Is accelerated oxidation of lactate required for dichloroacetate to lower the level of lactate in blood?

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We examined mechanisms by which dichloroacetate (DCA), an activator of pyruvate dehydrogenase (PDH), led to a decrease in the concentration of lactate in blood in a unique "metabolic setting," where the concentration of lactate in blood was 5.4 ± 0.5 mmol/L. Elevated levels of lactate were induced in anaesthetized rabbits by the administration of a large dose of insulin. The rate of consumption of oxygen was 1.2 ± 0.1 mmol/min, the respiratory quotient was close to unity, and close to half of the PDH was in its active form; therefore, virtually all ATP synthesis should require flux through PDH. Hence, we predicted that DCA should not cause a significant decrease in the concentration of lactate in blood in this model. In contrast, if DCA was effective, new insights could be obtained into its mechanisms of action, at least in this setting. During steady-state hyperlactatemia, DCA was given as its sodium salt, 2 mmol/kg ($n = 10$); a control group ($n = 5$) received equimolar NaCl. Forty minutes later, the level of lactate in blood in the DCA group was 1.3 ± 0.2 mmol/L, significantly lower than in the NaCl group (4.2 ± 0.6 mmol/L). To determine the organ(s) responsible for removing lactate, arteriovenous differences were measured in organs drained by the jugular, femoral, and hepatic veins. There was no net uptake of lactate in these drainage beds after DCA was administered. From a quantitative analysis of the rate of removal of lactate and the rate of consumption of oxygen, it seems unlikely that the majority of the decrease in lactate could be directly attributed to an increase in its oxidation.

Key words: lactic acidosis, dichloroacetate, pyruvate dehydrogenase, metabolism.

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Nous avons examiné les mécanismes par lesquels le dichloroacétate (DCA), un activateur de la pyruvate déshydrogénase (PDH), a provoqué une chute de lactate sanguin, alors que la concentration de lactate était de $5,4 \pm 0,5$ mmol/L. Les taux élevés de lactate ont été induits, chez des lapins anesthésiés, par l'administration d'une forte dose d'insuline. Le taux de consommation d'oxygène était de $1,2 \pm 0,1$ mmol/min, le quotient respiratoire se situait près de l'unité et environ la moitié de la PDH était sous sa forme active; dans un tel cas, presque toute la synthèse de l'ATP devrait impliquer la PDH. Par conséquent, nous avons émis l'hypothèse que le DCA ne devrait pas provoquer de diminution significative de la concentration de lactate sanguin chez ce modèle. Toutefois, si le DCA était efficace, de nouveaux aspects de ses mécanismes d'action seraient révélés, du moins pour cette situation métabolique. Durant une hyperlactacidémie continue, le DCA a été administré sous sa forme Na, 2 mmol/kg ($n = 10$); un groupe témoin ($n = 5$) a reçu une dose équimolaire de NaCl. Quarante minutes plus tard, le taux de lactate sanguin du groupe DCA a été de $1,3 \pm 0,2$ mmol/L, ce qui était significativement plus faible que celui du groupe NaCl ($4,2 \pm 0,6$ mmol/L). Pour déterminer le ou les organes à l'origine de l'élimination du lactate, on a mesuré les différences artérioveineuses dans les organes drainés par les veines hépatiques, fémorales et jugulaires. Après l'administration de DCA, il n'y a pas eu de capture de lactate dans ces lits de drainage. D'après les résultats d'une analyse quantitative du taux d'élimination de lactate et du taux de consommation d'oxygène, il semble peu probable que la majeure partie de la diminution de lactate puisse être directement attribuée à une augmentation de son oxydation.

Mots clés : acidose lactique, dichloroacétate, pyruvate déshydrogénase, métabolisme.

[Traduit par la rédaction]

Introduction

It is important to define the "metabolic setting" to evaluate the impact of a therapeutic modality in the treatment of a metabolic abnormality. The example studied in this paper is the ability of dichloroacetate (DCA) to lower the concentration of lactate in blood (Stacpoole 1989). DCA has been shown to

stimulate pyruvate dehydrogenase (PDH) (Stacpoole 1989; Whitehouse and Randle 1973) and, in certain circumstances, the oxidation of lactate. The metabolic setting to permit an increase in flux through PDH, however, requires an increase in demand for the products of this reaction (ultimately, an increase in turnover of ATP or the synthesis of fatty acids) or that lactate is used in preference to other fuels (e.g., fatty acids). It follows that DCA may not have the same impact or effect if it is given to a subject who is deriving this needed ATP primarily from the oxidation of carbohydrate. Moreover, it is important to perform a quantitative analysis that takes into account the rate of removal of lactate in conjunction with the rate of consumption of oxygen to evaluate whether oxidation of lactate was indeed responsible for the observations made *in vivo*.

The model we chose to address these issues was one where the target enzyme for DCA, PDH, was sufficiently active to permit enough flux to regenerate needed ATP.² Therefore, we expected that DCA would not have a significant impact or,

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²The quantity of PDH is potentially enormous relative to the flux it must catalyze at rest. For example, consider the quantity of pyruvate oxidized in muscle in humans during vigorous exercise. O₂ consumption is 160 mmol/min and glycogen is the major fuel oxidized (Cheetham *et al.* 1986). This rate of O₂ consumption is almost 13-fold higher than at rest. Hence if more than 10% of PDH were in its active form at rest in muscle, the activity of PDH would not be rate limiting for the flux of pyruvate to acetyl-CoA. Moreover, a further increase in the activity of the enzyme would not necessarily mean an increase in flux because of constraints of O₂ consumption and the rate of turnover of ATP, especially if carbohydrate is already the major fuel being utilized.

if effective, new insights could be gained into its mechanisms of action *in vivo*. Results to be reported show that DCA led to a rapid, significant reduction in the concentration of lactate in blood in our model with elevated levels of lactate in blood. This decrease in lactate did not appear to be due to an enhanced rate of oxidation of lactate because the measured arteriovenous (A–V) difference across three major venous beds, the jugular, the splanchnic area, and the femoral drainage systems, revealed net release rather than net uptake of lactate. In addition, from a quantitative analysis of the rate of removal of lactate and the rate of consumption of oxygen, it seemed unlikely that most of the lactate was removed via oxidation. To explain these data incorporating the known stimulation of PDH by DCA (Stacpoole 1989; Whitehouse and Randle 1973), we speculate that there was an enhanced removal of lactic acid via gluconeogenesis or lipogenesis in an organ(s) not drained by the jugular, splanchnic, or femoral venous beds, presumably the kidneys; in addition, there may have been a decreased rate of production of lactate to permit its level to fall so quickly.

Methods

Male New Zealand white rabbits (mean weight 4 kg) were obtained from Reimans Fur Ranch, St-Agathe, Que. They were fed Purina rabbit chow until the time of the experiments. Chlorpromazine (2.5 mg/kg) was administered by the intramuscular route to sedate the animals 30 min prior to anaesthesia. Anaesthesia was induced by infusing pentobarbital sodium (15 mg/kg body weight; Nembutal, Abbott Laboratories, Montréal, Que.) via an ear vein; small additional doses were given, as needed, to maintain a steady plane of anaesthesia. Animals were allowed to breathe spontaneously throughout the experiment. Teflon canulas were inserted in the carotid artery, the femoral vein, jugular vein, and hepatic vein for measurement of A–V differences. The catheter in the hepatic vein was inserted via the femoral vein, and the location was verified at the end of the experiment. A balloon-tipped, flow-directed catheter was inserted into the pulmonary artery via the jugular vein for measurement of cardiac output and sampling of mixed venous blood. The temperature was monitored throughout the experiment, and animals were warmed by a heating blanket to maintain body temperature between 37 and 38°C.

Experimental protocols

The first series of experiments was carried out to characterize the model of hyperlactatemia in which delivery of O₂ was not limiting and fatty acids were not a major fuel; hence virtually all ATP needed by organs should require flux through PDH. To achieve this aim, the protocol of Haynes *et al.* (1988) was used. Anaesthetized rabbits ($n = 5$) received 7 units of regular insulin as an intravenous bolus plus a continuous infusion of 7 units per hour. Samples of blood were obtained at 20-min intervals from the aorta for measurement of metabolites as described below. Blood was obtained from the aorta and pulmonary artery at 80 and 120 min for measurement of hematocrit and blood gases. The cardiac output was measured at 80 and 120 min.

A second series of experiments was carried out to measure PDH activity in the three phases of this model. A sample of liver, renal cortex, and muscle was freeze clamped in liquid nitrogen in control anaesthetized rabbits ($n = 4$), in rabbits treated with insulin as above for 120 min ($n = 5$), and 10 min after DCA (2 mmol/kg) was given as an intravenous bolus to a third group of rabbits ($n = 5$), who received insulin as described above.

The third series of experiments was performed to determine whether DCA, if administered during the steady state of hyperlactatemia, would cause a decrease in the concentration of lactate in blood and, if so, which organ(s) was (were) responsible for the removal of lactate. Rabbits were anaesthetized and received insulin as above. During the steady state with elevated levels of lactate in blood

FIG. 1. Effect of insulin on the concentration of lactate in blood. For details, see text. Insulin was administered at time 0. Data are reported as means $\pm$ SEM. A steady-state hyperlactatemia was achieved 80 min after the administration of insulin.

(120 min), rabbits received either the sodium salt of DCA (2 mmol/kg, $n = 10$) as an intravenous bolus or equimolar NaCl in the control series ($n = 5$). Samples of blood for measurement of metabolites were taken from the aorta at 80 and 120 min after insulin was begun and at 10-min intervals for 40 min after the administration of DCA or NaCl. Blood was obtained from the aorta and pulmonary artery at 80 and 120 min after insulin was begun and at 20 min after administration of DCA or NaCl for the measurement of hematocrit and blood gases. The cardiac output was measured at 80 and 120 min after insulin was begun and at 20 min after animals received DCA or NaCl.

To determine the organ(s) responsible for removal of lactate, blood for measurement of metabolites was taken from the aorta, jugular vein, femoral vein, and hepatic vein at 80 and 120 min after insulin was begun and at 5, 10, 20, and 30 min after the administration of DCA. It was not technically possible to obtain samples from the renal vein without excessive manipulations that led to a rise in the levels of fatty acids in the blood.

Analytical methods

The cardiac output was measured by a thermodilution method using a cardiac output computer (Swan-Ganz 93-132-SF, American Edward Laboratories, Calif.). The pH, P_{O_2} , and P_{CO_2} of the blood were measured anaerobically at 37°C with a digital acid–base analyzer. A portion of blood was deproteinized after collection with 1:1 v/v 10% ice-cold perchloric acid for measurement of metabolites. Hematocrit, glucose, lactate, and fatty acids were measured as previously described (Halperin *et al.* 1985). PDH activity was measured as described by Taylor *et al.* (1973) for adipose tissue with the following modifications: Smaller quantities of muscle, liver, and kidney were used (20 mg/mL) and muscle samples were homogenized in the presence of 1% Triton (Sigma Chemical Company, St. Louis, Mo.) The assay was linear between 1.5 and 6 min, and assays were conducted for 6 min.

Calculations

The rates of consumption of oxygen and production of CO₂ were calculated by multiplying the arterial minus mixed venous oxygen or CO₂ content by the cardiac output. The contents of oxygen and CO₂ were calculated as described by Kelman (1966, 1967). The respiratory quotient (RQ) was calculated as the ratio of production of CO₂ to consumption of O₂.

Statistical analysis

Results are reported as means $\pm$ SEM. Statistical analysis was formed by the Student's *t*-test. Values of <0.05 were considered statistically significant. Analysis was performed on a MacIntosh computer using Statsview II statistical software package.

TABLE 1. Effect of insulin and DCA on the activity of PDH

Treatment	Kidney	Liver	Muscle
Control ($n = 4$)	13 ± 2	12 ± 1	25 ± 7
Insulin ($n = 5$)	35 ± 10	34 ± 13	46 ± 6 ^a
Insulin + DCA ($n = 5$)	67 ± 16 ^a	59 ± 15 ^a	81 ± 5 ^{a,b}

NOTE: The activity of PDH was measured as described by Taylor *et al.* (1973). The results are presented as means ± SEM for the percentage of enzyme in the active form.

^a $p < 0.05$ compared with the control period.

^b $p < 0.05$ compared with the insulin period.

TABLE 2. Level of metabolites in blood in the NaCl and DCA groups

	NaCl ($n = 5$)	DCA ($n = 10$)
Glucose (mmol/L)		
Control	6.6 ± 0.4	6.2 ± 0.7
Insulin	4.0 ± 0.1 ^a	3.6 ± 0.2 ^a
Treatment	3.5 ± 0.2 ^a	3.7 ± 0.3 ^a
Fatty acids (μmol/L)		
Control	1570 ± 552	871 ± 199
Insulin	641 ± 165	418 ± 39
Treatment	317 ± 223 ^a	314 ± 15 ^a
Lactate (mmol/L)		
Control	1.5 ± 0.4	1.6 ± 0.2
Insulin	5.3 ± 0.5 ^a	5.9 ± 0.5 ^a
Treatment	4.2 ± 0.6	1.3 ± 0.2 ^{b,c}

NOTE: For details, see text. The data in the insulin period are means of measurements obtained at 80 and 120 min. The data in the treatment period are means of measurements obtained at 40 min after the administration of NaCl or DCA.

^a $p < 0.05$ compared with the control period.

^b $p < 0.05$ compared with the insulin period.

^c $p < 0.05$ compared with the NaCl group.

Results

Model of hyperlactatemia

In rabbits infused with insulin, there was a decline in the concentration of glucose in plasma from 6.3 ± 0.4 to 3.8 ± 0.1 mmol/L at 80 min after the insulin infusion was started; similarly the level of fatty acids declined from 1103 ± 233 to 483 ± 57 μmol/L. Hence there was evidence of the metabolic actions of insulin. The concentration of lactate in blood rose from 1.5 ± 0.2 to 5.4 ± 0.5 mmol/L after 80 min and remained in this range thereafter (Fig. 1). The rate of consumption of O_2 was 1.2 ± 0.1 mmol/min and did not vary significantly throughout the experiment. The RQ was close to unity (1.0 ± 0.1) throughout the experiment, indicating that carbohydrate rather than fatty acids was the primary fuel oxidized. Less than 25% of PDH was in its active form in liver, kidney, and muscle in control rabbits. There was almost a twofold increase in activity of the enzyme in muscle after the administration of insulin. As expected, DCA has led to a further increase in the activity of PDH (Table 1).

Effect of DCA

The concentration of lactate in blood was similar in both the DCA and NaCl groups (5.9 ± 0.5 vs. 5.3 ± 0.5 ; Table 2, Fig. 2). Although there was a small decrease in the concentration of lactate in blood in the saline group, this decrease was

FIG. 2. Effect of DCA on the concentration of lactate. For details, see text. Data are reported as means ± SEM for the DCA group (●) and the NaCl group (○). The concentration of lactate in blood was similar in both groups before the administration of DCA or NaCl. A marked decrease in the concentration of lactate in blood was noted in the DCA group.

TABLE 3. Measured A–V difference for lactate across the jugular, femoral, and splanchnic areas

	Time (min)	A–J	A–F	A–H
Insulin	+80	−0.23 ± 0.13	−0.39 ± 0.06	−0.04 ± 0.07
	+120	−0.11 ± 0.07	−0.35 ± 0.06	−0.04 ± 0.09
DCA	+5	−0.46 ± 0.14 ^a	−0.54 ± 0.16	−0.27 ± 0.21
	+10	−0.46 ± 0.08 ^a	−0.39 ± 0.17	−0.15 ± 0.14
	+20	−0.40 ± 0.11 ^a	−0.19 ± 0.24	−0.26 ± 0.16
	+30	−0.22 ± 0.12	−0.03 ± 0.22	−0.10 ± 0.08

NOTE: For details, see text. The measured A–V difference for lactate in rabbits ($n = 10$), at 80 and 120 min after starting the infusion of insulin and at 5, 10, 20, and 30 min after the administration of DCA (infusion began immediately after the 120-min time of the insulin infusion). J, jugular vein; F, femoral vein; H, hepatic vein; A, aorta.

^a $p < 0.05$ compared with insulin + 120.

much greater in the DCA group. At 40 min, the level of lactate in blood in the DCA group was 1.3 ± 0.2 mmol/L, significantly lower than that in the saline group (4.2 ± 0.6 mmol/L). The concentrations of glucose and fatty acids in blood were not different in the DCA group as compared with the saline group before or at 40 min after the administration of DCA or NaCl (Table 2). After the administration of DCA, the cardiac output was 0.6 ± 0.1 L/min and the rate of consumption of O_2 was 1.3 ± 0.1 mmol/min, values not significantly different from those before the administration of DCA (0.5 ± 0.1 L/min and 1.2 ± 0.1 mmol/min, respectively).

A–V measurements

To determine the organ(s) responsible for removal of lactate, the A–V difference for lactate was measured in organs drained by the jugular, femoral, and hepatic veins. It is noteworthy that there was net production of lactate in all these beds during the steady-state interval (Table 3). This suggests that one or more organs drained by other venous systems were removing lactate to maintain the steady state. Furthermore, there was no significant uptake of lactate in any of these beds after the administration of DCA (Table 3).

Discussion

The major finding of this study was that the level of lactate in blood decreased promptly when DCA was added, but in this model, the mechanism involved could not be explained solely by an enhanced rate of oxidation of lactate.

DCA, an activator of PDH (Stacpoole 1989; Whitehouse *et al.* 1974), has been shown to lower the level of lactate in blood in a variety of experimental models of lactic acidosis (Abu Romeh and Tannen 1986; Graf *et al.* 1985; Holloway and Alberti 1975; Johnson and Alberti 1977; Loubatières *et al.* 1976, 1978; Merrill *et al.* 1980; Park and Arieff 1982) and in some studies in humans (Blackshear *et al.* 1982; Stacpoole *et al.* 1983, 1988). The drug activates PDH and hence should stimulate the oxidation of lactate if the products of this reaction can be utilized. An increase in flux through PDH, however, requires an increase in demand for the regeneration of ATP, a need to synthesize fatty acids, or that carbohydrates replace another fuel (e.g., fatty acids) to regenerate the required amount of ATP. Therefore conclusions about the mechanism of action of DCA require that the metabolic setting under which the drug is used be clearly defined. Moreover, the stoichiometry of the rate of removal of lactate as compared with the rate of consumption of oxygen must be examined before deciding which metabolic action was responsible for the removal of lactate.

We examined the role of DCA in a specific metabolic setting characterized by a steady-state hyperlactatemia. In this model, the level of fatty acids in plasma was reduced, the RQ was close to unity, and a sufficient proportion of PDH was in its active form before the administration of DCA² (Table 1). Hence flux through PDH should already be the major metabolic pathway for regeneration of ATP. In such a model, we expected that DCA would have a little quantitative impact to enhance the rate of oxidation of lactate if there was no marked increase in oxygen consumption since flux through PDH should be limited by signals related to the rate of turnover of ATP. To achieve these aims, the protocol of Haynes *et al.* (1988), an infusion of insulin into anaesthetized rabbits, was utilized because these authors demonstrated that glycogen in liver and muscle were the major fuels consumed in this setting; this regeneration of ATP already required flux through PDH. We speculate that the basis for the steady-state lactatemia in this model was as follows: PDH was dephosphorylated, and hence in its active form owing to the administration of insulin (Table 1) (Cheng and Lerner 1987; Denton and Halestrap 1979; Jungas 1971); control of flux through PDH was now principally due to feedback inhibition by the products of the reaction it catalyzes, acetyl-CoA and NADH together with a decrease in ADP (Neely and Morgan 1974; Newsholme and Start 1977). Although changes in concentrations of these same intermediates, and also those of citrate and AMP, should also lead to inhibition of phosphofructokinase 1 (PFK1), a greater control strength exerted at PDH than PFK1 could lead to the accumulation of lactic acid.

To our surprise, DCA led to a marked decrease in the concentration of lactate in blood (Fig. 2). The other unanticipated result was that this was achieved although there was no net uptake of lactate across three accessible major venous drainage beds; in fact, there was production of lactate, across the jugular, femoral, and splanchnic venous drainage systems (Table 3). One possible explanation for the rise in A-V difference of lactate after the administration of DCA could be

the transport of lactate out of the cells in exchange for DCA entry on the monocarboxylate transporter (Halestrap and Poole 1988), at least at the earlier time points. Nevertheless, there was always a net release of lactate in all these drainage beds over the 30-min period (Table 3).

Although there was some decrease in the production of lactate (statistically insignificant), inhibition of glycolysis alone would not lead to removal of the existing pool of lactate, as net production of lactate still occurred in the jugular, hepatic, and femoral venous drainage systems. Therefore, although an inhibition of glycolysis in other organs might have contributed to the decrease in lactate, it does not explain the magnitude of effect of DCA to lower the level of lactate in blood. The data could be explained by an effect of DCA to enhance the removal of lactate by one or more organs other than those drained by the jugular, femoral, or splanchnic venous systems. It is noteworthy that a steady-state hyperlactatemia was maintained before the administration of DCA when there was net production of lactate across these venous beds and, therefore, one or more other organs were removing lactate to maintain the steady state.

The mechanism of removal of lactate could be via oxidation, lipogenesis, or gluconeogenesis (or conversion to alanine). It seems unlikely from a quantitative analysis of the rate of removal of lactate and the rate of consumption of oxygen that oxidation of lactate was the major mechanism for its removal (see below). The amount of lactate removed is equivalent to the reduction in its pool plus the amount of ongoing production of lactate. The concentration of lactate in blood in the DCA group fell from 5.9 to 2.3 mmol/L over 30 min. In the saline group the concentration of lactate in blood fell from 5.3 to 4.3 mmol/L over the same period of time (19%). Therefore it is possible that close to 20% of the drop in the concentration of lactate in the DCA group was due to dilution. The decrease in the concentration of lactate in blood that was due to the effect of DCA was then close to 2 mmol/L. If the volume of distribution of lactate in these rabbits was close to 50% of their body weight, i.e., close to 2 L (Roos 1975), then approximately 4 mmol of lactate was removed from its pool in this time interval. Removal of 4 mmol of lactate via oxidation would require 12 mmol of O₂ (Halperin *et al.* 1992); this is roughly equivalent to one-third the measured consumption of O₂ by the whole animal over 30 min (1.3 mmol/min × 30 min = 39 mmol). Furthermore, there was no uptake of lactate across the jugular, femoral, and splanchnic venous drainage systems. If one also considers the amount of lactate that was produced and removed³ after the administration of

³The amount of ongoing production of lactate after the administration of DCA can be estimated from the measured A-V difference across the jugular, femoral, and splanchnic areas and using the following assumptions: (i) the hind limb is representative of other muscular beds, (ii) the A-V difference at one point of time is representative of the time average A-V difference across that bed, and (iii) the splanchnic area receives 20% of the cardiac output, while the brain and the muscles receive 25% each. Applying the above, for example, one can calculate that there was production of 0.34 mmol of lactate by the brain in the first 5 min after the administration of DCA, i.e., $0.46 \times 0.6 \times 0.25 \times 5 = 0.34$. Using similar calculations, and keeping in mind the limitations of the above assumptions, we estimated that the amount of ongoing production of lactate over a 30-min period after the administration of DCA was approximately 4 mmol. Removal of this amount of lactate via oxidation would require another 12 mmol of O₂.

DCA, oxidation of lactate would seem to be a rather unlikely major mechanism for the effect of DCA to lower the level of lactate in blood.

The mechanism of the lactate-lowering effect of DCA in this model remains speculative. Lipogenesis is an unlikely mechanism, since it is a very slow metabolic process (Acheson *et al.* 1982; Hellerstein *et al.* 1991). It is possible that lactate was removed in this model via gluconeogenesis. The removal of 1 mmol of lactate via gluconeogenesis requires the consumption of 0.5 mmol of O₂ (Halperin *et al.* 1992). From A–V data across the splanchnic area, gluconeogenesis in the liver was not the site of removal of lactate (Table 3). This suggests that if gluconeogenesis was the pathway involved, it was stimulated in the kidney. Quantitatively, if the organ(s) that removed lactate (kidneys) consumed 25% of the total consumption of O₂ over 30 min (1.3 mmol/min $\times$ 30 min $\times$ 0.25, i.e., close to 10 mmol of O₂), it is possible that gluconeogenesis could be the pathway for the removal of lactate. In support of this view, the kidney is a major gluconeogenic organ in the rabbit (approximately 50% of oxygen consumption by the kidney of the rabbit is used for gluconeogenesis (K. S. Kamel, S. Cheema-Dhadli, and M. L. Halperin, unpublished observations). Nevertheless, an effect of DCA to stimulate gluconeogenesis in the kidney is not consistent with *in vitro* studies in which DCA was shown to inhibit gluconeogenesis in rat kidney slices (Lacey and Randle 1978). Notwithstanding, the metabolic setting *in vitro* and in our model differ. If DCA stimulated gluconeogenesis in the kidney *in vivo*, perhaps it did so by stimulating PDH and thereby with a small flux of carbon provided a number of signals that can lead to stimulation of gluconeogenesis (e.g., $\downarrow$ acetyl-CoA, $\downarrow$ NADH, $\downarrow$ ATP/ADP, $\downarrow$ AMP) (Hers and Hue 1983). Although we attempted to test this hypothesis directly by cannulating the renal vein percutaneously via the femoral or the jugular vein, we were unsuccessful. We could not use the abdominal approach of our previous studies because, in rabbits, this led to a very high level of fatty acids, which makes a major metabolic change in our model. Therefore the possibility that DCA has led to stimulation of gluconeogenesis in the kidney must remain a speculative one. However, it remains clear from the data that DCA led to the removal of lactate in this model via a mechanism other than simply promoting its oxidation.

To summarize, DCA was effective in lowering the level of lactate in blood in this model. The effect did not seem to be mediated via stimulation of oxidation of lactate. The results of our study emphasize the importance of defining the metabolic setting and performing a quantitative and biochemical analysis in evaluating the impact of a certain therapeutic modality in the treatment of a metabolic abnormality.

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