>
</tr>
<tr>
<td>1.37 ± 0.08</td>
<td>0.11 ± 0.02</td>
<td>12</td>
<td>Anesthetized, fasted adult swine</td>
<td>51</td>
</tr>
<tr>
<td>1.38 ± 0.05</td>
<td>0.17 ± 0.04</td>
<td>8</td>
<td>Anesthetized, fasted adult swine</td>
<td>51</td>
</tr>
<tr>
<td>1.40 ± 0.10</td>
<td>0.15 ± 0.02</td>
<td>9</td>
<td>Anesthetized, fasted adult swine</td>
<td>51</td>
</tr>
<tr>
<td>1.05 ± 0.16</td>
<td>0.13 ± 0.02</td>
<td>6</td>
<td>Anesthetized, fasted dog</td>
<td>40</td>
</tr>
</tbody>
</table>

Table 1. Serum lactate and pyruvate concentrations and the lactate-to-pyruvate ratio in humans and animals

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Fig. 1. Schematic indicating the metabolic pathways that were investigated in this study. $F_{gl}$, $F_{pa}$, $F_{py}$, and $F_{ba}$ are the fractions of acetyl-CoA originating from [1-13C]glucose, [U-13C]lactate, [2-13C]pyruvate, and unlabeled palmitate, respectively. The labeling pattern illustrated represents that used in the majority of the experiments. Substrates may enter the tricarboxylic acid (TCA) cycle either through the common pathway involving acetyl-CoA (citrate synthase) or by anaplerosis. OAA, oxaloacetate; 2KG, 2-ketoglutarate.

In addition to carbon entry into the TCA cycle metabolism via acetyl-CoA carbon, entry can also occur via so-called anaplerotic pathways. Such pathways include pyruvate carboxylase and malic enzyme, which catalyze the formation of oxaloacetate and malate, respectively, from pyruvate; the synthesis of succinyl CoA from propionyl CoA; or synthesis of anaplerotic pathways. Such pathways include pyruvate carboxylase via acetyl-CoA carbon, entry can also occur via so-called anaplerotic pathways. Such pathways include pyruvate carboxylase and malic enzyme, which catalyze the formation of oxaloacetate and malate, respectively, from pyruvate; the synthesis of succinyl CoA from propionyl CoA; or synthesis of 2-ketoglutarate from glutamate. In principle, the 13C-labeling pattern in glutamate will be different if [13C]pyruvate is metabolized via pyruvate carboxylase compared with PDH. Furthermore, the influx of unlabeled four-carbon units into the TCA cycle via other anaplerotic pathways results in decreased 13C-enrichment of C2- and C4-glutamate relative to enrichment of C4-glutamate. We have previously shown that glutamate isotopomer analysis can be used to determine the relative flux through pyruvate carboxylase as well as the relative contribution of unlabeled anaplerotic substrate to TCA cycle flux (30).

Therefore, we initially analyzed the glutamate isotopomer data using two models: one in which only unlabeled anaplerosis was included (as shown in Fig. 1), and the other as used previously (30), where both unlabeled and 13C-labeled anaplerosis via pyruvate carboxylase were included. Statistical comparison of the two models indicated that the more complex model did not significantly improve the overall fit of the data, and the values for pyruvate carboxylase were highly variable and not well defined (data not shown). Similar results were also obtained when all exogenous carbohydrates were uniformly labeled with carbon-13 (data not shown). Consequently, we concluded that under these experimental conditions, despite the presence of several sources of [13C]pyruvate, there was insufficient information content in the [13C]glutamate isotopomer distribution to reliably determine the relative flux through pyruvate carboxylase. Therefore, only unlabeled anaplerosis was included in the analyses presented here.

In addition to being oxidized, pyruvate can also be converted to alanine via alanine aminotransferase and to lactate via lactate dehydrogenase. Therefore, [U-13C]lactate can be metabolized to [U-13C]alanine, and [1-13C]glucose metabolism can result in [3-13C]alanine (from the generation of [3-13C]pyruvate), generating singlet and doublet C3-resonances of alanine, respectively. Thus, as previously described (11, 30), isotopomer analysis of the C3-resonances of alanine and lactate provides an index of the nonoxidative metabolism of exogenous glucose via glycolysis.

Experimental protocol. Four experimental groups (no insulin, $n = 5$; low insulin, $n = 8$; high insulin, $n = 7$; and DCA + low insulin, $n = 10$), as listed in Table 2, were used to assess the effect of insulin and direct stimulation of PDH on the relative contributions of glucose, lactate, pyruvate, and fatty acids to acetyl-CoA entry into the TCA cycle. There is the potential for insulin to bind to glassware and plastic tubing used in the perfusion apparatus. Therefore, in a subset of experiments from each group, perfusate samples were collected after being passed through the perfusion system and analyzed for insulin by radioimmunoassay (RI-13K, Linco Research, St. Charles, MO). These results are summarized in Table 2. In the no insulin group, there was apparently a low level of insulin, which presumably reflects a low level of contamination with insulin in the BSA in the perfusate or nonspecific binding in the radioimmunoassay. The low insulin concentration group, although a little higher than that seen in the fed state, is in the range used in insulin clamp studies (17, 22). The high insulin concentration is somewhat higher than that typically reported for insulin-resistant humans and animals (13, 18) but lower than values typically found in humans during GIK infusion trials (57). DCA (5 mM) was added along with insulin at the same level as in the low insulin group. The concentration of DCA used to activate PDH was based on that previously shown to increase glucose oxidation in the presence of fatty acids (61) and has been shown to increase the fraction of PDH in the active form to 70–80% (14, 62).

The labeling scheme used for the groups described above was as follows: [1-13C]glucose, [U-13C]lactate, [2-13C]pyruvate, and unlabeled palmitate. A small number of experiments were also conducted with low insulin ($n = 7$) and high insulin ($n = 3$) with the following labeling scheme: [U-13C]glucose, [U-13C]lactate, [U-13C]pyruvate, and unlabeled palmitate. The results for total carbohydrate oxidation and anaplerosis from these groups were similar to those of the other labeling scheme and were combined with data for these parameters from the other low and high insulin groups.

Statistical analysis. All data are presented as means ± SE. Unpaired and paired $t$-tests and ANOVA combined with Scheffe’s post hoc test were used where appropriate (Statview, Abacus Concepts; Berkeley, CA).

RESULTS

Cardiac function. Left ventricular contractile function was not different among the groups at the beginning or end of perfusion with 13C-labeled substrates.

Table 2. Experimental groups and experimentally determined insulin concentrations in buffer

<table>
<thead>
<tr>
<th>Insulin Concentration, µU/ml</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No insulin</td>
<td>2.1 ± 0.2</td>
</tr>
<tr>
<td>Low insulin</td>
<td>82.6 ± 8.2</td>
</tr>
<tr>
<td>High insulin</td>
<td>923.9 ± 90.1</td>
</tr>
<tr>
<td>DCA (5 mM) + low insulin</td>
<td>99.1 ± 12.6</td>
</tr>
</tbody>
</table>

Values are means ± SE. DCA, dichloroacetate.
The rate of left ventricular pressure development, left ventricular developed pressure, heart rate, and rate-pressure product for the four groups are summarized in Fig. 2. The lack of effect of insulin and DCA on contractile function seen here is consistent with other reports on the effects of these agents under normoxic conditions (8, 25, 41, 47). However, this is in contrast with the known beneficial effects of both insulin and particularly DCA on cardiac function after ischemia (39, 41, 48, 59).

Glutamate isotopomer analysis. Typical C4-glutamate resonances from each of the four groups are shown in Fig. 3A. It can be seen that in the absence of insulin the predominant resonances are from lactate. As the insulin concentration increases, the intensity of the resonances originating from glucose also increases. The addition of DCA also increases the contribution from glucose resonances compared with the low insulin group. A simple inspection of the C4-glutamate resonance provides insight into the relative contributions of glucose and lactate to acetyl-CoA entry into the TCA cycle. However, from a detailed analysis of all the glutamate resonances, we determined the effects of increasing insulin concentration and the addition of DCA on the contributions of glucose, lactate, pyruvate, and palmitate to acetyl-CoA entry into the TCA cycle (Figs. 4 and 5).

Not surprisingly, the no insulin group had the lowest total carbohydrate oxidation (Fig. 4A); however, inter-
of DCA. *

The low insulin group (Table 2). The low insulin data from Fig. 4 are repeated here to demonstrate directly the effects of DCA. *P < 0.05 vs. low insulin.

Interestingly, lactate contributed approximately twice as much to acetyl-CoA formation as glucose, and the contribution of pyruvate to acetyl-CoA was only about one-half that of glucose despite being present at a 50-fold less concentration (Fig. 4B). In the low insulin group, total carbohydrate oxidation increased by almost 70% (compared with the no insulin group), due almost entirely to a threefold increase in glucose oxidation. In the high insulin group, there was a further significant increase in both total carbohydrate oxidation and glucose oxidation. Interestingly, there was a more than twofold increase in the oxidation of exogenous pyruvate between the no insulin and high insulin groups. Paradoxically, there was a significant 40% decrease in lactate oxidation between the no insulin and high insulin groups. The effect of insulin on palmitate oxidation was the inverse of that for total carbohydrate and high insulin groups. ANOVA revealed a significant positive insulin effect on total carbohydrate oxidation, glucose, and pyruvate oxidation and a negative effect on lactate and palmitate oxidation.

DCA added in addition to low insulin resulted in a more than fourfold increase in anaplerosis between the no insulin and low insulin groups and a nearly sixfold increase between the no insulin and high insulin groups (Fig. 6A). There was no difference in anaplerosis between the low insulin and high insulin groups. DCA plus low insulin led to a small but non-significant increase (P = 0.082) in anaplerosis (Fig. 6B) compared with low insulin alone.

**Lactate and alanine isotopomer analysis.** Analysis of the contribution of glucose metabolism to tissue lactate and alanine allows an estimate of myocardial glycolytic activity. Representative spectra of the C3-lactate (methyl) resonance are shown in Fig. 3B for each experimental group. As the insulin concentration is increased, there is a clear increase in the singlet peak, indicating increased contribution from glucose to tissue lactate consistent with increased flux through glycolysis. The addition of DCA to the low insulin group also resulted in an increase in the resonance from glucose. To obtain an index of nonoxidative glucose oxidation (Fig. 5A) leading to almost complete inhibition of fatty acid oxidation. This increase in carbohydrate oxidation was due to an 80% increase in glucose and an almost threefold increase in pyruvate oxidation (P < 0.05 for both glucose and pyruvate), with no significant change in lactate oxidation (Fig. 5B).

To determine whether the relative contributions of exogenous substrates to acetyl-CoA formation were related to their concentrations, we normalized the contributions of each substrate to their relative concentrations, accounting also for the number of acetyl-CoA moieties per substrate molecule oxidized. The results of this analysis are summarized in Table 3. It is clear that under all conditions, pyruvate was more readily oxidized than all the other substrates and glucose was the least oxidized. Interestingly, lactate and palmitate were oxidized to a similar extent under all conditions except in the DCA + low insulin group. In the absence of insulin, pyruvate contributed 50 times more than glucose and almost threefold more than lactate; however, in the high insulin group, the gap between pyruvate and glucose use decreased by almost 50%, whereas the difference between pyruvate and lactate increased by a factor of 3.

Insulin had a significant effect on anaplerotic flux with a more than fourfold increase in anaplerosis between the no insulin and low insulin groups and a nearly sixfold increase between the no insulin and high insulin groups (Fig. 6A). There was no difference in anaplerosis between the low insulin and high insulin groups. DCA plus low insulin led to a small but non-significant increase (P = 0.082) in anaplerosis (Fig. 6B) compared with low insulin alone.

Table 3. **Contribution of exogenous substrates to acetyl-CoA entry into the tricarboxylic acid cycle normalized to relative substrate concentrations**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>No Insulin</th>
<th>Low Insulin</th>
<th>High Insulin</th>
<th>DCA + Low Insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>0.11 ± 0.02</td>
<td>0.30 ± 0.05a</td>
<td>0.48 ± 0.02ab</td>
<td>0.54 ± 0.02b</td>
</tr>
<tr>
<td>Lactate</td>
<td>2.2 ± 0.24d</td>
<td>1.8 ± 0.24d</td>
<td>1.4 ± 0.14ad</td>
<td>2.09 ± 0.07d</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>5.8 ± 0.54ac</td>
<td>9.5 ± 1.2bd</td>
<td>12.6 ± 1.1ac</td>
<td>27.1 ± 1.1bc</td>
</tr>
<tr>
<td>Palmitate</td>
<td>2.6 ± 0.1c</td>
<td>1.5 ± 0.1ac</td>
<td>0.9 ± 0.1a</td>
<td>0.15 ± 0.06b</td>
</tr>
</tbody>
</table>

Values are means ± SE. Relative concentrations are defined as follows: pyruvate = 1 (i.e., 1 acetyl-CoA formed per molecule oxidized); lactate = 10 (1 acetyl-CoA formed per molecule oxidized, but lactate concentration 10-fold higher than that of pyruvate); glucose = 100 (2 acetyl-CoA formed per molecule oxidized, but glucose concentration 50-fold higher than that of pyruvate); palmitate = 24 (8 acetyl-CoA formed per molecule oxidized, but palmitate concentration 3-fold higher than that of pyruvate). Statistical significance determined by paired t-tests: *P < 0.05 vs. lactate and glucose; **P < 0.05 vs. glucose only; ***P < 0.05 vs. pyruvate and glucose; ****P < 0.05 vs. lactate only.

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metabolism (i.e., glycolysis), the singlet representing \([3-^{13}\text{C}]\)lactate/alanine was corrected to account for the unlabeled lactate and alanine produced by metabolism of \([1-^{13}\text{C}]\)glucose, and the percent contribution of exogenous glucose to total tissue lactate and alanine formation was calculated (Fig. 7). The addition of insulin significantly increased the contribution of glucose to both tissue lactate and alanine resonances. There was a further increase in the contribution from glucose in the high insulin group compared with the low insulin group. The changes in the contribution of glucose to tissue alanine in response to insulin were comparable to the changes in \(F_{\text{gl}}\) (Fig. 4AB). By comparison, the changes in the contribution of glucose to tissue lactate were blunted; this is most likely due to the contribution from perfusate \([U-^{13}\text{C}]\)lactate. The addition of DCA also increased the contribution of glucose to tissue lactate compared with the low insulin group. In the DCA + low insulin group, the intensities of both the lactate and alanine resonances were lower than that seen in the other groups, presumably reflecting a decrease in tissue lactate and alanine levels after the addition of DCA. In all groups, the intensities of the alanine resonances were less than those of lactate. In the DCA + low insulin group, the intensity of the alanine resonance was too low to permit reliable isotope-pomer analysis.

**DISCUSSION**

It is well established that glucose, lactate, and pyruvate are readily oxidized by the heart. However, we believe that this is the first report on the contributions of each of these substrates to cardiac energy production when present in physiologically relevant concentrations over a range of insulin levels and in response to direct stimulation of PDH. These results clearly demonstrate that in addition to long-chain fatty acids and glucose, lactate and pyruvate can also contribute significantly to energy production in the isolated perfused rat heart. In fact, in the absence of insulin, glucose contributed only \(\sim 25\%\) of the total pyruvate oxidized. Even under conditions where carbohydrates represented \(>95\%\) of substrate entering the TCA cycle, we found that glucose contributed at most \(50\%–60\%\) of total carbohydrate oxidation. It is especially noteworthy that pyruvate contributed between 20\% and 30\% of the total carbohydrate oxidation despite being present at only 0.1 mM. That both insulin and DCA increased total carbohydrate oxidation as well as glucose oxidation is of little surprise. However, the fact that both had similar effects on increasing exogenous pyruvate oxidation suggests that insulin directly stimulates PDH. We have also shown here for the first time that physiologically relevant concentrations of insulin increased anaplerosis in the isolated perfused heart.

Despite evidence demonstrating that both in vivo and in vitro lactate can contribute significantly to cardiac energy production (3, 9, 20, 29, 35), the predominant view remains that fatty acids and glucose are the major substrates used for energy production with other fuels contributing to a much lesser extent. However, depending on the conditions, we have shown that pyruvate and lactate combined contributed between 40\% and 70\% of total carbohydrate oxidation. The lactate concentration used here was 1 mM and was chosen to represent typical resting levels found in vivo; however, as can be seen in Table 1, this is at the low end of reported values. Indeed, moderate exercise can lead to serum lactate levels of 3–4 mM (37), and even higher levels can be seen during vigorous exercise or in response to trauma (32, 33, 37). There are substantially less data available on serum pyruvate levels; however, as can be seen from Table 1, the use of 0.1 mM pyruvate, as used here, also represents a relatively conser-

![Fig. 6. The effect of insulin (A) and DCA (B) on total anaplerosis. Anaplerosis is determined as a percentage of the total TCA cycle flux. *P < 0.05 vs. no insulin.](image)

![Fig. 7. Percent contribution of exogenous glucose to total tissue lactate and alanine. The data represent the intensity of the C3-lactate and C3-alanine resonances resulting from metabolism of \([1-^{13}\text{C}]\)glucose corrected for the unlabeled glycolytic fragment relative to the total C3-lactate and alanine resonance intensity. This provides an index of nonoxidative glucose metabolism, i.e., glycolysis. Note that in the DCA + low insulin group, there was insufficient alanine observed in the spectrum for analysis. *P < 0.05 vs. no insulin; †P < 0.05 vs. low insulin.](image)
The predominant oxidative metabolic pathway for exogenous glucose, lactate, and pyruvate is the formation of acetyl-CoA via PDH. Thus one might anticipate that their contributions to acetyl-CoA formation may simply be proportional to their concentrations in the perfusate. However, as noted above, pyruvate was present at only 0.1 mM and yet contributed to a similar extent as lactate despite a 10-fold concentration difference. The disproportionate use of both lactate and pyruvate relative to glucose is emphasized further if their contributions to acetyl-CoA formation are normalized to the pyruvate equivalents produced by these substrates at their respective concentrations, as shown in Table 3. Insulin decreases the difference between glucose and lactate use to a much greater extent than the difference between glucose and pyruvate use. Furthermore, DCA added to low insulin increased the difference between pyruvate and both glucose and lactate use, whereas it had relatively little effect on the difference between glucose and lactate. This analysis emphasizes the fact that the contributions of different substrates to cardiac energy production cannot be inferred from their available concentration. Thus, even though a substrate may be present at relatively low levels, it may make a significant contribution. Jeffrey and colleagues (35) reached a similar conclusion when examining the contribution of ketone bodies to TCA cycle flux in the isolated perfused heart.

The stimulation of glucose metabolism by insulin is typically thought to be mediated primarily by increasing glucose entry into the cell via increased translocation of glucose transporters to the sarcolemma, whereas the main effect of DCA is the activation of PDH via inhibition of PDH kinase. If the effect of insulin on carbohydrate oxidation was solely mediated via increased glucose entry into the cell and increased flux through glycolysis, this should increase the amount of glucose-derived pyruvate that is oxidized at the expense of other exogenous pyruvate sources. However, we found that insulin stimulated the oxidation of exogenous pyruvate (Fig. 4B) in a similar but less-pronounced manner to DCA (Fig. 5B). These data are consistent with earlier reports of insulin activation of PDH in cardiomyocytes (16, 42, 53) and in adipocytes (34).

Stimulation of PDH activity should lead to increased oxidation of all exogenous sources of pyruvate assuming that they all contributed to a common cytosolic pyruvate pool. However, in contrast to pyruvate, insulin and DCA did not stimulate lactate oxidation. Although the addition of DCA almost doubled glucose oxidation and tripled pyruvate oxidation, lactate oxidation remained unchanged (Fig. 5B). With insulin, there was a negative correlation between insulin levels and lactate oxidation (Fig. 4B). These data suggest that lactate-derived pyruvate is oxidized via a pathway that is distinct from both endogenous pyruvate and glycolytically derived pyruvate. This would be consistent with the existence of mitochondrial lactate dehydrogenase and the lactate shuttle hypothesis proposed by Brooks and colleagues (4, 5) and is also in agreement with our earlier work on compartmentation of cardiac carbohydrate metabolism (7).

The predominant influx of substrates into the TCA cycle in the heart is via acetyl-CoA and citrate synthase. However, four- or five-carbon units can also enter the TCA cycle via so-called anaplerotic pathways. Historically, flux through anaplerotic pathways was considered to be a transient process leading to replenishment of TCA cycle intermediates in response to acute physiological or metabolic change (27). However, a recent series of elegant studies using gas chromatography-mass spectroscopy clearly demonstrated that both in vivo and in vitro there is a steady-state influx of carbons into the TCA cycle via pyruvate carboxylation that can be modulated by substrate availability and by physiological changes such as hibernation (15, 50, 51, 60). Although we were unable to identify the specific site of anaplerosis in our experiments, we found that total anaplerosis ranged from 2% to 8% of total TCA cycle flux, consistent with the values reported for flux through pyruvate carboxylase in the isolated perfused rat heart (15, 60). Interestingly, we found that the addition of insulin increased anaplerosis by approximately threefold. To our knowledge, this is the first time that anaplerosis has been shown to be sensitive to insulin. This is consistent with reports showing that increased mitochondrial substrate availability stimulates anaplerosis (60) as well the report of insulin growth factor 1 stimulation of anaplerosis in cultured vascular smooth muscle cells (30).

There has long been an interest in using metabolic modulators as therapy in a variety of cardiac diseases, including myocardial ischemia, infarction, and heart failure. GIK therapy for acute myocardial infarction was proposed in the 1960s, and several recent trials have shown promise in improving survival (19, 21, 46). There have also been several studies demonstrating the utility of the fatty acid oxidation inhibitors such as ranolazine and trimetazidine as antiischemic agents (23, 52). One of the most commonly proposed mechanisms for the beneficial effects of increasing glucose oxidation at the expense of fatty acid oxidation is that this improves the coupling between glycolysis and glucose oxidation leading to lower accumulation of cellular protons and hence less intracellular acidosis (43, 44). However, this is based primarily on studies where glucose was the only carbohydrate provided (43, 44). As we have shown here, when other substrates such as lactate and pyruvate are provided at physiologically relevant concentrations, glucose contributes at most 50–60% of total carbohydrate oxidation. Furthermore, direct stimulation of PDH resulted in a much greater increase in pyruvate oxidation than glucose. In addi-
tion to stimulating glucose oxidation, DCA paradoxically has also been reported to inhibit glycolysis (12, 54). Such an effect could explain the observation that DCA increased pyruvate oxidation to a greater extent than glucose oxidation. Because increasing pyruvate use alone improves contractile performance after ischemia (6, 58) and in heart failure (31), further studies into the mechanism(s) underlying the beneficial effects of increased carbohydrate use may need to consider substrates in addition to glucose.

An important factor that should be considered is that we used an isovolumetric Langendorff preparation, whereas the majority of in vitro studies of cardiac metabolism use a “working heart” or ejecting preparation. The main difference between these two preparations is that the overall oxidative energy consumption as defined by oxygen consumption is lower in the isovolumetric preparation. The oxygen consumption rates and workload in a working heart preparation are similar to that seen in vivo. Clearly, substrate oxidation rates are highly dependent on cardiac work; however, the relative contributions of different substrates to oxidative metabolism appear to be less sensitive to workload. For example, in a working heart preparation, Jeffrey et al. (35) showed that an almost twofold difference in workload had no significant effect on the relative contributions of lactate and fatty acids to acetyl-CoA formation. Indeed, in that study, the contributions of lactate and fatty acids to energy production were similar to those reported here. Goodwin et al. (29) also reported that in a working heart preparation, glucose oxidation was low and that lactate and oleate were the predominant sources of energy. However, a twofold increase in workload induced by epinephrine increased both lactate and glucose oxidation but not oleate oxidation. The presence of circulating catecholamines or other hormones certainly may influence energy metabolism. Our perfusion model involves a relatively simple hormonal environment, with only insulin present. Thus a more complex in vitro preparation, or an in vivo preparation, may yield results somewhat different from what we have found here.

It is important to note that in contrast to more traditional radioisotope measurements of substrate oxidation, the data presented here are not in the form of absolute oxidation rates but rather as relative contributions to acetyl-CoA entry into the TCA cycle. As previously demonstrated, this relative flux data can be converted to absolute oxidation rates if oxygen consumption rates are determined during perfusion with 13C-labeled substrates (9). Oxygen consumption was not routinely measured in these experiments, and thus we were unable to calculate absolute rates of glucose, lactate, and pyruvate oxidation. Because the rate-pressure product was similar among all four of the groups, total ATP utilization should also be similar. However, this may not mean that oxygen consumption is comparable because of the differences in ATP production per oxygen consumed for glucose and palmitate. As summarized by Opie (49), the amount of ATP produced per oxygen atom consumed is ~12–13% greater when glucose is the sole energy source compared with when palmitate is the sole source. The amount of ATP produced per oxygen consumed by oxidation of both lactate and pyruvate falls midway between glucose and palmitate. With the use of this analysis, we estimated that the fractional change in oxygen consumption between the no insulin and high insulin groups was 5 ± 9% and between the low insulin and low insulin + DCA groups was 4 ± 14%. Therefore, we predict that the shift toward greater carbohydrate use in these experiments would not result in a significant change in total oxygen consumption.

In conclusion, we found that in the isolated perfused rat heart, glucose, lactate, and pyruvate all contribute significantly to myocardial energy production when present at physiologically relevant concentrations. Even under conditions where carbohydrates represented >95% of substrate entering the TCA cycle, we showed that glucose contributed at most 50–60% of total carbohydrate oxidation. Despite being present at only 0.1 mM, pyruvate contributed between ~10% and 30% of total acetyl-CoA entry into the TCA cycle. We also found that insulin and DCA increased glucose and exogenous pyruvate oxidation with no increase in lactate oxidation. The differential effects of insulin and DCA on pyruvate and lactate oxidation provide further evidence for compartmentation of cardiac carbohydrate metabolism. These findings demonstrate that the regulation of cardiac carbohydrate oxidation is complex and indicate the need for more studies into the mechanisms underlying the beneficial effects of increasing cardiac carbohydrate metabolism.

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REFERENCES


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44. Lopaschuk GD, Wambolt RB, and Barr RL. An imbalance between glycolysis and glucose oxidation is a possible explanation for the detrimental effects of high levels of fatty acids during aerobic reperfusion of ischemic hearts. J Pharmacol Exp Ther 264: 135–144, 1993.


47. Mazzer CD, Cason BA, Stanley WC, Shnider CB, Wisneski JA, and Hickey RF. Dichloroacetate stimulates carbohydrate metabolism but does not improve systolic function in ischemic


