Hepatic pyruvate dehydrogenase activity in humans: effect of cirrhosis, transplantation, and dichloroacetate

ROBERT E. SHANGRAW, JOHN M. RABKIN, AND GARY D. LOPASCHUK

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1. Liver biopsy PDH activity was assayed by measuring $[^{14}C]$citrate synthesis from $[^{14}C]$oxaloacetate and served as controls. Liver biopsy PDH activity was assayed by measuring $[^{14}C]$citrate synthesis from $[^{14}C]$oxaloacetate and PDH-derived acetyl-CoA. PDH in the active form (PDHa) in cirrhotic and control liver was 5.6 ± 1.3 (SE) and 37 ± 0 nmol · g wet wt $^{-1}$·min$^{-1}$, respectively (P < 0.001). Total PDH activity (PDHt) was 21.5 ± 3.6 and 264 ± 27 nmol · g wet wt $^{-1}$·min$^{-1}$, respectively (P < 0.001). DCA increased PDHa in cirrhotic liver to 22.3 ± 4.1 nmol · g wet wt $^{-1}$·min$^{-1}$ (P < 0.05 vs. no DCA) without altering PDHt. Graft liver PDHa was 166 ± 19 nmol · g wet wt $^{-1}$·min$^{-1}$, which was not altered by DCA.

We conclude that decreased hepatic PDH activity secondary to decreased content may underlie lactic acidosis during OLT, which can be partially compensated by DCA administration. There is no apparent inhibition of graft liver PDH activity after reperfusion.

Lactic acidosis: liver disease

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Lactic acidosis: liver disease

Patients with cirrhosis, despite maintenance of a normal fasting plasma lactate concentration, are susceptible to clinically significant lactic acidosis. This lactic acidosis derives from both increased peripheral production of lactic acid in response to a glucose challenge (16) and decreased capacity to clear a lactic acid load (21, 33). The liver is the major organ responsible for clearing lactic acid from plasma, as it participates in the Cori cycle (14). There is evidence in animals that hepatic pyruvate dehydrogenase (PDH) activity may be decreased in cirrhosis (9). To what degree this contributes to lactic acidosis is unknown. However, stimulation of PDH activity with dichloroacetate (DCA) attenuates lactic acidosis during orthotopic liver transplantation (OLT), reducing the NaHCO$_3$ requirement by 80% (26). DCA stimulates PDH activity by inhibiting PDH kinase, an enzyme that phosphorylates and inhibits PDH (32). As a result, DCA increases the fraction of PDH in the active, dephosphorylated form (32). PDH activity controls the rate of glucose oxidation, which increases the efficiency of energy production from glucose metabolism. By stimulating PDH activity, DCA increases pyruvate oxidation to CO$_2$ and reduces plasma lactic acid concentration (6, 26, 32).

The tissue(s) responsible for the decrease in plasma lactic acid concentration in response to DCA remains uncertain. Skeletal muscle does not appear to play a major role in the hypolactatemic response to DCA, since lactate flux across the extremity of either anesthetized animals (11) or awake humans (2) is unaffected by DCA. This raises the possibility that a direct action of DCA on the liver may contribute to the hypolactatemic action of DCA. The first aim of our study was to determine total PDH activity (PDHt) and the quantity of PDH in the active, dephosphorylated form (PDHa) in native control and cirrhotic human liver, to test if PDH activity is reduced as a result of end-stage disease, the extent to which decreased activity occurs by PDH inhibition, and whether DCA can increase hepatic PDH activity in vivo.

PDH activity is inhibited in the posts ischemic myocardium, which is associated with poor glucose oxidation and impaired myocardial performance (13, 18). Stimulation of PDH with DCA in the posts ischemic myocardium increases glucose oxidation and augments myocardial performance (18). However, PDH activity in newly perfused graft liver, either under basal conditions or after DCA treatment, is unknown. Graft liver PDH activity may also be inhibited, because after harvesting the liver undergoes several hours of cold ischemia, followed by 1 h of warm ischemia before reperfusion in the recipient. Furthermore, after reperfusion in the recipient, the liver is exposed to a high circulating concentration of citrate from blood product transfusion (8), which could lead to continued end-product inhibition of PDH activity. The second purpose of our study was to determine whether PDH activity is inhibited in graft liver during OLT and whether an inhibition of graft liver PDH activity can be reversed with DCA.

This study was designed to assess PDH activity in control, cirrhotic, and graft liver to test the hypotheses that 1) liver disease decreases hepatic PDH activity, 2) graft PDH activity is inhibited due to protracted ischemia, and 3) DCA reverses functional PDH inhibition in cirrhotic and graft liver.

MATERIALS AND METHODS

Subjects

This randomized, double-blinded study was approved by the Institutional Review Boards at Oregon Health Sciences University and the Portland Veterans Affairs Medical Center,
as well as the U. S. Food and Drug Administration (IND no. 35,790). Forty-three patients with stable end-stage cirrhosis undergoing OLT were enrolled after providing written informed consent. Liver transplant patients were randomly assigned by coin toss to either receive intraoperative DCA (group 1, \( n = 28 \)) or an equal 150-ml volume of aqueous 5% glucose only (group 2, \( n = 15 \)). Of the 43 total patients, 12 (6 each from groups 1 and 2) were used for both study on the effect of DCA on native liver PDH activity and graft liver PDH activity, and the remainder were only used for study of graft liver PDH activity. Inclusion criteria for OLT patients were end-stage liver disease, preoperative hemodynamic stability, no history of insulin-dependent diabetes mellitus, and adequate renal function as determined by a plasma creatinine concentration \(<1.4 \text{ mg/dl}\). The severity of liver disease was assessed by the Pugh-Childs scoring system (25). In addition to the OLT patients, six patients without clinical or laboratory evidence of liver dysfunction were enrolled in the native liver biopsy study to serve as controls. These patients, who were undergoing subtotal liver resection, had no history of diabetes mellitus or other metabolic disease and were receiving no medications at the time of the study. All subjects were postabsorptive for at least 8 h, during which time they received no intravenous glucose or other sources of calories. Preoperative assessment of all patients included analysis of venous blood for determination of hematocrit, glucose, lactate and electrolyte concentrations, and liver function tests.

Anesthetic and Surgical Procedures

All patients were monitored by plethysmography blood pressure cuff, two-lead electrocardiography, end-tidal capnography, and arterial pulse oximetry. Patients undergoing OLT were also monitored by mixed venous oximetry and had indwelling pulmonary and bilateral radial artery catheters. A rapid transfusion system, consisting of two 7 French intravenous catheters, a 2 l/min pump (Sorin, Irvine, CA) coupled to a filtered 4-liter reservoir (Capiox; Therumo, Tokyo, Japan), a Gisch heat exchanger, and an in-line bubble trap, was used for OLT patients. This system warmed (38°C) and continuously circulated blood products in the reservoir. Shed blood from transplant patients was processed to salvage erythrocytes (Cell-Saver; Haemonetics, Braintree, MA), to limit the requirement for banked blood. Patients for subtotal hepatic resection received only peripheral intravenous catheters. Neither the rapid infusion system nor invasive hemodynamic monitoring was used for subtotal hepatic resection.

Anesthesia induction consisted of fentanyl (3 µg/kg) and thiopental (3–4 mg/kg). Pancuronium (0.15 mg/kg) was used to facilitate endotracheal intubation, and anesthesia was maintained with fentanyl (1–5 µg·kg\(^{-1}·\text{h}^{-1}\)) and isoflurane (0.3–0.8% end-tidal concn) in oxygen-air. Pancuronium was used as needed to maintain neuromuscular blockade. Patients undergoing OLT received intravenous methylprednisolone (1.0 g), ampicillin (1.0 g), and ceftriaxone (1.0 g) shortly after anesthesia induction. No other immunosuppressive medication was administered during the study. Venovenous bypass was not employed. Patients for subtotal liver resection received intravenous cefotetan (2.0 g) before surgical incision but did not receive immunosuppressive therapy.

Protocol

Liver transplantation. DCA (TCI America, Portland, OR) was prepared as described previously (26). Liver transplant patients in group 1 received DCA (265 \( \mu \text{mol/kg iv} \)) over 60 min, immediately after anesthesia induction (time 0) and repeated at either test clamping of the portal vein or at 240 min, whichever occurred first. This dosing schedule yielded a sustained plasma DCA concentration of \(<1 \text{ mmol/l}\) throughout the dissection, anhepatic, and early reperfusion stages of OLT (26). In human volunteers, a plasma DCA concentration of 1 mM produces a maximal decrease in plasma lactate concentration (6, 31). Patients in group 2 received an equal volume of aqueous 5% glucose (150 ml) without DCA at the same time intervals.

Blood product replacement in both groups was guided by hematocrit (packed erythrocytes), prothrombin time (fresh plasma), and platelet count (platelets). CaCl\(_2\) was administered as necessary to maintain a plasma ionized Ca\(^{2+}\) at 1.0 meq/l. A balanced salt solution (Normosol; Dupont Pharmaceuticals, Wilmington, DE) was used for crystalloid infusion to avoid lactate challenge. Intraoperative metabolic acidosis in both groups was treated in an identical fashion: metabolic acidosis (\( \text{pH } < 7.30 \), with [HCO\(_3\)] \(< 17 \text{ mmol/l} \)) was treated with \( \text{NaHCO}_3 \) (1 meg/ml iv) to half correct the HCO\(_3\) deficit, as calculated by the following equation: HCO\(_3\) deficit \( = 0.3 \times \) body wt (kg) \( \times \) base deficit (mmol/l). Where 0.3/l/kg was used as the HCO\(_3\) space. NaHCO\(_3\) utilization was recorded. An aggressive treatment of metabolic acidosis was employed because acidosis can theoretically impair cardiac performance (27).

Graft livers in both groups were preserved in ice-cold “University of Wisconsin” (UW) solution (Viaspan; Dupont Pharmaceuticals, Wilmington, DE) and flushed with 1.5 liters of heparinized 6% hetastarch (Hespain; Dupont Pharmaceuticals, Wilmington, DE) at 7°C before graft warming and surgical anastomosis.

Subtotal liver resection. Intraoperative lactated Ringer solution (2,500–3,500 ml) was infused to maintain hemodynamic stability and urine output of \( \geq 0.5 \text{ ml·kg}^{-1}·\text{h}^{-1} \). No DCA, CaCl\(_2\), NaHCO\(_3\), or blood products were administered to these patients.

Data Collection

Blood sampling and analysis in OLT patients. Arterial blood was collected before anesthesia induction, immediately before surgical incision, during the hepatic dissection at 60 min before portal vein clamping, 30 min and 60 min after portal vein clamping, and after portal vein unclamping at 15 min, 30 min, 60 min, 120 min, and 180 min. Arterial blood samples for blood pH, \( \text{PCO}_2, \text{PO}_2 \), base excess, and plasma lactate and glucose concentrations were analyzed immediately on collection. For lactate and glucose concentrations, collected blood was first centrifuged at 4°C to separate plasma. All tubes contained heparin except those for lactate determination, which contained sodium fluoride. Arterial blood gases were determined using an automated analyzer (model 1312 Blood Gas Manager; Instrumentation Laboratories, Lexington, MA), and whole blood base excess was calculated using the Siggaard-Anderson alignment nomogram (Radiometer, Copenhagen, Denmark). Plasma lactate concentration was determined by a lactate dehydrogenase assay with an autoanalyzer (Monarch 760, Instrumentation Laboratories). Plasma glucose concentration was measured by a glucose oxidase assay using an autoanalyzer (Glucose Analyzer 2; Beckman Instruments, Brea, CA).

Measurement of PDH Activity in Liver Biopsies

Open surgical biopsies of native liver were taken at the time of surgical excision in both the OLT and subtotal hepatic resection protocols. During OLT, the hepatic artery was clamped 12 ± 5 and 7 ± 2 min before portal vein clamping and hepatectomy in DCA-treated and untreated patients, respec-
tively (ND). In the hepatic resection protocol, the porta hepatis (hepatic artery and portal vein) was ligated intact immediately before surgical resection. Biopsy specimens from the hepatic resection were taken from a margin free of disease by visual and histological examination. In the OLT protocol, needle biopsies (8–35 mg) of graft liver were taken after reestablishment of both the portal and hepatic arterial circulations, at the time of biliary anastomosis. Time between portal vein reperfusion and hepatic artery reperfusion did not differ between OLT group (57 ± 5 and 58 ± 5 min for DCA-treated and untreated patients, respectively). No at-

Two-way analysis of variance (ANOVA), with Tukey’s post hoc test, was used for statistical comparisons of pH, Pco2, HCO3, base excess, lactate and glucose between groups at baseline and during OLT. One-way ANOVA with Tukey’s post hoc test was used to compare different temporal values within a group. Total, active, and fractional PDH activity among groups was compared by Kruskal-Wallis test followed by Mann-Whitney pair testing if justified. The two graft liver PDH groups were compared by both Mann-Whitney and Student’s t-test. Student’s t-test was used to compare surgical and graft ischemia times and use of blood products, CaCl2, and NaHCO3 between OLT groups. Severity of liver disease was compared by the Mann-Whitney test. Statistical tests were performed using a specialized software program (Crunch 4, Crunch Software, Oakland, CA). Differences were considered statistically significant at P < 0.05.

A post hoc power analysis revealed that six subjects per group for the native liver PDH study provided statistical power (1-β) of 0.8 to detect a difference in PDHα of 1.45 nmol·g wet wt⁻¹·min⁻¹ between groups, with a = 0.05, given cirrhotic liver PDHα mean and standard deviation values of 9.60 and 3.18 nmol·g wet wt⁻¹·min⁻¹, respectively, without DCA stimulation. In the graft liver study, the sample size of 15 subjects/group provided a 1-β of 0.8 with α = 0.05 to detect a difference in PDHα of 21 nmol·g wet wt⁻¹·min⁻¹ between groups, given a mean ± SD of 166 ± 74 nmol·g wet wt⁻¹·min⁻¹, respectively, without DCA stimulation (7).

**RESULTS**

Preoperative Baseline Characteristics: Native Liver Study

Table 1 shows demographic characteristics of the patients for biopsy of the native liver, with end-stage cirrhosis undergoing OLT, or without evidence of liver dysfunction undergoing subtotal hepatic resection. Age distribution was comparable among groups, although there were more men relative to women patients for OLT compared with the subtotal hepatic resection group. Both groups of OLT patients exhibited cirrhosis of different etiologies. A spectrum of localized hepatic pathology was observed in the hepatic resection group.

Table 2 lists preoperative laboratory values. Patients for OLT differed from the hepatic resection patients in that they were hyponatremic, hypoalbuminemic, hyperbilirubinemic, and coagulopathic (as evidenced by prolonged prothrombin time). Serum aspartate aminotransferase concentration was elevated in the OLT patients, but lactate dehydrogenase, alkaline phosphatase, or alanine aminotransferase concentrations did not differ from hepatic resection patients. The two OLT groups differed only in that the no DCA group showed a relative azotemia and less marked hyperbilirubinemia compared with the group selected to receive DCA. Preoperative plasma concentrations of glucose, lactate, venous CO2, and creatinine did not differ among the three groups.

Effect of DCA During Liver Transplantation: Native Liver Study

Table 3 shows that plasma glucose concentration increased compared with preoperative values in both
groups, although the arterial HCO$_3$ content was mod-

Table 2. Preoperative laboratory values: native liver study

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex, M/F</th>
<th>Liver Pathology</th>
<th>Pugh-Childs Score</th>
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<td></td>
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<tr>
<td>1</td>
<td>65</td>
<td>M</td>
<td>α1-Antitrypsin deficiency</td>
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<td>43</td>
<td>M</td>
<td>ETOH cirrhosis</td>
<td>14</td>
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<td>59</td>
<td>M</td>
<td>Cryptogenic cirrhosis</td>
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<td>F</td>
<td>Cryptogenic cirrhosis</td>
<td>8</td>
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<tr>
<td>5</td>
<td>4</td>
<td>M</td>
<td>ETOH cirrhosis</td>
<td>9</td>
</tr>
<tr>
<td>6</td>
<td>62</td>
<td>F</td>
<td>Cryptogenic cirrhosis</td>
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<tr>
<td>Average</td>
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<td>4/2</td>
<td></td>
<td>11 ± 1</td>
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<td>Liver Transplant with DCA</td>
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<td></td>
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<tr>
<td>1</td>
<td>45</td>
<td>M</td>
<td>ETOH cirrhosis</td>
<td>8</td>
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<td>2</td>
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<td>58</td>
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<td>M</td>
<td>Hepatitis C cirrhosis</td>
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<tr>
<td>Average</td>
<td>51 ± 3</td>
<td>3/3</td>
<td></td>
<td>11 ± 1</td>
</tr>
</tbody>
</table>

Averages are given as means ± SE. M, male; F, female. DCA, dichloroacetate. ETOH, ethanol. *P < 0.05 vs. subtotal hepatic resection (control). †P < 0.05 vs. orthotopic liver transplantation without DCA.

Cirulating concentrations

- Plasma glucose, mg/dl: 175 ± 17* vs. 193 ± 27*
- Plasma lactate (mM): 5.00 ± 0.63* vs. 1.59 ± 0.31†
- Arterial pH: 7.34 ± 0.01 vs. 7.35 ± 0.02
- Arterial HCO$_3$ (mM): 18.8 ± 1.0 vs. 21.0 ± 0.9†

Replacements

- Packed erythrocytes, U: 8 ± 1 vs. 9 ± 2
- Fresh frozen plasma, U: 21 ± 4 vs. 18 ± 4
- Platelets, U: 25 ± 6 vs. 17 ± 5
- CaCl$_2$, g: 4 ± 1 vs. 3 ± 1
- NaHCO$_3$, meq: 39 ± 17 vs. 7 ± 6†
- Crystalloid, liters: 4.8 ± 0.6 vs. 5.2 ± 0.7

Time for hepatectomy, min: 247 ± 26 vs. 160 ± 10†

Values are means ± SE; n = 6 for each transplant group. *P < 0.05 vs. baseline value (given in Table 2). †P < 0.05 vs. liver transplant without DCA.

Mostly increased in patients who received DCA. Blood product replacement, crystalloid requirement, and CaCl$_2$ administration did not differ between groups. In contrast, NaHCO$_3$ use required to correct metabolic acidosis during the hepatic dissection was decreased by 80% in the DCA-treated group compared with patients who did not receive DCA (P < 0.05). Surgical time from skin incision to hepatectomy was decreased in the DCA-treated patient group.

Hepatic PDH$_a$ at the time of hepatectomy in both patient groups undergoing OLT was about one-tenth of that in control livers from patients undergoing subtotal hepatic resection (Fig. 1, P < 0.001). Median PDH$_a$ was 264 nmol·g wet wt$^{-1}$·min$^{-1}$ (range 184–344 nmol·g wet wt$^{-1}$·min$^{-1}$) in controls, 20.4 nmol·g wet wt$^{-1}$·min$^{-1}$ (range 9.8–33.5 nmol·g wet wt$^{-1}$·min$^{-1}$) in OLT patients without DCA, and 15.9 nmol·g wet wt$^{-1}$·min$^{-1}$ (range 7.3–55.5 nmol·g wet wt$^{-1}$·min$^{-1}$) in OLT patients with DCA. PDH$_a$ did not differ between the two OLT patient groups. Compared with control liver, PDH$_a$ was also decreased in both patient groups undergoing OLT, by a factor of almost 3 in DCA-treated patients and by a factor of 10 in patients who did not receive DCA (P < 0.001). Median PDH$_a$ was 54.5 nmol·g wet wt$^{-1}$·min$^{-1}$ (range 27.0–94.0 nmol·g wet wt$^{-1}$·min$^{-1}$) in controls, 5.5 nmol·g wet wt$^{-1}$·min$^{-1}$ (range 0.9–11.0 nmol·g wet wt$^{-1}$·min$^{-1}$) in OLT patients without DCA, and 18.1 nmol·g wet wt$^{-1}$·min$^{-1}$ (range 4.1–84.7 nmol·g wet wt$^{-1}$·min$^{-1}$) in OLT patients with DCA. When the two OLT groups were compared, PDH$_a$ was increased almost fourfold in the DCA-treated group compared with that in patients undergoing OLT without DCA (Fig. 1, P < 0.05). The fraction of PDH$_a$ was 25.0 ± 6.0% in control liver, 34.6 ± 14.6% in cirrhotic liver from the untreated group undergoing OLT (ND vs. control), and 86.3 ± 13.1% in the DCA-treated group (P < 0.03 vs. no DCA). Respective median fractional PDH activity was 24.8% (range 8.5–44.7%) in controls, 24.6% (range...
6.7–68.9%) in OLT patients without DCA, and 90.6% (range 43.5–160%) in OLT patients with DCA.

**Effect of DCA During Liver Transplantation: Graft Liver Study**

Accumulation of arterial lactate with accompanying lactic acidosis occurred in all patients, with the nadir occurring shortly after unclamping of the portal vein with reperfusion of the graft liver (Fig. 2). DCA moderated the severity of lactic acidosis during the anhepatic and neohepatic stages of liver transplantation, decreasing the peak arterial plasma lactate concentration by 45% (3.47 ± 0.17 vs. 6.26 ± 0.28 mmol/l, P < 0.001). Patients who received DCA had a less-marked metabolic acidosis, as indicated by differences in arterial pH and base excess at several time points (Fig. 2), despite a smaller NaHCO₃ requirement compared with patients not receiving DCA (Table 4). There were no differences between groups in times for total surgical procedure, hepatectomy stage, anhepatic period, or warm or cold graft ischemia (Table 4). DCA-treated patients had a modestly increased requirement for plasma (fresh frozen plasma (FFP)) transfusion, but otherwise the two groups received similar quantities of CaCl₂, blood product transfusion, and crystalloid infusion.

Small biopsy mass available for PDH analysis precluded measurement of PDHₜ in graft liver. Graft liver PDHₐ did not differ between those patients treated or not treated with DCA (Fig. 1). Graft liver PDHₐ in both groups exceeded that in native diseased liver by an order of magnitude and was also 2.5-fold greater than that observed in freshly obtained control liver from the hepatic resection patients (P < 0.04, Fig. 1). Median PDHₐ was 161 nmol·g wet wt⁻¹·min⁻¹ (range 53–325 nmol·g wet wt⁻¹·min⁻¹) in graft liver without DCA and 129 nmol·g wet wt⁻¹·min⁻¹ (range 6–272 nmol·g wet wt⁻¹·min⁻¹) in graft liver with DCA.

**DISCUSSION**

Our study showed that both PDHₜ and PDHₚₐ are markedly decreased in human liver with end-stage cirrhosis. It further showed that DCA increases PDHₚₐ during OLT without altering PDHₜ, which allows partial compensation for the compromise in PDH activity resulting from end-stage liver disease. DCA-induced activation of PDH was accompanied by a decrease in the severity of lactic acidosis during OLT. Finally, our study found that PDH activity is not suppressed in the postischemic graft liver and that DCA does not increase graft liver PDH activity at 1 h after reperfusion in the recipient.

It could be argued that the additional plasma (FFP) transfusion in our OLT patients who received DCA provided an additional plasma buffer that decreased the need for NaHCO₃. However, FFP replacement was guided by intraoperative loss, as judged by coagulation parameters. No hyperproteinemia resulted from FFP transfusion in either OLT group: preoperative plasma albumin concentration, the principal plasma protein buffer, was 3.1 ± 0.1 and 3.0 ± 0.1 g/dl in DCA-treated and nontreated patients, respectively. Corresponding albumin values on conclusion of the OLT were 2.8 ± 0.1 and 3.0 ± 0.1 g/dl, respectively. Furthermore, the transfusate pH was 6.8, consequent to lactic acid production from the contained erythrocytes and HCO₃ depletion from the cell salvage procedure (26), which meant that any supplemental buffer capacity of FFP was more than counteracted by the H⁺ challenge associated with the transfusion. The moderating effect of DCA on acid-base stability in our study cannot be explained on the basis of increased plasma transfusion.

Decreased PDH activity in end-stage cirrhosis was due to decreased PDHₜ rather than suppression of its activation state. Although PDHₚₐ was much greater in control liver, the fraction of total PDH in the active
Fig. 2. Effect of DCA on circulating lactate concentration and acid-base parameters during orthotopic liver transplantation. Control, patients who did not receive DCA (n = 15); DCA, patients treated with 40 mg/kg DCA before surgical incision and again 4 h later (n = 28). Data are means ± SE. * Significant difference from group that did not receive DCA at the same time interval (P < 0.001). + Significant difference from basal value within the same group (P < 0.01). II, stage 2 (anhepatic period); III, stage 3 (reperfusion).
state was similar (both median values were 25%). Our finding in human cirrhotic liver of decreased total PDH activity with unaltered fractional activation state compared with controls is consistent with results in rats with experimentally induced cirrhosis (9). Giardina et al. (9) reported that total PDH density in mitochondria from cirrhotic liver was decreased by 44%. The larger loss of PDHt in the present study compared with that of Giardina et al. (9) may be explained by 1) tissue edema and scar formation in the livers of our patients, superimposing decreased mitochondrial content per gram of wet weight on decreased mitochondrial PDH density per mitochondrion, and/or 2) possibly more severe hepatic disease in our patients undergoing OLT than in the experimental animals in the study by Giardina et al. (9). Decreased PDH activity observed in our OLT patients may underlie the propensity of cirrhotic patients for lactic acidosis during surgery or other insults such as infection. The normal liver responds to lactic acid challenge by disproportionately increasing lactate oxidation, which is regulated by PDH, more than gluconeogenesis (19, 23). Consequently, decreased PDHt in cirrhotic liver may compromise its ability to respond to a lactic acid challenge. It is noteworthy that cirrhosis causes a defect in PDH activity in the liver but not in skeletal muscle (9, 15), which illustrates the importance of hepatic PDH activity to whole body functions associated with PDH activity, such as the impaired response to lactic acid challenge (33).

Our study showed that DCA activates PDH in native, cirrhotic liver and moderates lactic acidosis during OLT. The threefold activation of native liver PDH likely increases net hepatic lactate uptake during OLT, although this was not specifically evaluated in the present study. DCA has been shown to increase hepatic lactate uptake in dogs with lactic acidosis produced by hypoxia or phenformin intoxication (10, 22). Thus DCA may facilitate the ability of the cirrhotic liver to better respond to the lactic acid challenge associated with massive blood transfusion during OLT or other major surgery. Plasma lactic acid sources during OLT are a confluence of direct infusion of lactic acid contained in blood products (26) and increased conversion of the transfusion-related glucose challenge to lactic acid by peripheral tissues in patients with liver disease (16, 26). Coupled with an impaired (hepatic) disposal of lactic acid (21, 33), this leads to clinical lactic acidosis during surgery. In the case of OLT, there is also a 1-h anhepatic period during which no hepatic uptake of lactic acid can occur. Decreased PDH activity compared with control liver persisted even with DCA stimulation, although the difference in functional PDH activity between cirrhotic and control liver was reduced. The result was that the severity of lactic acidosis during OLT in the present study, as measured by both plasma lactate accumulation and acid-base stability, was limited by DCA treatment. This finding confirms our previous observation in patients with end-stage liver disease undergoing OLT (26).

Our rationale for examining graft liver PDH activity was that if PDH activity is inhibited in the newly perfused graft liver and if this inhibition could be reversed by DCA, then it would be reasonable to test whether DCA can also accelerate recovery of graft hepatic function. Data from several laboratories have indicated that PDH activity is inhibited in the postischemic heart and that this inhibition compromises metabolic activity, energy production, and cellular function for the postischemic heart (13, 17, 18, 30). Reversal of PDH inhibition with DCA also leads to an improvement of contractile function in the postischemic heart (17, 18, 30). However, in the present study, PDH activity in postischemic, newly perfused graft liver was very high, ~25 times greater than that in native liver and three times greater than in control liver not exposed to prolonged ischemia or hypercitratemia. Coupled with the finding that DCA failed to further increase PDH in the active form in graft liver, the most likely explanation is that graft liver PDH is already largely in the active form, without DCA stimulation. If we assume that DCA causes an 80% activation of the PDH, then the DCA-treated group would have had a total enzyme content of ~138/0.8 = 170 nmol·g wet wt⁻¹·min⁻¹. This value is slightly decreased compared with the 264 nmol·g wet wt⁻¹·min⁻¹ for total PDH activity in control liver. It may be that tissue edema in the preserved graft liver is responsible for this calculated modest decrease in total PDH activity expressed on the basis of wet weight. A definitive conclusion regarding the true (fractional) activation state of graft liver PDH, however, cannot be made on the basis of our data, because we were unable to determine PDHt concomitantly with PDHt in graft liver.

The mechanism producing an apparent activation of PDH in postischemic graft liver, which is opposite from the finding in the postischemic heart, is uncertain and warrants further study. There are no data on donor liver PDH activity at the time of organ procurement, which would allow for evaluation of possible effects of

<table>
<thead>
<tr>
<th>Liver Transplant Without DCA</th>
<th>Liver Transplant With DCA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Operative stage, min</td>
<td></td>
</tr>
<tr>
<td>Hepatic dissection (Stage 1)</td>
<td>250 ± 12</td>
</tr>
<tr>
<td>Anhepatic period (Stage 2)</td>
<td>66 ± 10</td>
</tr>
<tr>
<td>Reperfusion (Stage 3)</td>
<td>329 ± 18</td>
</tr>
<tr>
<td>Replacement</td>
<td></td>
</tr>
<tr>
<td>Packed erythrocytes, U</td>
<td>12 ± 1</td>
</tr>
<tr>
<td>Fresh frozen plasma, U</td>
<td>29 ± 2</td>
</tr>
<tr>
<td>Platelets, U</td>
<td>31 ± 6</td>
</tr>
<tr>
<td>CaCl₂, g</td>
<td>6 ± 1</td>
</tr>
<tr>
<td>NaHCO₃, meq</td>
<td>47 ± 16</td>
</tr>
<tr>
<td>Crystallaid, liters</td>
<td>4.4 ± 0.7</td>
</tr>
</tbody>
</table>

| Graft ischemia time, min     |                           |
| Cold                        | 493 ± 37                  |
| Warm                        | 55 ± 3                    |

Values are means ± SE; n = 15 for liver transplant without DCA and n = 28 for liver transplant with DCA. *P < 0.05 vs. liver transplant without DCA.
intrahepatic HCO₃ content, since the liver is an important source of plasma HCO₃. It has recently been demonstrated that HCO₃ is a potent stimulator of PDH activity in liver mitochondria (20). Therefore, activation of PDH in graft liver may be sustained even if it is not initiated by the hepatic HCO₃ production as citrate and lactate are metabolized. HCO₃-stimulated PDH activity may underlie the observation of Beech et al. (1) of increased lactate uptake and nonglucosegenic disposal by livers exposed to NaHCO₃ infusion. Our measured hepatic PDH activity is lower than our rat heart PDH activity using a similar method (4). In contrast, we have also recently found intraoperative 15-min postsischemic human cardiac PDH₄ to be 225 ± 62 nmol·g dry wt⁻¹·min⁻¹ or ~50 nmol·g wet wt⁻¹·min⁻¹ (28). This value is comparable to that for PDH₄ in control liver we observed in the present study and may reflect decreased PDH₄ in freshly postsischemic heart. Lower PDH activity in liver compared with heart was reported by Bryson et al. (3), who found a 10-fold greater mitochondrial density of PDH activity in mouse heart compared with liver. Similarly, Priestman et al. (24) reported a threefold increase in PDH E₁ subunit content in rat heart compared with liver. They also found greater basal content of the PDH kinase, which inhibits PDH activity, in rat liver compared with heart (24). Our values for the fraction of total PDH in the active form are two to three times the 11% reported in livers of pentobarbital-anesthetized rats (29). It is possible that the lactate and glucose challenge of blood transfusion could stimulate hepatic PDH in the OLT patients, but this explanation is less likely for the control livers of partial-hepatectomy patients in the present study, who received neither intraoperative glucose nor blood transfusion. Differences in PDH activation state may alternatively be due to species variation or different anesthetic techniques.

In conclusion, hepatic PDH activity is markedly diminished in cirrhotic patients, as a result of decreased PDH, without apparent change of its relative phosphorylation state. DCA can partially compensate for the decreased total PDH activity and improve acid-base homeostasis during OLT, but a functional decrease in hepatic PDH activity persists in these patients. In contrast, graft liver PDH total activity appears to be comparable to that in liver without hepatic dysfunction, and its PDH activity is consistent with a stimulation rather than inhibition of its phosphorylation state.

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REFERENCES

12. Jones, C. G., and M. A. Titheradge. Measurement of metabolic fluxes through pyruvate kinase, phosphoenolpyruvate carboxyki-


