Mechanism of Dichloroacetate-Induced Hypolactatemia in Humans With or Without Cirrhosis

Robert E. Shangraw and Farook Jahoor

Dichloroacetate (DCA) has been used as an experimental treatment for lactic acidosis because it lowers plasma lactic acid concentration. Three potential mechanisms could underlie the hypolactatemic action of DCA, but the dominant mechanism in vivo remains unclear. This study tested whether DCA-induced hypolactatemia occurs via decreased lactate production, increased lactate clearance, or decreased rate of glycolysis in healthy humans and in patients with end-stage cirrhosis. Cirrhosis is associated with decreased hepatic pyruvate dehydrogenase (PDH) content. Six healthy volunteers and 7 cirrhotic patients received a primed, constant infusion of 1-^{13}C-pyruvate and ^{15}N-alanine for 5 hours. DCA (35 mg/kg intravenously) was administered at 2 hours. Plasma isotopic enrichment was measured by gas chromatography/mass spectrometry (GC/MS), and exhaled CO_2 enrichment by isotope ratio mass spectrometry. Pyruvate and alanine production rates (Ra) were determined by isotope dilution, and pyruvate oxidation calculated as $^{13}CO_2$ production from ^{13}C-pyruvate. Ra lactate was calculated as the difference between Ra pyruvate and its disposal by oxidation to CO_2 and conversion to alanine. Baseline plasma lactate kinetics in cirrhotic patients did not differ from controls. DCA decreased lactate concentration in both groups by approximately 53%. DCA decreased glycolysis (Ra pyruvate) by 24%, increased the fraction of pyruvate oxidized to CO_2 by 26%, and decreased pyruvate transamination to alanine by 25%. DCA also inhibited lactate production by 85%, but decreased plasma lactate clearance by 60% in both groups. DCA reduces plasma lactic acid concentration by inhibiting production, via stimulating pyruvate oxidation and inhibiting glycolysis, rather than increasing clearance. In addition, end-stage cirrhosis does not alter either the mechanism or the magnitude of the metabolic response to DCA.

THE ANHEPATIC PHASE of liver transplantation is characterized by development of metabolic acidosis, largely due to plasma accumulation of lactic acid. Dichloroacetate (DCA) has been used as an experimental alternative to $NaHCO_3$ for treatment of lactic acidosis, including that which occurs during liver transplantation.[1] DCA offers a more physiological treatment of lactic acidosis because it increases arterial pH by directly reducing plasma lactic acid concentration. The exact mechanism(s) by which DCA lowers plasma lactate concentration in vivo is uncertain. Based on the combined findings of studies performed both in vitro and in vivo, it is possible that DCA exerts its hypolactatemic effects through three different mechanisms.

First, it has been proposed that DCA lowers circulating lactic acid concentration by increasing the oxidation of pyruvate to CO_2 by stimulating pyruvate dehydrogenase (PDH), the key regulatory enzyme that controls pyruvate oxidation.[2,3] PDH activation in peripheral tissues would decrease peripheral formation and release of lactic acid, as the pyruvate supply from which lactate is formed is preferentially oxidized in the Krebs cycle.[2] Second, activation of hepatic PDH stimulates hepatic uptake of lactic acid from the circulation.[4] Both mechanisms are effective in vitro.[2,4,5] Third, some studies have shown that DCA can slow the rate of glycolysis in skeletal muscle, thereby reducing the production of pyruvate from which lactate is formed.[6-8] The putative effect of DCA on glycolysis is controversial, as other studies in healthy human volunteers[9] and perfused rat hindquarter[10] have not yielded a DCA-induced inhibition of glycolysis. The extent to which these 3 mechanisms contribute to the action of DCA on plasma lactic acid concentration in vivo remains unclear.

Cirrhosis is associated with a marked decrease in hepatic, but not skeletal muscle, content of PDH.[11,12] It is possible that both the efficacy and dynamic mechanism by which DCA affects lactic acid metabolism may be altered by end-stage cirrhosis. This study was designed to determine the mechanism(s), and compare the efficacy, by which DCA reduces plasma lactic acid concentration in humans with or without end-stage cirrhosis.

MATERIALS AND METHODS

Subjects

This study was approved by the Institutional Review Boards at Oregon Health and Science University (OHSU) and the Portland (OR) Veterans Affairs Medical Center. Seven patients with stable end-stage cirrhosis, awaiting liver transplantation, were enrolled after written informed consent. Inclusion criteria for cirrhotic patients were end-stage liver disease; hemodynamic stability; no history of insulin-dependent diabetes mellitus; no recent history of cigarette smoking; medications that did not include β-adrenergic agonists, β-adrenergic antagonists, or corticosteroids; and adequate renal function, as indexed by plasma creatinine concentration less than 1.4 mg/dL. Severity of liver disease was assessed by Pugh-Child scoring system.[13] Six healthy volunteers without clinical or laboratory evidence of disease, and taking no medications, were enrolled as controls. All subjects were

From the Department of Anesthesiology and Perioperative Medicine, Oregon Health and Science University and Veterans Administration Medical Center, Portland, OR; and the USDA/ARS Children's Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine, Houston, TX.

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Address reprint requests to Robert E. Shangraw, MD, PhD, Department of Anesthesiology and Perioperative Medicine, UHS-2, Oregon Health and Science University, 3181 SW Sam Jackson Park Rd, Portland, OR 97239-3098.

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studied in the OHSU General Clinical Research Center, were awake and alert, and postabsorptive for 8 hours, during which time they received no intravenous glucose or other sources of calories.

Venous blood was sampled before the start of the stable isotope infusion protocol. Blood for assessment of coagulation status was collected into tubes containing sodium citrate and analyzed immediately. Samples for analysis of serum electrolytes, total CO_2 content, creatinine, glucose, insulin and glucagon concentrations were collected into chilled tubes without additives. Samples for assessment of plasma lactate were collected into tubes containing sodium fluoride.

Infusion Protocol

The protocol began at 7 AM, when an intravenous catheter was placed in an upper extremity for stable isotope tracer infusion, and a second intravenous catheter was placed in the dorsum of the contralateral hand for sampling of "arterialized" blood. The sampling hand was warmed with a heating pad.

After baseline blood and breath sampling, each subject received a primed, constant infusion of 1-^{13}C-pyruvate (prime: 12 μmol/kg; infusion: 0.8 μmol/kg/min) and ^{15}N-alanine (prime:12 μmol/kg; infusion: 0.15 μmol/kg/min) for 5 hours. After a 2-hour basal period, DCA (35 mg/kg intravenously) was administered over 30 minutes, and the isotope infusions continued for an additional 3 hours. Blood and breath samples were taken at 90, 100, 110, 120, 210, 240, 270, and 300 minutes of isotope infusion. Indirect calorimetry (Deltatrac, Sensormedics, Fullerton, CA) was performed on each subject during the basal period and again during the final 60 minutes of isotope infusion.

Blood for measurement of isotopic enrichment was collected into prechilled tubes containing sodium fluoride. Blood was immediately centrifuged, and plasma stored at −70°C until isotope analysis. In addition, an aliquot of blood was immediately centrifuged and analyzed for plasma glucose and lactate concentrations at the bedside, using an autoanalyzer. Breath samples were collected by facemask through a 1-way Phillips valve into a sealed 3-L anesthesia bag, immediately aspirated into 10-mL vacuum tubes, and stored at room temperature until analysis.

Analytical Procedures

Plasma insulin, C-peptide, and glucagon concentrations were determined by radioimmunoassay.[14,15] Isotope ratios of plasma alanine and lactate were measured on a Hewlett-Packard 5989B gas chromatography/mass spectrometry (GC/MS) system (Hewlett Packard, Fullerton, CA). Isotope ratio for plasma lactate was determined by negative chemical ionization GC/MS on its pentafluorobenzyl derivative with ion monitoring at m/z 89 and 90.[16] Since 1-^{13}C-pyruvate contributes to the M + 1 isotopomer of alanine (which includes both ^{13}C and ^{15}N atoms), the isotope ratio of plasma alanine due to ^{13}C-pyruvate was determined as the difference between the enrichment of the M + 1 isotopomer of the whole alanine derivative, and a fragment of the alanine derivative that contained the ^{15}N but not the ^{13}C of alanine. To accomplish this, plasma free amino acids were purified by cation exchange chromatography, and −acetyl, −propyl ester derivatives were prepared.[17] Total isotopic enrichment of alanine (due to both ^{13}C and ^{15}N atoms present) was measured by positive chemical ionization GC/MS, monitoring ions at m/z 174 and 175. Alanine ^{15}N enrichment was determined by electron impact ionization GC/MS, monitoring ions at m/z 44 and 45.[17] Isotopic enrichment of alanine due to the ^{13}C atom was obtained by subtraction. Breath $^{13}CO_2$ content was determined by gas isotope ratio mass spectrometry (Europa Scientific, Crewe, UK).

Calculations

Pyruvate and alanine production rates (Ra) were determined by the equation:

$$\text{Ra} = \left(\frac{\text{IE}_{\text{infusate}}}{\text{IE}_{\text{plateau}}} - 1\right) \times \text{F}$$

where F is the infusion rate of tracer substrate (μmol/kg/min), $IE_{infusate}$ is the isotopic enrichment of the infused tracer (mole % excess), and $IE_{plateau}$ is the plasma isotopic enrichment of substrate (mole % excess) at isotopic steady-state. Plasma lactate isotopic enrichment was used to calculate Ra pyruvate because plasma lactate is in isotopic equilibrium with pyruvate. Alanine production from pyruvate (Ra Pyr→Ala, μmol/kg/min) was calculated using the plasma ^{13}C isotopic enrichments of alanine and lactate:

$$\text{Ra Pyr} \rightarrow \text{Ala} = (^{13}\text{C-alanine}/^{13}\text{C-lactate}) \times \text{Alanine Flux}$$

Pyruvate oxidation was calculated as:

$$\text{Pyruvate oxidation} = \left(\frac{^{13}\text{CO}_2\text{excretion/R}}{\text{IE}_{\text{plateau}}}\right)$$

where $^{13}CO_2$ excretion is product of total CO_2 production (V_{CO_2}) and the ^{13}C isotopic enrichment of CO_2 (μmol/kg/min), R is the recovery of $^{13}CO_2$ from $NaH^{13}CO_3$, and $IE_{plateau}$ is the isotopic enrichment of plasma lactate (mole % excess) at isotopic steady-state.

Lactate production (Ra lactate) was calculated as the difference between Ra pyruvate and pyruvate disposal by oxidation to CO_2 and conversion to alanine. Lactate clearance (Cl, mL/kg/h) was obtained from Ra lactate divided by its plasma concentration.

Statistical Analysis

Data are expressed as the mean ± SE. Student's *t*test was used to make between-group comparisons of plasma substrate and hormone concentrations; Ra values for pyruvate, alanine, and lactate; pyruvate oxidation rates; and lactate clearance. Paired *t* test was used to compare data before and after DCA within groups. Statistical tests were performed using a specialized software program (Crunch 4, Crunch Software, Oakland, CA), with significance set at $P < .05$.

RESULTS

Baseline Characteristics

The control group consisted of 5 men and 1 woman. Their mean age was 30 ± 3 (SE) years, body weight was 66 ± 3 kg, and body mass index (BMI) was 22.2 ± 0.9 kg/m^2. The cirrhosis group consisted of 5 men and 2 women. Their age was 47 ± 3 years, body weight was 77 ± 6 kg, and BMI was 25.5 ± 1.7 kg/m^2. The etiology of end-stage liver disease in the cirrhosis group was ethanol abuse combined with hepatitis C viral infection in 4 patients, ethanol abuse without viral infection in 2 patients, and cryptogenic cirrhosis in 1 patient. The median severity of liver disease, by Pugh-Child score, was 10 with a range of 7 to 13.[13] No patient with cirrhosis had consumed alcohol for a period over 1 year. Compared to controls, the cirrhotic group was older ($P < .001$), but had comparable body weight and BMI.

Table 1 lists screening laboratory values. Compared to controls, cirrhotic patients were hyponatremic, hypoalbuminemic, hyperglycemic, hyperbilirubinemic, and coagulopathic as evidenced by prolonged prothrombin time. There was no difference between groups in basal V_{O_2}, resting energy expenditure, or respiratory quotient (RQ).

Table 1. Biochemical and Indirect Calorimetry Parameters

	Controls (n = 6)	Cirrhosis (n = 7)
Serum albumin (g/dL)	4.5 ± 0.1	3.3 ± 0.2*
Serum bilirubin (mmol/L)	0.7 ± 0.1	4.0 ± 1.5*
Prothrombin time (s)	12.3 ± 0.5	14.8 ± 0.3*
Plasma glucose (mmol/L)	4.73 ± 0.28	5.23 ± 0.55*
Plasma urea (mmol/L)	4.00 ± 0.25	5.82 ± 1.18
Serum creatinine (mg/dL)	1.2 ± 0.1	1.2 ± 0.1
Total blood CO_2 (mmol/L)	27.8 ± 0.9	24.6 ± 1.1
REE % predicted	96 ± 4	99 ± 5
RQ	0.82 ± 0.01	0.82 ± 0.02

Abbreviations: REE, resting energy expenditure, measured by indirect calorimetry, expressed as percentage of that predicted by the Harris-Benedict equation; RQ, respiratory quotient, equal to V_{CO_2}/V_{O_2}.
*$P < .05$ *v* controls.

Effect of Cirrhosis on Basal Lactate Metabolism and Substrate Kinetics

Figure 1 illustrates that the basal dynamics of alanine metabolism in cirrhotic patients did not differ from those of controls. For pyruvate, the basal Ra, oxidation, and conversion to alanine were similar between cirrhotic patients and controls (Fig 2). Basal pyruvate oxidation, calculated as a percent of pyruvate flux, was 61 ± 2 and 65 ± 2 in cirrhotic patients and controls, respectively. Plasma lactate concentration, and the underlying balance between its rate of appearance and clearance were also not different between groups (Fig 3).

Response of Healthy Volunteers and Patients With Cirrhosis to DCA

DCA decreased the overall Ra for plasma alanine by 17% in controls and 21% in patients with cirrhosis (Fig 1). DCA also decreased Ra pyruvate by approximately 25% in both controls and cirrhotic patients (Fig 2). The DCA-induced reduction in Ra pyruvate did not differ between the 2 groups. Although DCA did not alter the absolute pyruvate oxidation rate in either group, DCA increased the percent of generated pyruvate that was oxidized to 81% ± 3% in cirrhotic patients and to 97% ± 4% in controls ($P < .02$). DCA elicited parallel reductions

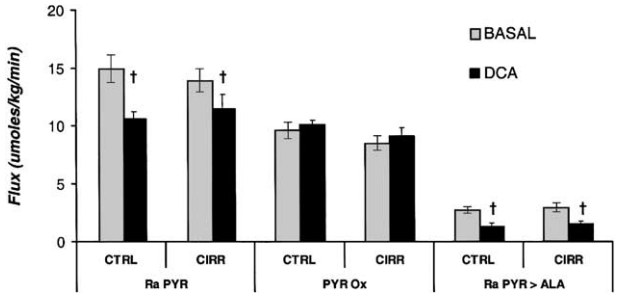

Fig 2. Effect of DCA on pyruvate metabolism in healthy volunteers (CTRL, n = 6) and in patients with cirrhosis (CIRR, n = 7). Pyruvate production rate (Ra) and transamination rate (Ra pyr > ala) were determined by isotope dilution, and pyruvate oxidation calculated as $^{13}CO_2$ production from ^{13}C pyruvate. There were no differences between volunteers and cirrhotic patients in any parameter. †Differences between basal and DCA treatment within groups ($P < .05$).

(47% to 59%) in the rate of conversion (transamination) of pyruvate to alanine in cirrhotic patients and controls. It is noteworthy that DCA decreased the fraction of alanine flux coming from pyruvate from 33% to about 20% in both groups. As shown in Fig 3, DCA inhibited lactate production by approximately 85% in both subject groups. Inhibition of lactate production by DCA was associated with a reduction in plasma lactate concentration by 53% and a decrease in lactate clearance by 60% in both subject groups.

Hormonal Profile and Its Response to DCA

Cirrhotic patients exhibited a marked basal hyperinsulinemia, with serum insulin concentration 5-fold higher than in controls (Table 2). Hyperinsulinemia was paralleled by a 3-fold increase in circulating C-peptide concentration. Concomitantly, basal serum glucagon concentration in cirrhotic patients was more than 7 times higher than the control value. The response to DCA in healthy volunteers was a 35% decrease in serum insulin and C-peptide concentrations, without alteration of serum glucagon concentration. In cirrhotic patients, DCA de-

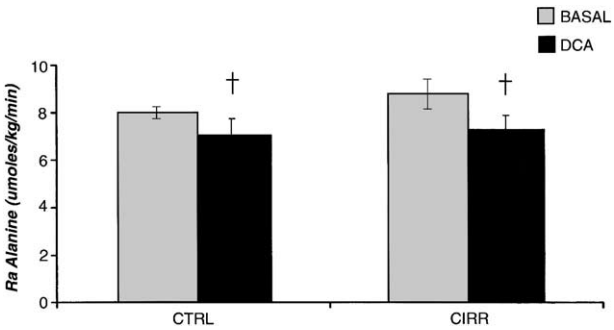

Fig 1. Effect of DCA on alanine flux in healthy volunteers (CTRL, n = 6) and in patients with cirrhosis (CIRR, n = 7). Alanine production rate (Ra) was determined by isotope dilution. There were no differences between volunteers and cirrhotic patients at any time. †Differences between basal and DCA treatment within groups ($P < .05$).

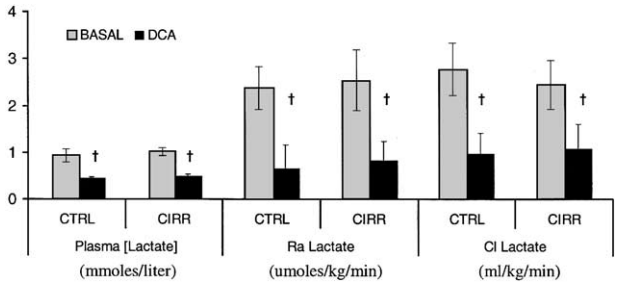

Fig 3. Effect of DCA on lactate metabolism in healthy volunteers (CTRL, n = 6) and in patients with cirrhosis (CIRR, n = 7). Ra lactate was calculated as the difference between Ra pyruvate and its disposal by oxidation to CO_2 and conversion to alanine. There were no differences between volunteers and cirrhotic patients in any parameter. †Differences between basal and DCA treatment within groups ($P < .05$).

Table 2. Hormonal Profile in Cirrhotic Patients and in Healthy Volunteers Before and After DCA

	Basal	(+) DCA
Insulin (μU/mL)		
Controls	9.60 ± 2.64	6.24 ± 0.96†
Cirrhosis	53.8 ± 11.5*	30.2 ± 8.4*†
C-peptide (ng/mL)		
Controls	1.11 ± 0.11	0.70 ± 0.08†
Cirrhosis	3.68 ± 0.45*	3.17 ± 0.25*
Glucagon (pg/mL)		
Controls	37 ± 2	38 ± 3
Cirrhosis	272 ± 38*	340 ± 42*

*$P < .01$ *v* controls.
†$P < .05$ *v* basal.

creased serum insulin concentration by 44%, but did not appreciably affect either C-peptide or glucagon concentrations.

DISCUSSION

Our results show that (1) end-stage cirrhosis does not alter basal whole-body lactate kinetics, and (2) DCA administration in both healthy and cirrhotic subjects lowers plasma lactate concentration by 50%, inhibits the rate of glycolysis (Ra pyruvate) by about 25%, and concurrently stimulates pyruvate oxidation, resulting in a marked increase in the percent of pyruvate Ra oxidized. Based on these results we conclude that the primary mechanism by which DCA induces hypolactatemia in healthy and cirrhotic humans is by reducing the quantity of pyruvate available for synthesis of lactate, by both decreasing the rate of pyruvate synthesis via glycolysis and augmenting pyruvate oxidation in the Krebs cycle. A diagram of the metabolic pathways in involved in the present study in the basal state and after DCA treatment is shown in Fig 4.

The hypolactatemic effect of DCA could have been secondary to either decreased lactate production, increased rate of removal, or a combination of the two. Lactate production rate is determined by the availability of pyruvate, and by the cytoplasmic NADH/NAD^+ ratio, which influences the equilibrium relation between lactate and pyruvate.[18,19] Most pyruvate is metabolized by the PDH reaction to acetyl-coenzyme A (CoA), and subsequently oxidized to CO_2 in the TCA cycle.[20] The PDH reaction requires NAD^+ and adenosine triphosphate

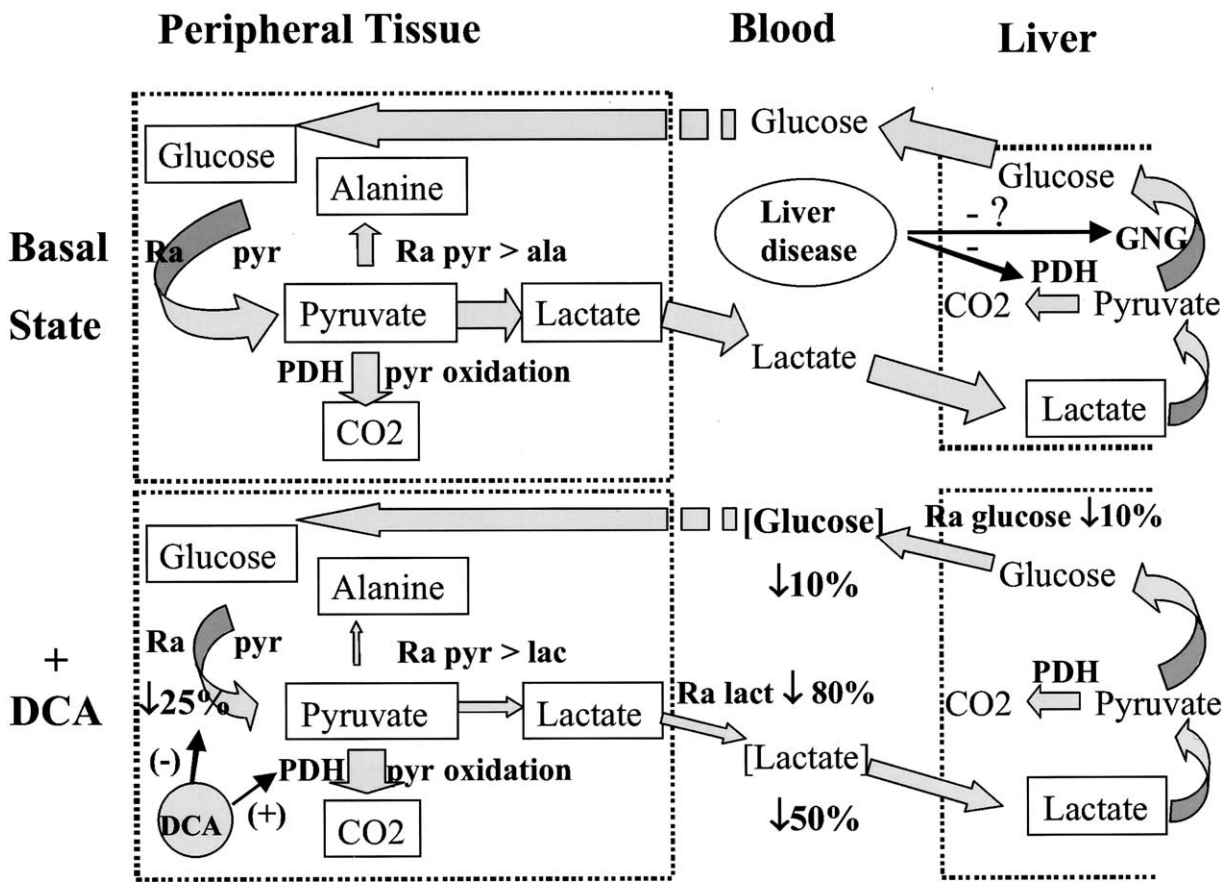

Fig 4. Illustration of the metabolic pathways involved in the present study, in the basal state (top) and after treatment with DCA (bottom). Liver disease decreases PDH content in liver but not extrahepatic tissues. Liver disease may have an inhibitory effect on gluconeogenesis (GNG) by liver but in vivo in the presence of hyperglucagonemia there is no apparent effect on Ra glucose. Treatment with DCA, which activates PDH, increases the efficiency of pyruvate oxidation; and decreases pyruvate production, conversion of pyruvate to alanine, alanine production, and lactate production in both volunteers and patients with end-stage liver disease.

(ATP).[20] Therefore, conditions such as diabetes mellitus that inhibit PDH activity,[2] or such as hypoxia that impair mitochondrial reoxidation of NADH to NAD^+,[18] will slow the movement of pyruvate into the Krebs cycle and increase the availability of pyruvate for conversion to lactate. Pyruvate availability is also determined by the rate of glycolysis. Stimulation of glycolysis will generate pyruvate faster than it can be utilized by the PDH reaction, which in turn will favor its conversion to lactate.[18-20] Hence, inhibiting the rate of glycolysis and/or stimulating pyruvate oxidation will reduce lactate production. Our present findings indicate that DCA uses both mechanisms to lower plasma lactic acid concentration. Overall, there is an increase in the proportion of pyruvate oxidized or at least committed to the Krebs cycle to almost 100%, which decreases the proportion disposed via the nonoxidative pathways to lactate and alanine. Simultaneously the overall rate of glycolysis is inhibited, leading to a marked reduction in the production rate of lactate.

Our findings agree with those of Wolfe et al,[9] which demonstrated that DCA stimulates whole-body pyruvate oxidation. This is expected because DCA stimulates PDH activity, a rate-limiting step for glucose oxidation, in many tissues.[2,5,12,21] Our results confirm the data of Wolfe et al[9] showing that DCA stimulates pyruvate oxidation and decreases lactate production in healthy human volunteers. The modest increase (~5%) in absolute pyruvate oxidation observed in the present study cannot alone account for the marked reduction (~85%) in whole-body lactate production. On the other hand, when combined with the approximately 25% reduction of Ra pyruvate by DCA in the cirrhotic patients and controls, the increased movement of pyruvate into the oxidative pathway secondary to the apparent PDH stimulation leaves little free pyruvate available for either transamination to form alanine or reduction to form lactate.

Our observed DCA-induced inhibition of whole-body glycolysis is in agreement with the effect reported in burned patients, burned rats, and guinea pigs with lactic acidosis.[6-9] In burned patients, for example, the reduced conversion of pyruvate to lactate by DCA was associated with an increased rate of pyruvate oxidation and an 18% reduction in rate of glycolysis.[9] Clark et al[7] concluded that the DCA-induced reduction in lactate output by incubated skeletal muscle of burned rats was mostly accounted for by the reduction in pyruvate production. Although the mechanism by which DCA inhibits glycolysis is unknown, Clark et al[7] speculated that it may be due to inhibition of phosphofructokinase secondary to a decrease in intracellular adenosine monophosphate (AMP) concentration. Phosphofructokinase activity in vitro is decreased by an increase in intracellular ATP or citrate concentrations, or by a decrease in AMP concentration. Clark et al[7] found lower AMP concentrations but no change in ATP or citrate when rat muscle was incubated in the presence of DCA. On the other hand, Howlett et al[22] did not observe a DCA-induced decrease in skeletal muscle free AMP content. The possibility that the DCA-induced decrease in glycolysis is mediated by cumulation of intracellular citrate is diminished by the finding that the citrate concentration in skeletal muscle decreases in concert with that of lactate after DCA administration.[23]

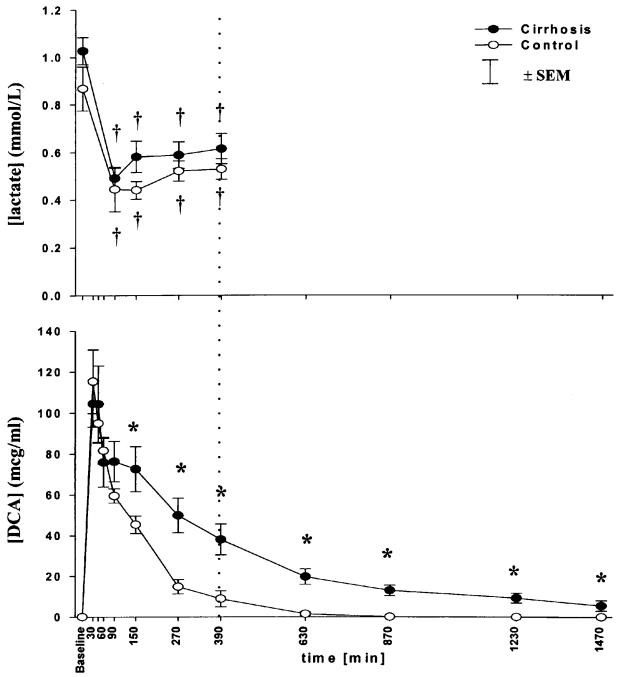

Fig 5. Time course for plasma concentrations of lactate (top) and DCA (bottom) after DCA 35 mg/kg intravenously in healthy volunteers (○, n = 6) and patients with cirrhosis (●, n = 7). DCA was administered over 30 minutes starting at time 0. Dotted vertical line is the end of the present study. Data for plasma DCA concentrations are from Shangraw and Fisher.[24] *Differences between controls and the cirrhosis group ($P < .05$). †Differences between basal and DCA treatment within groups.

Our present and previous findings[24] and those of others[25,26] indicate that the hypolactatemic effect of DCA is prompt and outlasts the DCA plasma concentration by several hours. In the present study kinetic measurements were made for only 3 hours post-DCA. Nevertheless, we observed a maximum reduction (~50%) in plasma lactate at 1.5 hours (Fig 5), demonstrating that the effect of DCA on pyruvate and lactate metabolism is prompt. Evidence that the hypolactatemic effect of DCA is long-lasting comes from an earlier study with a 24-hour sampling period.[25] Curry et al[25] administered DCA 25 mg/kg to healthy volunteers and produced an almost identical reduction (50%) in plasma lactate concentration which was maintained for 16 hours, returning to pre-DCA levels only after 20 to 24 hours. A 50-mg/kg dose decreased plasma lactate concentration for over 24 hours.[24] In a previous study in which we administered DCA 35 mg/kg to groups of cirrhotic patients and healthy controls, we observed an exponential fall in plasma DCA concentration that returned to near baseline after 10 to 15 hours in both groups (Fig 5).[24] Together, these findings suggest that the hypolactatemic effect of DCA occurs promptly and extends long after plasma DCA concentration returns to baseline. The exact molecular mechanism for such a sustained effect remains unclear.[26]

It is possible that we have overestimated the DCA-induced activation of pyruvate oxidation by using the appearance of

$^{13}CO_2$ from 1-^{13}C-pyruvate. This is true because additional production of acetyl-CoA secondary to DCA-induced stimulation of PDH may exceed the rate at which Krebs cycle activity can fully oxidize acetyl-CoA after the C1 carbon of pyruvate is cleaved during the PDH reaction. As a result, unlabeled acetyl-CoA not only accumulates but is also taken up in other intracellular pools. For example, increased acetylation of the muscle intracellular pool of free carnitine occurs following DCA administration.[22] Nevertheless, results similar to those of the present study were obtained when 3-^{13}C-lactate rather than 1-^{13}C-pyruvate was used as tracer before and throughout DCA administration,[9] which makes a gross error in estimation of pyruvate oxidation less likely. Further, whether acetyl-CoA produced by the PDH reaction is subsequently oxidized fully or acetylates carnitine, the more important point is that the flux of pyruvate through the PDH reaction eliminates it as a carbon source for production of lactate.

There is evidence that DCA can reduce plasma lactate concentration by stimulating lactate removal from the circulation.[27,28] McAllister et al[27] reported that DCA administration to dogs increased lactate extraction by the heart, while Graf et al[28] showed that dogs with hypoxic lactic acidosis responded to DCA by concomitantly inhibiting lactate production and increasing its hepatic uptake, to decrease circulating lactate concentration. The marked decrease in hepatic PDH content caused by cirrhosis[11,12] does not appear to compromise the whole-body hypolactatemic response to DCA. Rather, the results of this study show that DCA reduces plasma lactic acid concentration by inhibiting lactate production, a metabolic capacity shared by many tissues, rather than by increasing lactate clearance, a largely hepatic function.

There is uncertainty regarding the tissue(s) responsible for the DCA-induced decrease in plasma lactic acid concentration. The most likely site of action for DCA on plasma lactate concentration is peripheral tissue. Erythrocytes are not a candidate tissue because, despite their copious lactate production, they do not contain PDH—the target for DCA. Most circulating lactate is believed to be produced by skeletal muscle.[19,28] DCA decreases lactate release by skeletal muscle in vitro[2] and also decreases muscle intracellular lactate concentration in vivo both at rest and during exercise.[22] However, in recumbent (such as with our subjects) healthy human volunteers, the marked DCA-induced hypolactatemia occurs without substantial participation by the leg, composed largely of skeletal muscle.[29] A failure of skeletal muscle to participate in the in vivo hypolactatemic response to DCA has also been observed in anesthetized dogs.[30] The apparent discrepancy with regard to the effect of DCA on lactate balance across skeletal muscle may be explained by the relatively low perfusion of skeletal muscle at rest or during general anesthesia. Based on its large mass and very high metabolic activity it is very likely that the site of DCA-sensitive lactate production is visceral tissues.

Our observations from liver transplant intraoperative data, that basal lactate concentration was minimally higher in cirrhotics than in healthy controls, indicated to us that basal lactate clearance may be slower in the cirrhotic group. This supposition was supported by our previous study of liver biopsies in patients similar to those of the present study, which demonstrated that hepatic PDH is decreased by about 90% on a per-weight basis in cirrhosis.[12] We reasoned that if hepatic PDH facilitates the hepatic uptake and disposal of plasma lactate, lactate clearance would be impaired to some degree. This, coupled with the report that the plasma half-life of administered lactate is prolonged in patients with cirrhosis even exhibiting a normal plasma lactate concentration,[31] made it reasonable to assume that basal lactate clearance is slower in the cirrhotic group. The present study showed that this is not the case. On the other hand, our data that basal lactate clearance is not altered by end-stage cirrhosis does not imply that the response to a lactate challenge would be normal in cirrhosis. During liver transplantation, patients with end-stage liver disease accumulate an excessive quantity of lactic acid, associated with metabolic acidosis, which persists until the graft liver has been perfused for over 1 hour. The intraoperative lactate challenge is due in part to massive blood transfusion.[1] In addition, cirrhotics exhibit an exaggerated hyperlactatemic response to a glucose challenge.[32] Woll and Record[31] demonstrated that despite a normal resting lactate concentration, cirrhotic patients exhibit a prolonged clearance of a moderate lactate challenge. They found an impaired lactate clearance in patients whose disease was not end-stage, and liver perfusion not compromised by surgery.[31] Nosadini et al[33] reported that cirrhotic patients without a portosystemic shunt had splanchnic lactate uptake that did not differ from that of healthy controls, while splanchnic lactate uptake in those with a portosystemic shunt was 66% lower than in controls. None of our cirrhotic patients had either surgical portocaval or splenorenal shunts, but 3 of the 7 had an indwelling transjugular intrahepatic portocaval shunt (TIPS) catheter. It is noteworthy that whole-body lactate clearance of our cirrhotic subjects with a TIPS catheter (2.54 mL/kg/min) did not differ from the 2.38 mL/kg/min of patients without the TIPS catheter, although the *hepatic* lactate clearance could have been lower than in healthy controls in either (or both) cirrhotic subgroups. We did not test the response of our subjects to a lactate challenge. It would be of interest to test whether the balance of lactate production versus disposal is different in either cirrhotic or healthy humans when DCA administration occurs in the setting of a lactate clamp,[34] and whether end-stage cirrhosis alters the balance when plasma lactate concentration is either maintained or increased.

The elevated plasma concentrations of insulin and glucagon in our cirrhotic group is consistent with reports that marked hyperinsulinemia and hyperglucagonemia are part of the pathophysiology of end-stage cirrhosis.[32] The high plasma hormone concentrations are due more to increased secretion than impaired clearance. Hyperinsulinemia in healthy volunteers inhibits hepatic glucose production and stimulates muscle glycolysis,[35] which we indexed in the present study by Ra pyruvate. Hyperglucagonemia has an opposing effect, at least in liver, increasing hepatic glucose production by stimulating gluconeogenesis.[36] It is possible that cirrhotic patients maintain a normal rate of hepatic glucose production due to concomitant hyperglucagonemia.[24,37] It is further possible that our failure to detect a slower rate of glycolysis (Ra pyruvate) in cirrhotic patients was due to the influence of pre-existing hyperinsulin-

emia, which could have exerted a stimulatory effect. We previously reported that DCA induces a small (10%) but significant reduction in plasma glucose concentration in both healthy and cirrhotic humans.[24] Our observation was associated with a comparable reduction in endogenous glucose production, and is consistent with a minor inhibitory effect of DCA on gluconeogenesis by incubated liver.[4,24] The modest decreases in glucose production and its plasma concentration after DCA are consistent with data reported in rats[38] and dogs.[39] In both animal studies, the DCA-induced decrease in endogenous glucose production was secondary to an inhibition of gluconeogenesis rather than glycogenolysis.[38,39]

In summary, we found that DCA decreases plasma lactic acid concentration by decreasing lactate production, rather than increasing clearance, in healthy and cirrhotic humans. Decreased lactate production is due to a combination of increased flux of pyruvate into the oxidation pathway and decreased pyruvate production through an inhibition of glycolysis. End-stage liver disease did not alter either the mechanism or the magnitude of the metabolic response to DCA.

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