Increased Serum D-Lactate Associated With Diabetic Ketoacidosis

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We hypothesized that serum D-lactate may be increased in vivo in diabetes mellitus as a result of increased glucose flux through the glyoxalase pathway and/or via hepatic ketone metabolism. Levels of D-lactate and related metabolic intermediates were measured in 30 cats with spontaneous diabetes mellitus and in one ketoacidotic nondiabetic cat. Serum D-lactate was significantly ($P = .0051$) elevated in cats with ketoacidosis (337.2 ± 70.2 μmol/L) as compared with nonketoacidotic diabetic (140.3 ± 58.8) and control (25.0 + 6.5) cats. Two nonketoacidotic cats also had high levels of D-lactate. There was a significant linear correlation ($r = .684$, $P = .0001$) between D-lactate and β-hydroxybutyrate concentrations. Serum D-lactate did not correlate with serum glucose ($r = .078$, $P = .6825$), and in vitro erythrocyte D-lactate formation did not increase in the presence of hyperglycemia. These data suggest that hepatic ketone metabolism, rather than hyperglycemia, may be a major source of serum D-lactate in diabetics.

METHYLGLYOXAL METABOLISM via the glyoxalase pathway, a biochemical process that is widely distributed in tissues,[1] produces D-lactate. Bacterial production of D-lactate has also been observed in patients with short-bowel syndrome.[2] Hyperglycemia increases the rate of D-lactate production in tissues with insulin-independent glucose uptake through increased methylglyoxal formation and metabolic flux through the glyoxalase pathway.[3,4] Because methylglyoxal is also an intermediate in the metabolism of acetone by hepatic cytochrome P-450 2E1 (the acetone-glucose pathway), we hypothesized that increased D-lactate in diabetes mellitus may be associated with ketosis, as well as with hyperglycemia.[5,6]

In a preliminary study to assess the relation of D-lactate with blood glucose and ketone levels, we measured these and related biochemical intermediates in cats with diabetes mellitus. D-Lactic acidosis had previously been documented in cats that ingested 1,2-propanediol, another substrate of the acetone-glucose pathway.[7] Our results indicate that increased serum D-lactate is associated in vivo with ketoacidosis rather than with hyperglycemia, and suggest that ketone metabolism by hepatic cytochromes may be a significant source of methylglyoxal in diabetes.

MATERIALS AND METHODS

Cats with spontaneous diabetes mellitus were categorized as ketoacidotic or nonketoacidotic based on clinical presentation and/or the presence of urine ketones. Nonketoacidotic cats included those newly diagnosed as diabetic (based on fasting hyperglycemia, glucosuria, and typical clinical signs) and those previously diagnosed as diabetic but presenting for recheck examinations or other clinical problems. One nondiabetic ketoacidotic cat was also sampled. Cat owners provided informed consent for removal of 10 mL whole blood by jugular venipuncture. Dipstick analysis (Multistix; Ames Laboratories, Elkhart, IN) was used to evaluate ketonuria. Healthy control cats were sampled in an identical manner.

Blood was collected into tubes containing sodium fluoride and into serum tubes. Plasma and serum were separated and rapidly frozen using dry ice and acetone. Samples were stored at −20°C for up to 2 weeks for all assays except pyruvate, which was performed immediately. L-Lactate, pyruvate, and β-hydroxybutyrate levels were determined using commercial assays (Sigma Chemical, St Louis, MO). Lactate to pyruvate ratios were calculated. Glucose level was measured using a hexokinase method on an automated chemistry analyzer (Ciba Corning, Oberlin, OH).

Serum samples for D-lactate analysis were first deproteinized by centrifugation in a micropartition column (Amicon, Beverly, MA). D-Lactate content was determined using a kinetic assay[8] modified for a Beckman DU-70 spectrophotometer (Brea, CA). Chemicals were obtained from Sigma Chemical Co. Serum or 1.0 mmol/L D-lactate standard (75-μL aliquots) were placed in 30°C reaction cuvettes containing glycine-hydrazine buffer (pH 9.2), 15 U D-lactate dehydrogenase, and 6.3 mmol/L β-NAD, and the change in absorbance at 340 nm was determined over a 5-minute period.[8] Assay precision was established using control samples of pooled feline serum that contained high (1,000 μmol/L), medium (250), and low (20) concentrations of D-lactate (n = 7 each). Coefficients of variation ranged from 3.8% to 16.6% for within-run analyses and from 5.0% to 16.3% for between-run analyses, with greater variation at low concentrations of D-lactate. Analytic recovery of 250 μmol/L D-lactate added to serum (n = 7) was 99.13% ± 5.0%, with a range of 92.0% to 106.5%. The reaction was linear between 0 and 1,000 μmol/L (Fig 1).

For in vitro studies, 10.0 mL heparinized blood was collected by jugular venipuncture from three healthy nondiabetic cats. Blood was centrifuged, buffy coats were removed, and red blood cells (RBC) were washed three times with modified Hanks balanced salt solution that contained sodium bicarbonate, pH 7.3 (Sigma Chemical). A final wash was performed in buffer that contained either 5.0 (normoglycemic) or 25.0 mmol/L (hyperglycemic) glucose. Sodium chloride was added to the normoglycemic buffer so that the osmolality of the two buffers was the same. RBC were resuspended in respective buffers to approximately 20% hematocrit. RBC suspensions were incubated for 24 hours at 37°C in a shaking water bath. D-Lactate level was measured in the supernatant at 0, 2, 4, 8, and 24 hours.

Data were analyzed using ANOVA. Differences were consid-

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Submitted December 3, 1993; accepted June 14, 1994.

Supported by a grant from the Robert H. Winn Foundation.

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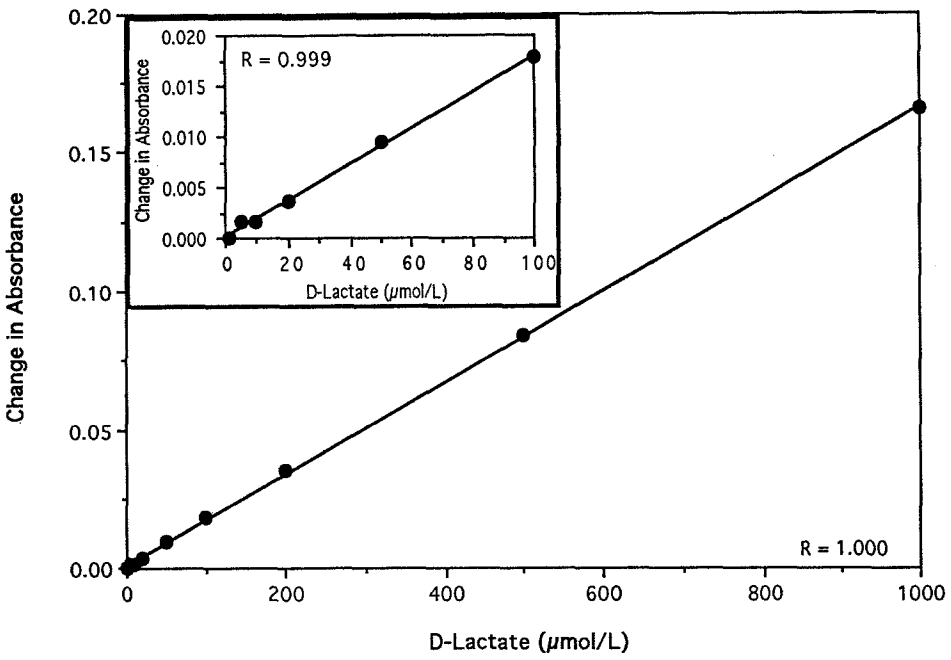

Fig 1. Linearity of a modified kinetic assay for D-lactate, using an aqueous standard solution. Each point represents the mean of duplicate experiments.

ered significant when P was less than .05. Data are expressed as the mean ± SEM.

RESULTS

Mean serum glucose levels were elevated in cats with diabetes, regardless of the presence of ketoacidosis (Table 1). Mean β-hydroxybutyrate concentrations were significantly higher in ketoacidotic cats. The β-hydroxybutyrate concentration in the nondiabetic ketoacidotic cat was 6.9 mmol/L, with a normal blood glucose value of 5.1 mmol/L (92.6 mg/dL). Although L-lactate levels and lactate to pyruvate ratios were increased in cats with ketoacidosis, considerable variation was observed among cats and differences were not significant (Table 1).

Cats with ketoacidosis (n = 9) had significantly (P = .0051) greater serum D-lactate levels (337.2 ± 70.2 μmol/L) than nonketoacidotic diabetic cats (140.3 ± 58.8) and healthy control cats (25.0 ± 6.5) (Fig 2). Two of 22 nonketoacidotic cats also had high D-lactate concentrations, which were greater than those of ketoacidotic cats (Fig 2). In the absence of these outliers, nonketoacidotic diabetic cats had a mean D-lactate value of 56.8 ± 12.9 μmol/L, which did not differ significantly from values in control cats. Interestingly, both nonketoacidotic cats with elevated serum D-lactate values showed signs of posterior paresis and generalized limb weakness; however, the relationship of neurologic to metabolic abnormalities was not ascertained.

D-Lactate values were significantly correlated with plasma β-hydroxybutyrate concentrations (r = .457, P = .0165). With the exclusion of the outliers noted before, the correlation was notably stronger (r = .684, P = .0001; Fig 3). One ketoacidotic cat with trace ketonuria had only a mild increase in plasma β-hydroxybutyrate concentration, despite a greatly increased D-lactate level (Fig 3). There was no correlation between D-lactate and serum glucose concentrations in diabetic cats (r = .078, P = .6825). In vitro

Table 1. Concentrations of Metabolic Intermediates (mean ± SEM) in Control, Nonketoacidotic Diabetic, and Ketoacidotic Diabetic Cats

	Control (n = 14)	Nonketoacidotic (n = 22)	Ketoacidotic (n = 8)
Glucose (mmol/L)	5.2 ± 0.2*	18.5 ± 2.5	23.6 ± 4.7
β-Hydroxybutyrate (mmol/L)	0.03 ± 0.01	0.68 ± 0.16	6.14 ± 1.76*
Pyruvate (mmol/L)	0.08 ± 0.02	0.12 ± 0.04	0.09 ± 0.05
L-Lactate (mmol/L)	1.67 ± 0.33	1.25 ± 0.22	2.15 ± 0.90
Lactate to pyruvate ratio	29.0 ± 7.2	35.7 ± 11.5	103.7 ± 89.1

*P < .001.

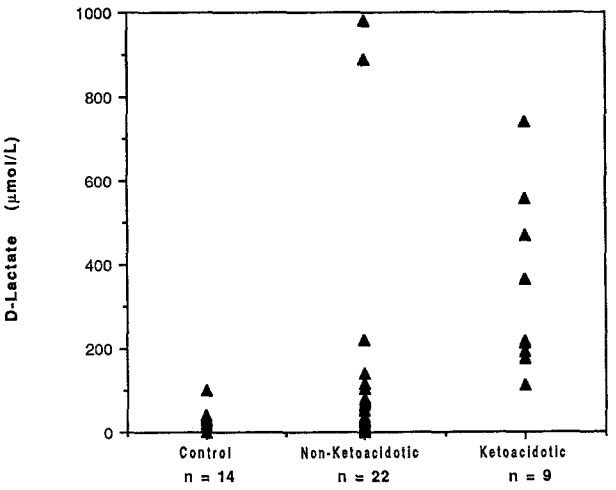

Fig 2. Serum D-lactate concentrations in healthy control cats, diabetic cats without ketoacidosis, and diabetic cats with ketoacidosis. Each triangle indicates an individual animal.

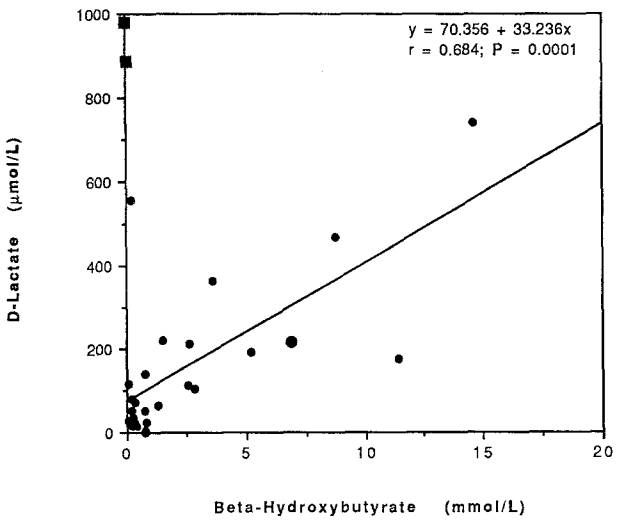

Fig 3. Positive linear correlation between serum D-lactate and β-hydroxybutyrate concentrations in 30 diabetic cats (●) and one ketoacidotic nondiabetic cat (●). Values from two nonketoacidotic cats with high D-lactate levels (■) were considered outliers.

D-lactate production by feline RBC was linear over time and did not differ significantly under normoglycemic and hyperglycemic conditions (Fig 4).

DISCUSSION

Increased D-lactate generation in diabetes mellitus has been associated with enhanced metabolic flux through the glyoxalase pathway as a result of increased methylglyoxal formation from hyperglycemia.[3,4,9] As the end product of this pathway, D-lactate serves as a marker of methylglyoxal

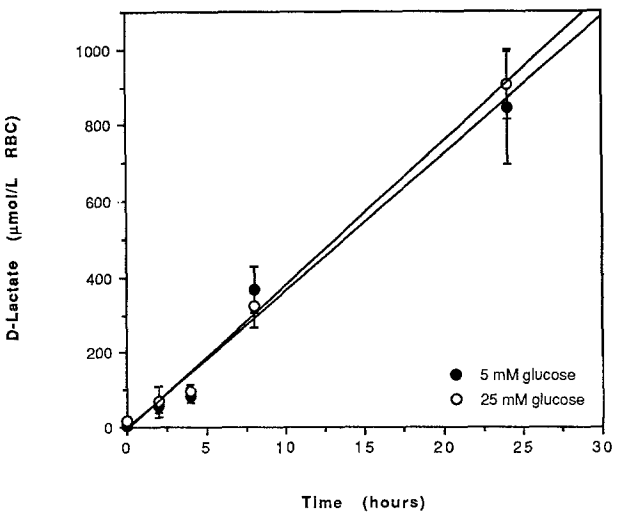

Fig 4. In vitro D-lactate formation by feline RBC incubated under normoglycemic (●, 5 mmol/L glucose) or hyperglycemic (○, 25 mmol/L glucose) conditions. Each point represents the mean ±SEM of duplicate samples from three different normal cats.

production, the latter of which may be of pathologic significance in the development of diabetic complications.[1,9] Hyperglycemia-induced methylglyoxal formation occurs largely in tissues with insulin-independent glucose uptake, ie, RBC, lens, and renal tissue, although increased D-lactate has also been detected in the liver and skeletal muscle of diabetic rats.[3,4,9]

In the present study, the rate of D-lactate formation by RBC in vitro was unaffected by external glucose concentration, such that RBC circulating in hyperglycemic diabetic cats do not appear to contribute to increased serum D-lactate, as has been suggested for other species.[3,4,9] This may be due to the fact that feline RBC are less permeable to external glucose than RBC of other species.[10] These data are supported by the lack of correlation between blood glucose and D-lactate values in diabetic cats in vivo. Although RBC have been considered the major source of blood D-lactate in diabetes, other tissues with insulin-independent glucose uptake such as lens and kidney may also contribute. Indeed, the approximately twofold increase in serum D-lactate in nonketoacidotic diabetic cats as compared with control cats was similar to the 1.7- to 2.3-fold increases described in diabetic rats.[4,9] A lack of increased serum D-lactate generation by feline RBC under hyperglycemic conditions may fortuitously increase the sensitivity of the feline model for identifying other sources of D-lactate.

Ketoacidotic cats had markedly increased serum D-lactate concentrations, which strongly correlated with the degree of ketoacidosis. This association suggests that the mild increase in D-lactate in most nonketoacidotic diabetic cats may have been due to the mild ketosis also present in these cats as compared with control cats. The relation between ketones and D-lactate was further supported by values from a nondiabetic ketoacidotic cat, which were similar to values from cats with diabetic ketoacidosis. Although ketoacidotic cats had slightly higher blood glucose values than nonketoacidotic cats, this difference was not significant, nor was there a correlation between glucose and D-lactate values. These findings indicate that in ketoacidosis, methylglyoxal formation from ketone metabolism may be more significant than that from hyperglycemia. Methylglyoxal is formed from the metabolism of acetone by hepatic cytochrome P-450 2E1. Induction of cytochrome P-450 2E1 in ketosis, diabetes, and starvation has been well described, and D-lactate production from acetone has been demonstrated in vitro.[5,6] Further studies to assess directly the contribution of hepatic ketone metabolism to serum D-lactate are currently under way.

Unusually elevated D-lactate values in two nonketoacidotic cats suggest a different mechanism of D-lactate accumulation in these animals, such as dietary factors or intestinal microbial overgrowth.[7,11] Neurologic signs have previously been observed in people and cats with D-lactic acidosis; however, they generally occur at D-lactate concentrations of 7 to 8 mmol/L, considerably higher than levels observed in these cats.[2,7,8]

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