Fatty Acid Metabolism and Contractile Function in the Reperfused Myocardium

Multinuclear NMR Studies of Isolated Rabbit Hearts

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The hypothesis that substrate availability can alter contractile function in reperfused myocardium after global ischemia was investigated in this study. Isolated rabbit hearts were placed in a dual tuned (1H/13C) NMR probe with a 9.4-T magnet and perfused with the following substrates given individually or in combination: 10 mM glucose, 2 mM palmitate, and 2.5 mM [3-13C]pyruvate. Glucose was the sole substrate present for all groups of hearts before the onset of 10 or 20 minutes of zero-flow ischemia. Contractility (dP/dt) was significantly higher in hearts reperfused with glucose compared with hearts reperfused with palmitate or the combination. In addition, myocardial oxygen consumption/unit of work at reperfusion was more efficient with glucose than with palmitate. ATP content during reperfusion was similar with glucose and palmitate and did not account for improved function with glucose. To determine if inhibition of pyruvate metabolism by palmitate might result in altered posts ischemic function, additional hearts were reperfused with 2.5 mM [3-13C]pyruvate provided alone or in combination with palmitate. Using 13C NMR spectroscopy, it was shown that with the addition of palmitate, pyruvate oxidation was decreased in control and 10-minute ischemic hearts as is consistent with inhibition of pyruvate dehydrogenase by fatty acids. However, palmitate/pyruvate did not worsen posts ischemic function as compared with palmitate or pyruvate alone. Tricarboxylic acid cycle activity was slowed in reperfused pyruvate hearts, but no further reduction was observed when palmitate was present. In conclusion, palmitate reduces the mechanical function of the reperfused isolated rabbit heart as compared with glucose. This effect of palmitate does not appear to be caused by suppression of pyruvate oxidation or by a change in high energy phosphate content. (Circulation Research 1991;68:714–725)

With the advent of thrombolytic therapy for the treatment of acute myocardial infarction, attention has been directed to possible therapeutic interventions that may enhance the mechanical performance of reperfused myocardium.1 The beneficial effects derived from various interventions generally have been attributed to a reduction in the cell injury produced by ischemia or the prevention of further injury caused by reperfusion. A number of different types of pharmacological interventions have been studied, including β-blockade and calcium channel blockade and the use of free radical scavengers.1 Other studies have examined the role of substrate metabolism and its effects on cardiac performance. For example, long-chain fatty acids have been shown to impair left ventricular function and cause ventricular arrhythmias in humans.2 Perfusion of isolated rat hearts with palmitate before the induction of global ischemia produced a greater reduction in left ventricular function during ischemia than did perfusion of hearts under similar conditions with glucose or a lower concentration of fatty acid.3 Studies in the isolated, in situ swine heart model during regional myocardial hypoperfusion demonstrated a decrease in cardiac contractility in association with an increase in tissue acyl coenzyme A and acylcarnitine.4 The rise in tissue amphiphiles suggested the possibility that long-chain fatty acid metabolites may contribute to ischemic myocardial dysfunction during hypoperfusion. Indeed, inhibition of fatty acid metabolism with the palmitoylcarnitine transferase I inhibitor oxfenicine reduced the amphiphile concentration and improved function.5 However, with reperfusion, cardiac function was largely unaffected by an increase in myocardial amphiphiles.6,7 It has been clearly established that long-chain fatty acids, when present in high concentrations, are the preferred substrate metabolized by normal8 and reperfused myocardium.8,9 Fatty acid metabolism in-
hibits the pyruvate dehydrogenase complex by a direct feedback effect on the active form of the enzyme. 

Studies of normal hearts have demonstrated that fatty acids inhibit glucose transport in the cell, as well as phosphofructokinase and glucose phosphorylation through an increase in citrate, Positron emission tomography studies of reperfused myocardium have demonstrated delayed [13C]palmitate clearance in association with an increase in [18F]deoxyglucose uptake. These findings are consistent with a shift in substrate utilization from palmitate to glucose. However, Myears et al found that despite a doubling in [1H]glucose uptake in the reperfused myocardium, much of the substrate was not immediately used for energy production. A small increase in anaerobic metabolism was noted in the reperfused myocardium, but most of the energy produced was derived from palmitate. The authors postulated that enhanced membrane transport of glucose and repletion of glycogen might account for the increase in glucose uptake observed with positron emission tomography. Lopaschuk et al showed that by inhibiting palmitate metabolism with the carnitine palmitoyltransferase I inhibitor etomoxir, glucose oxidation was increased and contractile function was improved in the reperfused myocardium. Renstrom et al found a twofold increase in glucose oxidation in reperfused myocardium when oxefinicine, a carnitine palmitoyltransferase I inhibitor, was given to the in situ swine heart preparation. Enhanced glucose oxidation was associated with a significant improvement in cardiac function during hypoperfusion but not during reperfusion. In both of these studies it was apparent that the switch to glucose utilization by the reperfused myocardium could be elicited only if inhibitors of long-chain fatty acid metabolism were used. The investigation of glucose utilization by nonoxidative and oxidative pathways was not attempted in these studies.

Previous studies have demonstrated the utility of NMR spectroscopy for the direct study of substrate metabolism in the nonischemic myocardium. Chance et al determined metabolic flux parameters from neutralized perchloric acid extracts of perfused rat hearts and suggested that such measurements could be made in living systems. Sherry et al observed spin–spin coupling between 13C-enriched nuclei of glutamate isomers in the intact, nonischemic guinea pig heart preparation and showed that lactate was preferred as a substrate over glucose. Malloy et al reported that 31C NMR spectroscopy could be used to assess substrate selection and relative metabolic flux through anaplerotic and oxidative pathways in the intact Langendorff rat heart preparation. Early studies demonstrated that fatty acids inhibit pyruvate dehydrogenase activity. Recently, Weiss et al used 13C NMR with [1-13C]glucose to show that hexanoic acid, a short-chain fatty acid, caused a decrease in 13C-labeling of glutamate. In our laboratory, 13C NMR methods have been used to investigate substrate utilization and flux within the tricarboxylic acid (TCA) cycle in reperfused intact rabbit hearts.

The present study was undertaken to determine 1) if the availability of physiologically relevant fatty acids, in particular palmitate, could alter cardiac function in the isolated perfused rabbit heart after global ischemia; 2) if altered contractile function might be caused by changes in high energy phosphate content; and 3) if regulation of oxidative pyruvate utilization by palmitate alters postischemic myocardial function. To achieve these goals, high energy phosphate content and pyruvate oxidation in the presence of palmitate were examined with NMR spectroscopy.

Materials and Methods

Dutch-belted rabbits (500–750 g) were anesthetized with an intraperitoneal injection of pentobarbital sodium (200 mg/kg). The study was approved by the Subcommittee for Animal Use of Baylor College of Medicine and conforms to the position of the American Heart Association on research animal use. The heart was removed at the ascending aorta through a midternal incision and immediately washed with ice-cold cardioplegic solution (120 mM NaCl + 30 mM KCl). The heart was retrogradely perfused at a constant pressure of 100 cm water with a modified Krebs-Henseleit buffer (116 mM NaCl, 4 mM KCl, 1.5 mM CaCl2, 1.2 mM MgSO4, 1.2 mM NaH2PO4, 25 mM NaHCO3). The buffer was gassed with 95% O2–5% CO2 and maintained at 37°C at the level of the aortic cannula. No insulin was added to the perfusate. Perfusate supply was circulated from a 2-l reservoir containing 5 mM glucose. A catheter was placed into the pulmonary artery to collect coronary effluent. Myocardial oxygen consumption (MV02) was determined as previously described. A water-filled latex balloon was attached to a pressure transducer and inserted into the left ventricle through the left atrium to measure developed pressure and contractility (dP/dt). Volume in the latex balloon was increased to achieve an end-diastolic pressure of approximately 10 mm Hg. All data were recorded on a physiological monitoring system (model 2400S, Gould, Cleveland). The reservoir was enclosed in a heating pad, and temperature was held constant with a temperature-controlling device (model 2157, Cole Palmer, Chicago). The heart preparation was contained in a 20-mm glass tube and placed inside the bore of the magnet. To switch the substrate provided to the heart, a stopcock was turned to direct perfusate from the larger reservoir to a smaller (400 ml) reservoir. Since albumin was shown to cause a slight decrease in dP/dt and rate-pressure product in a preliminary study (high energy phosphates were unchanged in the preliminary study), all substrates were combined with 0.67 mM albumin. Oxygenation was achieved without foaming by continuously circulating the perfusate through an oxygenated glass chamber designed to distribute the fluid over a large surface area. The fluid was also
oxygenated by passing it through gas-permeable Silastic tubing held within a Tygon oxygen jacket enroute to the heart. With this technique, PO₂ of the perfusate was ≥400 mm Hg.

**NMR Spectroscopy**

The heart preparation was situated within a 20 mm, ³¹P/¹³C-tuned NMR probe (Bruker Instruments, Inc., Billerica, Mass.). NMR data were acquired using a wide-bore AM 400 spectrometer (Bruker) equipped with an Aspect 3000 series computer and a superconducting magnet (9.4 T). The sample chamber was tuned and the field homogeneity optimized using the proton signal of water. The probe was maintained at 37°C throughout the study. A composite ¹³C-free induction decay was acquired at 100.6 MHz in 6 minutes (180 acquisitions) using a 45° pulse angle and a 2-second repetition time. Sample heating was prevented by power-gating the broadband proton decoupling (0.5–5 W). To eliminate background ¹³C signal, naturally abundant ¹³C was obtained before ¹³C enrichment and then digitally subtracted from subsequent spectral data. Frequency induction decays were zero-filled to improve digital resolution, and exponential multiplication was performed to enhance the signal-to-noise ratio. Frequency domain spectra were produced by Fourier transformation. Peak assignments were made with reference to dioxane at 67.4 ppm. Pulse parameters were selected to prevent partial saturation of the resonances.²⁴ Separate ¹³C spectra were acquired of heart extracts with and without inverse-gated decoupling to correct for contributions of nuclear overhauser enhancement to signal intensity. ³¹P spectra were obtained at 162 MHz over 2 minutes (60 acquisitions) with a 45° pulse angle and a 2-second repetition time. An exponential function was introduced to improve signal-to-noise ratio. After Fourier transformation, resonance peak assignments were made relative to phosphocreatine (PCr) at 0 ppm. Relative ATP levels were determined from the signal intensity of the β-phosphate peak.

**Protocol**

To ensure that conditions were similar before and during ischemia, all hearts were perfused with 5 mM glucose as the sole substrate until the time of reperfusion. Two sets of experiments were performed; one involved the use of glucose and palmitate and the other involved the use of palmitate and pyruvate. All groups consisted of at least six hearts.

**Palmitate/glucose reperfusion.** Hearts were perfused from the 2-l reservoir until developed pressure and dP/dt were stable. After 20 minutes of ischemia, reperfusion was begun from a 400-ml reservoir containing one of the following substrates or substrate combinations: glucose (10 mM), palmitate (2 mM), or palmitate/glucose (2 mM/10 mM). To ensure that simply changing perfusion to a different reservoir did not contribute to a change in the performance of the preparation, some hearts were perfused successively with 5 mM glucose from the 2-l and the 400-ml reservoir. No changes in dP/dt, developed pressure, heart rate or high energy phosphates were induced in these hearts by changing perfusion to the smaller reservoir. A relatively high concentration of palmitate (2 mM) was chosen to maximize the metabolic effects of the substrate without compromising function in normal hearts (established in preliminary studies during a 30-minute period of normal flow). A comparable serum-free fatty acid concentration has been demonstrated in some human subjects after myocardial infarction.²⁵ The potassium salt of palmitic acid (Sigma Chemical Co., St. Louis) was complexed to essentially fatty acid–free (<0.005%) albumin (Sigma) and dialyzed before use. The number of hearts used in each group was as follows: glucose, 11; palmitate, 10; palmitate/glucose, 6.

³¹P spectra were obtained at 19 minutes (mid-acquisition) of ischemia and at 1, 3, 5, 13, 21, and 29 minutes of reperfusion. No ¹³C spectra were acquired during this portion of the study. Hemodynamic data were obtained continuously. MVO₂ was measured (CIBA-Corning) before and at 5 minutes (early) and 30 minutes (late) of reperfusion. In a separate set of experiments, lactate content (Sigma) was determined in the coronary effluent of hearts reperfused with 10 mM glucose or 2 mM palmitate after 20 minutes of ischemia. Samples for lactate determination were obtained before ischemia, and at 1, 3, 5, 15, and 30 minutes of reperfusion.

**Palmitate/pyruvate reperfusion.** To determine whether inhibition of pyruvate oxidation by palmitate might contribute to the impairment of functional recovery during reperfusion, additional experiments were performed in which hearts were reperfused with [³-¹³C]pyruvate (2.5 mM) (Merck Sharp & Dohme Isotopes, Montreal), palmitate (2 mM), or palmitate/[³-¹³C]pyruvate (2 mM/2.5 mM). The duration of ischemia was varied (10 or 20 minutes) to provide additional information about the effects of the severity of ischemia on hemodynamic and metabolic parameters. ¹³C spectra were acquired at 3, 11, 19, and 27 minutes of reperfusion (mid-acquisition), and ³¹P spectra were acquired at 9 or 19 minutes of ischemia, and at 7, 15, 23, and 31 minutes of reperfusion. MVO₂ was measured at 5 and 30 minutes of reperfusion. The number of hearts used in each group was as follows: 1) control: pyruvate, 7; palmitate, 10; palmitate/pyruvate, 6; albumin, 6; 2) 10 minutes of ischemia: pyruvate, 9; palmitate, 6; palmitate/pyruvate, 9; 3) 20 minutes of ischemia: pyruvate, 7; palmitate, 6; palmitate/pyruvate, 6.

**Data Analysis**

To determine the relative changes in signal intensities of ³¹P and ¹³C metabolites, the spectra were analyzed by both planimetry and an integration subroutine within the NMR dedicated software (Bruker). Oxidation of [³-¹³C]pyruvate resulted in ¹³C enrichment of the glutamate pool. As pyruvate en-
tered oxidative metabolism the initial labeling site within glutamate (GLU) was the C-4 position. Therefore, \([4-13C]GLU\) signal intensity was used as a measure of pyruvate utilization by the TCA cycle. The percent maximum \([4-13C]GLU\) was determined as the \([4-13C]GLU\) intensity at a single acquisition time divided by the maximum \([4-13C]GLU\) intensity recorded for that heart. Since quantitative analysis was not performed, only relative changes in utilization could be measured.

Multiple sites of \(^{13}\text{C}\) enrichment within the GLU pool eventually occur because of recycling of label

**Figure 1.** Effects of glucose and palmitate on myocardial contractility (dP/dt) after 20 minutes of ischemia. GLU, glucose; PAL, palmitate; rep, reperfusion. \(*p<0.05\) and \(+p<0.01\) compared with glucose.

**Figure 2.** Effects of pyruvate and palmitate on dP/dt for control hearts (panel A), after 10 minutes of ischemia (panel B), and after 20 minutes of ischemia (panel C). PY, pyruvate; PAL, palmitate; rep, reperfusion.
within the TCA cycle. Therefore, the ratio of [2-\textsuperscript{13}C]GLU/[4-\textsuperscript{13}C]GLU reflects TCA cycle activity until isotope distribution reaches a steady state.\textsuperscript{22} Referencing to the C-4 signal provides a control for differences in substrate entry rates into the TCA cycle. Thus, the cycle activity alone was examined independent of substrate delivery and entry at the pyruvate dehydrogenase step. [3-\textsuperscript{13}C]GLU signal intensity was not measured in the study since the exogenous [3-\textsuperscript{13}C]pyruvate signal observed overlaps the [3-\textsuperscript{13}C]GLU signal. The contribution of the [2,3-\textsuperscript{13}C]succinate pool to the [4-\textsuperscript{13}C]GLU signal was not considered in these measurements since high resolution \textsuperscript{13}C NMR analysis of tissue extracts demonstrated that less than 10% of the signal intensity arose from labeled succinate.\textsuperscript{22} Alanine (ALA) reflects the nonoxidative metabolism of pyruvate under these aerobic conditions.\textsuperscript{26} Thus, the [4-\textsuperscript{13}C]GLU/[3-\textsuperscript{13}C]ALA ratio served as a measure of pyruvate oxidation relative to nonoxidative metabolism of the available substrate.\textsuperscript{22} ALA signal intensity did not change over the time due to the steady-state conditions achieved with \textsuperscript{13}C-labeled pyruvate. [3-\textsuperscript{13}C]Lactate was considered in the ratio, but its signal was seldom detected within reperfused myocardium.

**Statistical Analysis**

Repeated measures analysis of variance with a univariate design (Complete Statistical System, StatSoft, Inc., Tulsa, Okla.) was used to determine differences in NMR measurements, hemodynamics, and oxygen consumption among and within groups of hearts. To determine overall effects, multivariate analyses were performed using the Wilks Lambda test. A value of \( p < 0.05 \) was considered significant.
Unless otherwise stated, all values are shown as mean±SEM.

Results

Hemodynamic Measurements

Palmitate/glucose reperfusion. The degree of contractile dysfunction (dP/dt) was greater for hearts reperfused with palmitate (p<0.01) and palmitate/glucose (p<0.05) than with glucose alone (Figure 1). Rate/pressure product measurements (not shown) were also performed and the results were identical to dP/dt.

Palmitate/pyruvate reperfusion. A small increase (p<0.05 compared with baseline) in dP/dt was noted at 3 minutes with palmitate/pyruvate in control hearts (Figure 2). Albumin (0.67 mM) with 5 mM glucose caused an initial decrease (p<0.05) in dP/dt of control hearts followed by stable function. Significant contractile dysfunction occurred after 10 minutes of ischemia and reperfusion. There were no significant differences in function among the three substrate groups. More pronounced contractile dysfunction occurred after more severe ischemia. dP/dt after 20 minutes of ischemia was lower (p<0.05) for pyruvate alone compared with palmitate and palmitate/pyruvate. Measurements of rate/pressure product provided essentially similar results to dP/dt.

High Energy Phosphates

Palmitate/glucose reperfusion. Figure 3 demonstrates in a single heart the changes found in high energy phosphate content after 20 minutes of ischemia and reperfusion with palmitate. Figure 4 depicts the changes in high energy phosphates after 20 minutes of ischemia and reperfusion with glucose, palmitate, and palmitate/glucose. These values were not significantly different from baseline at completion of reperfusion. A small increase (p<0.05 compared with 19 minutes of ischemia) in ATP occurred during early reperfusion with palmitate. However, ATP remained lower (p<0.001) than baseline during reperfusion in all substrate groups (Figure 4B).

Palmitate/pyruvate reperfusion. For control hearts, PCr increased (p<0.001 compared with baseline) for all substrates. PCr for palmitate/pyruvate control hearts was higher (p<0.05) than for palmitate for the duration of perfusion. After 10 minutes of ischemia, PCr increased (p<0.01, compared with baseline) for all substrates. After 20 minutes of ischemia, PCr increased (p<0.01 compared with baseline) for palmitate and palmitate/pyruvate. ATP content was decreased (p<0.001 compared with baseline) at the end of 10 and 20 minutes of ischemia; the decrease was greater (p<0.001) after 20 minutes than after 10 minutes. A small increase (p<0.05 compared with 19 minutes of ischemia) in ATP occurred during early reperfusion with palmitate/pyruvate and palmitate. However, ATP remained lower than baseline during reperfusion in all substrate groups after 10 and 20 minutes of ischemia. ATP content was reduced (p<0.05) compared with palmitate and palmitate/pyruvate during reperfusion in the pyruvate group, but this appeared to be due to a lower ATP value at 19 minutes of ischemia and not to an effect of pyruvate itself.

13C NMR Spectroscopy

Pyruvate utilization. Figure 6 is an example of spectra obtained from control hearts with 32 minutes of ischemia and 60 minutes of reperfusion.
of normal perfusion with pyruvate (top) and palmitate/pyruvate (bottom). Compared with the heart perfused with pyruvate, [2-\(^{13}\)C]GLU and [4-\(^{13}\)C]GLU signal of the palmitate/pyruvate heart was markedly reduced and indicated a decrease in pyruvate oxidation relative to [3-\(^{13}\)C]ALA. \(^{13}\)C-labeled TCA cycle intermediates also yielded [2-\(^{13}\)C, 3-\(^{13}\)C]aspartate signal via the aspartate aminotransaminase reaction. The decrease in aspartate signal observed in the bottom spectrum reflected the decrease in TCA cycle intermediates in a fashion similar to \(^{13}\)C-labeled glutamate.

Pyruvate reperfusion. The top portion of Figure 7A shows the changes in percent maximum [4-\(^{13}\)C]GLU for hearts reperfused with pyruvate alone. After 10 minutes of ischemia and 3 minutes of reperfusion, the ratio was decreased compared with the control group (0.14 versus 0.7; \(p<0.001\)). The ratio for the 20-minute ischemic hearts was zero. Steady-state \(^{13}\)C enrichment was reached at 11 minutes in the control hearts, whereas for the 10- and 20-minute ischemic hearts control levels were not reached during the reperfusion period. Overall, these measurements demonstrate that in the reperfused myocardium, oxidative utilization of pyruvate is decreased according to the severity of the preceding period of ischemia.

Palmitate/pyruvate reperfusion. The bottom portion of Figure 7A shows the changes in percent maximum [4-\(^{13}\)C]GLU signal for hearts reperfused with combined palmitate/pyruvate. After 10 and 20 minutes of ischemia and 3 minutes of reperfusion, the percent maximum [4-\(^{13}\)C]GLU was decreased compared with control (zero versus 34%; \(p<0.001\)). All three substrate groups reached similar steady-state enrichment at 27 minutes. Since ALA signal remained constant during the reperfusion period, the ratio of [4-\(^{13}\)C]GLU to [3-\(^{13}\)C]ALA could be used to compare pyruvate utilization in the reperfused heart in the presence and absence of palmitate. The changes in the [4-\(^{13}\)C]GLU/[3-\(^{13}\)C]ALA ratio caused by palmitate are shown in the bottom portion of

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**FIGURE 5.** Effects of pyruvate and palmitate on phosphocreatine (PCr) and ATP during control (A and D), after 10 minutes of ischemia (B and E), and after 20 minutes of ischemia (C and F). PY, pyruvate; PAL, palmitate; rep, reperfusion. *p<0.05 compared with palmitate.
Figure 7B. The ratio for the palmitate/pyruvate control hearts was decreased compared with the pyruvate control hearts (0.22 versus 0.7; \( p < 0.05 \)). The ratio was also decreased for the 10-minute ischemic hearts reperfused with palmitate/pyruvate compared with pyruvate alone (zero versus 0.14; \( p < 0.05 \)). These findings indicate that palmitate suppresses oxidative pyruvate utilization in the normal and mildly post-ischemic myocardium. Since pyruvate utilization was already markedly reduced after 20 minutes of ischemia, no additional effect could be observed with palmitate/pyruvate.

**Tricarboxylic Acid Cycle Activity**

**Pyruvate reperfusion.** Figure 7C shows the changes in the TCA cycle flux after 10 and 20 minutes of ischemia. The top portion of the figure describes the changes in the \([2-^{13}C]GLU/[4-^{13}C]GLU\) ratio for hearts reperfused with pyruvate alone. After 10 minutes of ischemia and 3 minutes of reperfusion, the \([2-^{13}C]GLU/[4-^{13}C]GLU\) ratio was decreased compared with control (zero versus 0.17; \( p < 0.05 \)). After 20 minutes of ischemia and 27 minutes of reperfusion, TCA cycle activity remained decreased compared with control (0.12 versus 0.6; \( p < 0.01 \)). Steady-state \(^{13}C\) enrichment was reached at 19 minutes for control and 19 minutes for the 10-minute ischemic hearts.

**Palmitate/pyruvate reperfusion.** The bottom portion of Figure 7C describes the changes in TCA cycle flux associated with the addition of palmitate to the perfusate. Palmitate had no effect on the \([2-^{13}C]GLU/[4-^{13}C]GLU\) ratio. Thus, the effect of palmitate on control and reperfused hearts was to decrease pyruvate utilization without an effect on TCA cycle activity.

**Lactate Measurements**

A large quantity of lactate washed out in the first 5 minutes of reperfusion in both the glucose- and palmitate-reperfused hearts (Table 1). Lactate content in the coronary effluent thereafter remained low in both groups and no differences occurred between the two substrates. These findings were consistent with intact (although delayed) oxidative utilization of pyruvate by the reperfused myocardium.

**TABLE 1. Lactate Release From Reperfused Hearts After 20 Minutes of Ischemia**

<table>
<thead>
<tr>
<th>Reperfusion time (min)</th>
<th>Glucose 10 mM (( n = 6 )) ( \mu \text{mol/min/g wet wt} )</th>
<th>Palmitate 2 mM (( n = 6 )) ( \mu \text{mol/min/g wet wt} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preischemia</td>
<td>0.45±0.27</td>
<td>0.36±0.09</td>
</tr>
<tr>
<td>1</td>
<td>13.00±1.63</td>
<td>13.91±2.31</td>
</tr>
<tr>
<td>3</td>
<td>1.27±0.18</td>
<td>1.14±0.24</td>
</tr>
<tr>
<td>5</td>
<td>1.44±0.39</td>
<td>0.45±0.14</td>
</tr>
<tr>
<td>15</td>
<td>0.80±0.45</td>
<td>0.37±0.09</td>
</tr>
<tr>
<td>30</td>
<td>0.78±0.23</td>
<td>1.12±0.31</td>
</tr>
</tbody>
</table>

Values are mean±SD.
**MVO₂ Measurements**

MVO₂ measurements for the 20-minute ischemic groups are shown in Table 2. Preischemic values in this table refer to MVO₂ measured during perfusion with 5 mM glucose. MVO₂ was significantly (p<0.05–0.01) decreased at 5 minutes of reperfusion compared with control. However, by 30 minutes, only pyruvate hearts had a lower (p<0.05) MVO₂. Thus, despite significant contractile dysfunction after 20 minutes of ischemia, MVO₂ was not significantly reduced compared with control hearts perfused with similar substrates. These findings indicated a change in the work relation to MVO₂ in postischemic hearts.

A measure of work efficiency, MVO₂/unit of work (micromoles O₂ per minute per rate/pressure product) was not significantly different among substrates at 7 minutes of reperfusion (Figure 8). However, at 31 minutes of reperfusion, MVO₂/unit of work was lower (p<0.05) for glucose than for palmitate and palmitate/glucose (0.17 versus 0.29 and 0.24, respectively).

**Figure 7.** Results of ¹³C NMR spectroscopy for hearts reperfused with pyruvate or palmitate/pyruvate. Panel A: Percent maximum (% max) [4-¹³C]glutamate (pyruvate [PY] utilization) for hearts reperfused with PY (top) and palmitate-PY (PAL)/PY (bottom). Panel B: [4-¹³C]Glutamate (GLU)/[3-¹³C]Alanine (ALA) ratio (PY utilization relative to non-oxidative metabolism) for hearts reperfused with PY (top) and PAL/PY (bottom). Panel C: [2-¹³C]GLU/[4-¹³C]GLU ratio (tricarboxylic acid cycle activity) for hearts reperfused with PY (top) and PAL/PY (bottom). *p<0.05, **p<0.01, and ***p<0.001 compared with control hearts.
This beneficial effect of glucose on M\(\text{VO}_2\)/unit of work occurred gradually over the period of reperfusion with M\(\text{VO}_2\)/unit of work being lower \((p<0.05)\) at 31 minutes than at 7 minutes. The reduction was due mostly to an increase in rate/pressure product without a corresponding rise in M\(\text{VO}_2\). For palmitate, M\(\text{VO}_2\)/unit of work was higher \((p<0.05)\) at 7 minutes compared with preischemia but was not significantly different compared with preischemia at 31 minutes.

Discussion

We found significant contractile dysfunction when isolated hearts were reperfused with palmitate and palmitate/glucose as compared with glucose alone. By measuring M\(\text{VO}_2\)/unit of work, it was observed that glucose produced a more efficient metabolic state in which to support contractility during reperfusion. These findings verify other experiments performed in the isolated working rat heart using an inhibitor of fatty acid oxidation.\(^7\) If the adverse effect of palmitate in the present study was due to inhibition of substrate oxidation at the level of pyruvate dehydrogenase, hearts reperfused with pyruvate alone should have demonstrated function comparable to the glucose reperfused hearts. However, since the degree of contractile dysfunction was similar with pyruvate versus palmitate/pyruvate, suppressed recovery with palmitate might occur with inhibition of glycolysis “upstream” of pyruvate dehydrogenase. Previously, in a study with positron emission tomography, Schwaiger et al.\(^27\) found enhanced anaerobic glucose utilization after a 3-hour coronary occlusion and 24 hours of reperfusion in the dog model. However, the relation of glycolysis to improved mechanical function cannot be determined from their work since cardiac function was not reported in these experiments.

A concentration of pyruvate up to 10 mM has been shown to activate pyruvate dehydrogenase\(^10\) and may overcome some of the deleterious effects of ischemia and palmitate. Also, Buenger et al.\(^28\) showed that pyruvate produced a concentration-dependent, positive inotropic effect on reperfused myocardium but that the effect was only apparent in the presence of glucose.\(^28\) The lower concentration of pyruvate used in the present study and the lack of supplemental glucose in the perfusate most likely accounted for the lack of a significant inotropic effect from pyruvate.

In the present study, ATP content was significantly reduced at 9 and 19 minutes of ischemia. The uniformity of reduction in ATP content was a reflection of the fact that all hearts were made globally ischemic during glucose perfusion. The pertinent finding of this study was that ATP content remained below baseline throughout the reperfusion period and was not significantly affected by substrate availability. In contrast to these findings, Lopaschuk et al.\(^7\) found that a decrease in ATP produced by 25 minutes of global ischemia was partially reversed in hearts reperfused with glucose but not with palmitate. In their study, palmitate was present in the perfusate during ischemia as well as reperfusion, whereas in the current study, palmitate was present only at reperfusion. Since fatty acids have been shown to have an adverse effect on high energy phosphate content during ischemia,\(^3,4\) the different findings may have been caused by the differences in substrate availability.

**Figure 8.** Myocardial oxygen consumption (M\(\text{VO}_2\))/unit of cardiac work (micromoles oxygen per minute per rate/pressure product) for 20-minute ischemic hearts reperfused (rep) with glucose (glu) and palmitate (pal). \(^*p<0.05\) compared with palmitate.
Taegtmeyer et al.\(^{29}\) demonstrated the disassociation between tissue content of high energy phosphates and recovery of cardiac function after an ischemic event. Later, others found that by increasing the inotropic state in normal\(^{30}\) and reperfused\(^{31}\) isolated hearts, turnover of myocardial high energy phosphate compounds and not tissue content best matched cardiac performance. This would also explain our findings during reperfusion with glucose, in which ATP content remained low during reperfusion but contractile function returned to baseline. Zweier and Jacobus,\(^{32}\) in a study of normal guinea pig hearts perfused with large concentrations of pyruvate (5–20 mM), demonstrated that contractile function was increased as was PCR content and flux of ATP synthesis from PCR. In the present study, a large increase in PCR occurred with a low concentration of pyruvate in control hearts, but only a slight increase in function occurred (compared with albumin/glucose). Previous studies have demonstrated that function is not necessarily related to static levels of high energy phosphates. From et al.\(^{33}\) studied the Langendorff perfused rat heart and found that when exogenous carbon sources varied, supposed regulatory parameters like ATP/ADP, cytosolic phosphorylation potential, and cytosolic ADP level did not uniquely relate to MVO\(_2\.\)\(^{33}\) Balaban et al.\(^{34}\) reported that the relative amounts of PCR and ATP remained constant in in vivo canine hearts over a wide range of rate/pressure products. Also, without glucose in the perfusate, the positive inotropism of pyruvate was presumably blunted in the current set of experiments. Measurement of energy flux may provide a better understanding of the relation between energy and contractile function. Sako et al.\(^{35}\) determined unidirectional rate of ATP synthesis with \(^{31}P\) NMR and found that the net rate of ATP synthesis was not altered in reperfused myocardium. These investigators suggested instead that inefficient utilization of ATP may contribute to altered energy metabolism under these conditions.

In our laboratory, percent maximum \([4.13^C]GLU\) enrichment from pyruvate was used to demonstrate that substrate oxidation was delayed relative to control hearts in the reperfused myocardium.\(^{22}\) The present study also showed that by increasing the duration of ischemia from 10 to 20 minutes, pyruvate utilization was further delayed. The use of the \([4.13^C]GLU/[3.13^C]ALA\) ratio in the present study allowed us to demonstrate that palmitate caused a marked reduction in pyruvate oxidation when the two substrates were provided together. This effect was observed both in control hearts and after 10 minutes of ischemia. For the 20-minute ischemic hearts, however, no additional effect of palmitate was observed on the already severe reduction in pyruvate utilization. Since alanine intensity remained constant throughout reperfusion, the change in the \([4.13^C]GLU/[3.13^C]ALA\) ratio was caused by a reduction in pyruvate entry into TCA cycle. This most likely resulted from inhibition of pyruvate dehydrogenase by palmitate\(^{36,21}\) and occurred in both normal and reperfused myocardium. Renstrom et al.\(^{36}\) demonstrated that \([2.14^C]\)pyruvate utilization was decreased in a hyperperfused swine preparation and failed to recover during reperfusion. Similar results were noted when serum fatty acids were increased by adding a triacylglycerol emulsion. In the present study, as in the study of Renstrom et al, pyruvate utilization was reduced when palmitate was added to the perfusate.

The effect of ischemia in the present study was to reduce cycle activity at early reperfusion. This effect was greater after 20 minutes of ischemia. Palmitate had no additional effect on this ratio in control or reperfused myocardium. Use of the \([2.13^C]GLU/[4.13^C]GLU\) ratio is a relative measure of TCA cycle metabolites. It reflects the rise to steady-state enrichment and is not a quantitative measurement of flux. The reduced substrate oxidation in the presence of competitive substrates like palmitate may have further reduced signal arising from the secondary sites of labeling within glutamate, resulting in a lowering of the \([2.13^C]GLU/[4.13^C]GLU\) ratio.

Armbrecht et al.\(^{37}\) recently measured TCA cycle flux in a canine preparation using positron emission tomography and \([1.13^C]\)acetate. These data were corroborated by coinjecting \([1.14^C]acetate\) into the coronary artery and measuring \(^{13}CO_2\) activity in blood samples. The results indicated that \(^{13}C\) clearance corresponded to \(^{12}CO_2\) production and therefore TCA cycle activity. Data concerning contractile function was not reported. In the present study, a reduction in TCA cycle flux as measured by the \([2.13^C]GLU/[4.13^C]GLU\) ratio in the 20-minute ischemic hearts reperfused with pyruvate was in contrast to the relatively high MVO\(_2\) at the end of reperfusion. The data obtained in this study do not provide further explanation of this phenomenon, which has previously been observed in our laboratory.\(^{22}\) However, reduced pyruvate oxidation has been observed in the reperfused working swine heart,\(^{36}\) while oxygen wasting relative to contractility has been reported in reperfused myocardium.\(^{5,38}\)

The observation in the present study that lactate release was low during reperfusion indicated that significant latent postischemic hypoxia was absent during reperfusion. Although we demonstrated that oxidative metabolism was slowed in the reperfused myocardium, the absence of continued high lactate release indicates that there was no large scale shift toward anaerobic metabolism during reperfusion. These data also indicate that the redox state within the reperfused myocardium was maintained at approximately control levels.

In summary, we have shown in this study that palmitate, when given only during reperfusion after reversible global ischemia in the isolated rabbit heart, was less effective than glucose in enhancing contractile recovery. Palmitate reperfusion was not associated with a reduction in high energy phosphate content, which bore no relation to the various de-
degree of contractile dysfunction observed in this study. Palmitate reduced pyruvate utilization in control and reperfused hearts, probably by inhibiting pyruvate dehydrogenase. However, this reduction of pyruvate metabolism had no effect on function in the reperfused myocardium.

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References


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