Acid-base and hormonal abnormalities in dogs with naturally occurring diabetes mellitus

Lawren L. Durocher, DVM, MS, DACVIM; Kenneth W. Hinchcliff, BVSc, PhD, DACVIM; Stephen P. DiBartola, DVM, DACVIM; Susan E. Johnson, DVM, MS, DACVIM

Objective—To examine acid-base and hormonal abnormalities in dogs with diabetes mellitus.

Design—Cross-sectional study.

Animals—48 dogs with diabetes mellitus and 17 healthy dogs.

Procedures—Blood was collected and serum ketone, glucose, lactate, electrolytes, insulin, glucagon, cortisol, epinephrine, norepinephrine, nonesterified fatty acid, and triglyceride concentrations were measured. Indicators of acid-base status were calculated and compared between groups.

Results—Serum ketone and glucose concentrations were significantly higher in diabetic than in healthy dogs, but there was no difference in venous blood pH or base excess between groups. Anion gap and strong ion difference were significantly higher and strong ion gap and serum bicarbonate concentration were significantly lower in the diabetic dogs. There were significant linear relationships between measures of acid-base status and serum ketone concentration, but not between measures of acid-base status and serum lactate concentration. Serum insulin concentration did not differ significantly between groups, but diabetic dogs had a wider range of values. All diabetic dogs with a serum ketone concentration >1,000 µmol/L had a serum insulin concentration <5 µU/mL. There were strong relationships between serum ketone concentration and serum glucagon-insulin ratio, serum cortisol concentration, and plasma norepinephrine concentration. Serum β-hydroxybutyrate concentration, expressed as a percentage of serum ketone concentration, decreased as serum ketone concentration increased.

Conclusions and Clinical Relevance—Results suggested that ketosis in diabetic dogs was related to the glucagon-insulin ratio with only low concentrations of insulin required to prevent ketosis. Acidosis in ketotic dogs was attributable largely to high serum ketone concentrations. (J Am Vet Med Assoc 2008;232:1310–1320)

In dogs with metabolically unstable diabetes mellitus, production of ketone bodies exceeds utilization of them for energy, resulting in an increase in serum ketone concentration. Most diabetic dogs have serum ketone concentrations that are higher than those in healthy dogs. Although mild ketonemia might not be clinically apparent, the accumulation of ketones in serum is associated with metabolic acidosis that, in its most extreme form, manifests as diabetic ketoacidosis. Organic anions believed to contribute to acidosis in ketotic dogs include ketone and lactate, although the relative contributions of ketone and lactate to acid-base status have not been examined in dogs with spontaneous diabetes mellitus. Ketoacidosis is proposed to result from a relative lack of insulin and an increase in counter-regulatory hormones, such as glucagon, cortisol, epinephrine, and norepinephrine. These changes have been documented in humans with diabetic ketoacidosis, but hormonal abnormalities in dogs with naturally occurring diabetes mellitus and any degree of ketonemia have not been elucidated.

Hormones other than insulin that play an important role in glucose regulation include glucagon, cortisol, epinephrine, and norepinephrine. Glucagon increases glycogenolysis and gluconeogenesis in the liver, but has little or no effect on peripheral tissues such as muscle or fat. Cortisol increases blood glucose concentrations by stimulating synthesis of gluconeogenic enzymes and facilitating mobilization of gluconeogenic precursors. It also promotes ketogenesis by increasing lipolysis, which releases ketogenic substrates from fat. Epinephrine and norepinephrine activate glycogen phosphorylase and glucose-6-phosphatase in the liver and increase gluconeogenesis and peripheral lipolysis, resulting in increases in serum glycerol, NEFA, and ketone concentrations. Although these hormones have been shown to be associated with ketonemia in humans, their relative contributions to ketosis remain to be elucidated.

From the Department of Veterinary Clinical Sciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH 43210. Dr. Durocher’s present address is Carolina Veterinary Specialists, 1600 Hanes Mall Blvd, Winston Salem, NC 27103. Dr. Hinchcliff’s present address is Veterinary Clinical Centre, Faculty of Veterinary Science, University of Melbourne, Princes Highway, Werribee, Victoria, Australia, 3030. Supported by a grant from the Iams Company. Presented in part at American College of Veterinary Internal Medicine Annual Forum, Seattle, June 2007. Address correspondence to Dr. Hinchcliff.

Abbreviation

| NEFA | Nonesterified fatty acid |

Unauthenticated | Downloaded 08/22/23 05:26 AM UTC
and dogs with experimentally induced diabetes mellitus, their role in dogs with naturally occurring diabetes mellitus is unknown.

Nonesterified fatty acid is used for production of ketones in the liver. Thus, serum NEFA concentration is an important indicator of metabolic status in dogs with diabetes mellitus, but the relationship between increases in serum NEFA concentration and severity of ketonemia in dogs with naturally occurring diabetes mellitus is unclear.

The purposes of the study reported here were to document acid-base abnormalities in dogs with naturally occurring diabetes mellitus, to determine the relative contributions of serum lactate and ketone concentrations to acidosis in dogs with hyperketonemia, and to compare serum ketone and hormone concentrations in diabetic dogs with concentrations in dogs without diabetes mellitus to identify the potential role of these hormones in the development of ketonemia. We hypothesized that diabetic dogs would have lower concentrations of insulin and higher concentrations of glucagon, cortisol, epinephrine, and norepinephrine than would healthy dogs and that in diabetic dogs, the degree of ketonemia would correlate with serum insulin, glucagon, cortisol, epinephrine, norepinephrine, glucose, and NEFA concentrations.

Materials and Methods

Study design—The study was a prospective, observational study involving a convenience sample of diabetic and healthy dogs examined at The Ohio State University between July 2005 and July 2006. The study design was approved by the Hospital Executive Committee of The Ohio State University College of Veterinary Medicine. Owners provided informed consent prior to enrollment of dogs in the study.

Dogs—Forty-eight dogs with diabetes mellitus and 17 healthy dogs were included in the study. Only those dogs that were ≥ 1 year old and weighed ≥ 8 kg (17.6 lb) were eligible for inclusion in the study. For dogs with diabetes mellitus, the diagnosis had been based on the basis of compatible clinical signs and laboratory findings, including polyuria, polydipsia, polyphagia, glucosuria, and hyperglycemia. Diabetic dogs must have had a blood glucose concentration ≥ 200 mg/dL at the time of examination at The Ohio State University to be eligible for inclusion in the study. Thirty-seven of the 48 dogs with diabetes mellitus were being treated with insulin at the time of the study. Diabetic dogs were classified by the attending clinician at the time of examination for inclusion in the study as stable and clinically normal or as unstable with clinical abnormalities such as vomiting, lethargy, dehydration, diarrhea, or diabetic ketoacidosis. Dogs with stable diabetes mellitus had not received insulin for at least 12 hours prior to collection of blood samples, and dogs with unstable diabetes mellitus had not received insulin for at least 6 hours prior to collection of blood samples. Diabetic ketoacidosis was defined as pH < 7.3, bicarbonate concentration < 15 mEq/L, ketonemia (serum ketone concentration > 200 µmol/L), and physical illness. Six dogs were determined to be diabetic ketoacidotic, whereas 5 dogs were determined to be ketotic but not acidic on the basis of this definition. Owners of diabetic dogs enrolled in the study were asked to complete a questionnaire about insulin dose, time of insulin administration, diet, and clinical signs, specifically the occurrence of seizures, polyuria, polydipsia, lethargy, and polyphagia.

Healthy dogs did not have any clinical signs compatible with diabetes mellitus and were considered healthy on the basis of history and results of a physical examination. All healthy dogs were owned by hospital staff members and clinicians. None had been identified as having any endocrine disorders.

Blood sample collection—A single jugular venous blood sample (1.2 mL) was collected from each dog enrolled in the study. A single drop of blood was immediately used to measure blood glucose concentration with a cage-side glucometer. Within 60 seconds after collection of each blood sample, 0.5 mL was transferred to a 3-mL heparinized syringe for blood gas analysis, and 3.5 mL was placed in a cold, 7-mL tube containing potassium EDTA and 25 µL of 10% sodium metabisulfite for determination of plasma epinephrine and norepinephrine concentrations, and the remainder was placed in a sterile evacuated glass tube that did not contain anticoagulant. Within 15 minutes after blood sample collection, the portions placed in tubes containing EDTA and in plain evacuated tubes were centrifuged in a refrigerated centrifuge at 1,300 X g for 10 minutes, and plasma and serum were obtained. Aliquots (500 µL) of plasma and serum were stored in plastic storage vials at –70°C until analyzed.

Sample analysis—Blood gas analyses were performed with a point-of-care blood gas analyzer, and serum biochemical profiles, including measurement of serum glucose, electrolyte, and triglyceride concentrations, were performed with a commercial analyzer. Serum total ketone concentrations were determined with a commercial kit, and β-hydroxybutyrate concentrations were determined with a microwell kit. Serum β-hydroxybutyrate concentration was expressed as a percentage of total ketone concentration by dividing the β-hydroxybutyrate concentration by the total ketone concentration and multiplying by 100. Serum NEFA concentrations were measured with a microtitration kit.

Serum lactate concentrations were determined by means of a spectrophotometric method. All samples were assayed in triplicate and incubated with reagent for 9 minutes at 37°C; optical density was measured at 540 nm. Sample concentrations were determined by comparison with a standard curve obtained by analyzing serial dilutions of a standard lactate solution.

Serum glucagon concentrations were measured with a commercial radioimmunoassay validated for use in dogs. The limit of sensitivity of the assay was 20 pg/mL, and the limit of linearity was 400 pg/mL. Serum insulin concentrations were also measured with a commercial radioimmunoassay. The limit of sensitivity was 4.6 µU/mL, and the limit of linearity was 315 µU/mL. Serum cortisol concentrations were measured with a commercial analyzer.

Plasma epinephrine and norepinephrine concentrations were determined by means of high-performance liquid chromatography with electrochemical detection.
For norepinephrine concentration, the intra-assay coefficient of variation was 3% and the interassay coefficient of variation was 6%. For epinephrine concentration, the intra-assay coefficient of variation was 3% and the interassay coefficient of variation was 6%. Sensitivity was 15 pg/mL for norepinephrine concentration and 6 pg/mL for epinephrine concentration.

Anion gap was calculated as the sum of serum sodium and potassium concentrations minus the sum of serum chloride and bicarbonate concentrations. The strong ion difference was calculated as the sum of serum sodium and potassium concentrations minus the sum of serum chloride and lactate concentrations. The strong ion gap was calculated by means of the following equation: strong ion gap = (albumin concentration [g/L] × (0.348 + 0.469/[1+10^(-7.77-pH)])) – anion gap (mEq/L).

**Statistical analysis**—Data were summarized as mean and SD or as median and range. The Kolmogorov-Smirnov test, examination of normality plots, and examination of residuals during regression analysis were used to determine whether data were normally distributed. For data that were normally distributed, the Student t test was used to compare values between diabetic and healthy dogs. The Mann-Whitney test was used for data that were not normally distributed.

For the diabetic dogs, linear regression was used to test for linear relationships between serum ketone concentration and acid-base variables, hormone concentrations (ie, serum insulin and glucagon concentrations and plasma epinephrine and norepinephrine concentrations), serum energy substrate concentrations (glucose and NEFA), and serum electrolyte concentrations. Serum glucose concentrations, instead of blood glucose concentrations, were used for all analyses, to be consistent. For all variables except serum insulin concentration, bivariable plots and plots of the residuals were visually examined.

Forward stepwise regression was used to test for associations between serum ketone concentration and serum cortisol, plasma epinephrine, and plasma

<table>
<thead>
<tr>
<th>Variable</th>
<th>Healthy dogs</th>
<th>Diabetic dogs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum ketone (µmol/L)</td>
<td>18 (7, 27)</td>
<td>162 (12, 23,800)*</td>
</tr>
<tr>
<td>Serum β-hydroxybutyrate (µmol/L)</td>
<td>12 (1,161)</td>
<td>133 (15, 3,385)*</td>
</tr>
<tr>
<td>Blood glucose (mg/dL)</td>
<td>101 (76, 108)</td>
<td>343 (261, 727)*</td>
</tr>
<tr>
<td>Serum insulin (µU/mL)</td>
<td>3.3 (1.1, 18)</td>
<td>3.9 (0.28, 146)</td>
</tr>
<tr>
<td>Serum glucagon (pg/mL)</td>
<td>39 (25, 55)</td>
<td>69 (19, 414)*</td>
</tr>
<tr>
<td>Glucagon-insulin ratio</td>
<td>0.27 (0.04, 0.82)</td>
<td>0.38 (0.02, 0.26)</td>
</tr>
<tr>
<td>Serum cortisol (µg/dL)</td>
<td>2.0 (0.5, 3.9)</td>
<td>2.9 (0.5, 33.2)</td>
</tr>
<tr>
<td>Plasma epinephrine (pg/mL)</td>
<td>154 (48, 672)</td>
<td>106 (7, 718)</td>
</tr>
<tr>
<td>Plasma norepinephrine (pg/mL)</td>
<td>318 (161, 738)</td>
<td>453 (97, 1,211)</td>
</tr>
<tr>
<td>Serum NEFA (mEq/L)</td>
<td>0.37 (0.16, 0.99)</td>
<td>1.45 (0.15, 3.7)*</td>
</tr>
<tr>
<td>Serum triglyceride (mg/dL)</td>
<td>62 (31, 131)</td>
<td>148 (41, 2,697)*</td>
</tr>
<tr>
<td>Serum cholesterol (mg/dL)</td>
<td>223 (162, 379)</td>
<td>389 (160, 2,067)*</td>
</tr>
<tr>
<td>Venous blood pH</td>
<td>7.37 (7.29, 7.44)</td>
<td>7.38 (7.15, 7.46)</td>
</tr>
<tr>
<td>Base excess (mEq/L)</td>
<td>–1.7 (–5.5, 2.9)</td>
<td>–2.65 (–19.8, 3)</td>
</tr>
<tr>
<td>Serum bicarbonate (mEq/L)</td>
<td>23.0 (19.9, 28.2)</td>
<td>22.5 (8, 27.9)*</td>
</tr>
<tr>
<td>Anion gap (mEq/L)</td>
<td>21 (19, 23)</td>
<td>23 (16, 48)*</td>
</tr>
<tr>
<td>Strong ion difference (mEq/L)</td>
<td>42 (39, 46)</td>
<td>45 (38, 61)*</td>
</tr>
<tr>
<td>Strong ion gap (mEq/L)</td>
<td>–3 (–5, –1)</td>
<td>–5.7 (–38, 1.4)*</td>
</tr>
<tr>
<td>PVCO2 (mm Hg)</td>
<td>40.4 (35.2, 54.3)</td>
<td>37.9 (20, 52.3)*</td>
</tr>
<tr>
<td>Serum sodium (mEq/L)</td>
<td>151 (148, 157)</td>
<td>144 (125, 154)*</td>
</tr>
<tr>
<td>Serum potassium (mEq/L)</td>
<td>4.2 (3.8, 4.8)</td>
<td>4.6 (2.5, 6.2)*</td>
</tr>
<tr>
<td>Serum phosphorus (mg/dL)</td>
<td>4.1 (2.9, 4.8)</td>
<td>4.5 (1.5, 10)</td>
</tr>
<tr>
<td>Serum chloride (mEq/L)</td>
<td>119 (111, 120)</td>
<td>108 (74, 119)*</td>
</tr>
<tr>
<td>Serum albumin (g/dL)</td>
<td>3.7 (3.4, 4)</td>
<td>3.7 (1.5, 4.7)</td>
</tr>
<tr>
<td>Serum calcium (mEq/L)</td>
<td>1.5 (1.4, 1.6)</td>
<td>1.4 (1.1, 1.8)*</td>
</tr>
<tr>
<td>Serum creatinine (mg/dL)</td>
<td>1.3 (1, 1.8)</td>
<td>1.1 (0.4, 5.4)*</td>
</tr>
<tr>
<td>SUN (mg/dL)</td>
<td>16 (10, 26)</td>
<td>16 (8, 58)</td>
</tr>
<tr>
<td>Serum lactate (mmol/L)</td>
<td>1.0 (0.6, 1.3)</td>
<td>2.0 (0.5, 5.8)</td>
</tr>
</tbody>
</table>

Data are given as median [minimum, maximum].

*Values were significantly (P < 0.05) different between groups. †One outlier value was removed. PVCO2 = Venous partial pressure of CO2.

Figure 1—Scatterplot of serum ketone versus serum lactate concentrations in 48 dogs with diabetes mellitus. There was not a significant linear relationship between serum ketone and serum lactate concentrations (adjusted R² = 0; P = 0.826).
epinephrine concentrations and between serum ketone concentration and serum NEFA and triglyceride concentrations. Because of the small number of dogs enrolled in the study, regression analyses were limited to biologically important variables, with no more than 2 variables included in any model.

The glucagon-insulin ratio was calculated as serum glucagon concentration (pg/mL) divided by

Figure 2—Scatterplots of serum ketone concentration versus venous blood pH (A), anion gap (B), serum bicarbonate (HCO$_3^-$) concentration (C), strong ion difference (SID; D), base excess in extracellular fluid (BEECF; E), and strong ion gap (SIG; F) in 48 dogs with diabetes mellitus. There were significant linear relationships between serum ketone concentration and venous blood pH ($\text{pH} = 7.38 - [0.000006 \times \text{ketone concentration}]$; adjusted $R^2 = 0.35; P < 0.001$), anion gap ($\text{anion gap} = 22 + [0.001 \times \text{ketone concentration}]$; adjusted $R^2 = 0.73; P < 0.001$), bicarbonate concentration ($\text{bicarbonate concentration} = 23 - [0.0006 \times \text{ketone concentration}]$; adjusted $R^2 = 0.65; P < 0.001$), SID ($\text{SID} = 40 + [0.0005 \times \text{ketone concentration}]$; adjusted $R^2 = 0.48; P < 0.001$), BE ($\text{BE} = -2.2 - [0.0007 \times \text{ketone concentration}]$; adjusted $R^2 = 0.68; P < 0.001$), and SIG ($\text{SIG} = -4.6 - [0.001 \times \text{ketone concentration}]$; adjusted $R^2 = 0.78; P < 0.001$).
serum insulin concentration, which was converted from µU/mL to pg/mL. The glucose-insulin ratio was calculated as serum glucose concentration (mg/dL) divided by serum insulin concentration, converted from µU/mL to µg/mL by use of appropriate conversion factors.

All analyses were performed with standard software. Values of *P* < 0.05 were considered significant.

**Results**

**Dogs**—Body weight of the 48 dogs with diabetes mellitus (mean ± SD, 25 ± 14.4 kg [55 ± 31.7 lb]) was not significantly (*P* = 0.2) different from body weight of the 17 healthy dogs (30 ± 9.5 kg [66 ± 20.9 lb]). However, dogs with diabetes mellitus (8.3 ± 2.7 years) were significantly (*P* = 0.03) older than the healthy dogs (6.7 ± 2.5 years). The 2 groups did not differ significantly with regard to sex distribution.

**Acid-base status**—Serum ketone, lactate, β-hydroxybutyrate, and glucose concentrations; anion gap; and strong ion difference were significantly higher in the diabetic dogs than in the healthy dogs (Table 1), and serum bicarbonate concentration and strong ion gap were significantly lower in the diabetic dogs than in the healthy dogs. In addition, serum sodium, chloride, creatinine, and calcium concentrations and venous partial pressure of CO₂ were significantly lower in diabetic dogs than in healthy dogs, whereas serum potassium, triglyceride, NEFA, and cholesterol concentrations were significantly higher in diabetic dogs than in healthy dogs. Venous blood pH, base excess; and serum phosphorus, albumin, and urea nitrogen concentrations were not significantly different between groups.

![Figure 3](image3.png) **Figure 3**—Scatterplots of serum lactate concentration versus venous blood pH (A) and anion gap (B) in 48 dogs with diabetes mellitus. There was no significant linear relationship between serum lactate concentration and venous blood pH (adjusted *R*² = 0.043; *P* = 0.089) or between serum lactate concentration and anion gap (adjusted *R*² = 0.004; *P* = 0.28).

![Figure 4](image4.png) **Figure 4**—Scatterplots of serum ketone concentration versus serum sodium (A), potassium (B), and chloride (C) concentrations in 48 dogs with diabetes mellitus. There were significant linear relationships between serum ketone concentration and serum sodium concentration (sodium concentration = 149 – (0.00046 × ketone concentration) – (0.0099 × glucose concentration); adjusted *R*² = 0.337; for ketone concentration, *P* < 0.001; for glucose concentration, *P* = 0.003; for sodium concentration, *P* = 0.14), serum potassium concentration (potassium concentration = 3.5 – (0.00004 × ketone concentration) + (0.0026 × glucose concentration); adjusted *R*² = 0.25; for ketone concentration, *P* = 0.009; for glucose concentration, *P* = 0.019), and serum chloride concentration (chloride concentration = 115 – (0.0009 × ketone concentration) – (0.02 × glucose concentration); adjusted *R*² = 0.61; for ketone concentration, *P* < 0.001; for glucose concentration, *P* = 0.019).
We did not detect a significant linear relationship between serum ketone concentration and serum lactate concentration in the diabetic dogs (Figure 1). However, there were significant linear relationships between serum ketone concentration and venous blood pH, anion gap, serum bicarbonate concentration, strong ion difference, base excess, and strong ion gap (Figure 2). Serum lactate concentration in the diabetic dogs was not significantly associated with any of the acid-base variables that were examined, including venous blood pH and anion gap (Figure 3). Serum creatinine concentration in the diabetic dogs was not significantly associated with venous blood pH (adjusted $R^2 = 0.00$; $P = 0.69$), serum bicarbonate concentration (adjusted $R^2 = 0.00$; $P = 0.62$), base excess (adjusted $R^2 = 0.00$; $P = 0.74$), anion gap (adjusted $R^2 = 0.00$; $P = 0.38$), strong ion difference (adjusted $R^2 = 0.03$; $P = 0.068$), or strong ion gap (adjusted $R^2 = 0.00$; $P = 0.47$).

In the diabetic dogs, serum sodium, potassium, and chloride concentrations had significant linear associations with serum ketone concentration (Figure 4). Hormone concentrations—Serum glucagon concentration was significantly higher in the diabetic dogs than in the healthy dogs (Table 1); however, serum insulin and cortisol concentrations and plasma epinephrine and norepinephrine concentrations were not significantly different between groups.

In the diabetic dogs, serum ketone concentration was significantly associated with serum insulin and glucagon concentrations and with the glucagon-insulin ratio (Figure 5). The relationship between serum ketone concentration and serum insulin concentration was not linear. Rather, examination of a plot of serum insulin concentration versus serum ketone concentration revealed that all dogs with serum ketone concentrations

![Graphs](image1.png)

![Graphs](image2.png)

**Figure 5**—Scatterplots of serum ketone concentrations versus serum insulin concentration (A), serum glucagon concentration (B), and the glucagon-insulin ratio (C) in 48 dogs with diabetes mellitus. There were significant linear relationships between serum ketone concentration and serum insulin concentration ($\text{ketone concentration} = -2.013 + [46 \times \text{serum insulin concentration}]$; adjusted $R^2 = 0.45$; $P < 0.001$) and between serum ketone concentration and the glucagon-insulin ratio ($\text{ketone concentration} = 311 + [883 \times \text{glucagon-insulin ratio}]$; adjusted $R^2 = 0.76$; $P < 0.001$).

**Figure 6**—Scatterplots of serum ketone concentration versus blood glucose concentration (A) and the glucose-insulin ratio (B) in 48 dogs with diabetes mellitus. There were significant linear relationships between serum ketone concentration and blood glucose concentration ($\text{ketone concentration} = -5,613 + [19 \times \text{blood glucose concentration}]$; adjusted $R^2 = 0.12$; $P = 0.008$) and between serum ketone concentration and the glucose-insulin ratio ($\text{ketone concentration} = -767 + [0.05 \times \text{glucose-insulin ratio}]$; adjusted $R^2 = 0.79$; $P < 0.001$).
In addition, there were significant linear relationships between serum ketone concentration and plasma norepinephrine concentration and serum cortisol concentration but not between serum ketone concentration and plasma epinephrine concentration (Figure 7). Serum glucose concentration was significantly associated with serum cortisol concentration (glucose concentration = 420 + [6 × cortisol concentration]; adjusted $R^2 = 0.10; P = 0.018$) and plasma norepinephrine concentration (glucose concentration = 360 + [0.2 × norepinephrine concentration]; adjusted $R^2 = 0.15; P = 0.003$) in the diabetic dogs, but not with plasma epinephrine concentration (glucose concentration = 449 + [0.016 × epinephrine concentration]; adjusted $R^2 = 0; P = 0.9$).

Finally, there were significant linear relationships between serum ketone concentration and serum NEFA concentration (adjusted $R^2 = 0.38; P < 0.001$) and between serum ketone concentration and serum triglyceride concentration (adjusted $R^2 = 0.09; P = 0.02$; values for a Miniature Schnauzer with dyslipidemia and diabetes mellitus were excluded).

Examination of a scatterplot of serum ketone concentration versus serum β-hydroxybutyrate concentration as a percentage of serum ketone concentration (Figure 8) revealed that in diabetic dogs with low serum ketone concentrations, serum β-hydroxybutyrate concentration was approximately 60% of the serum ketone concentration, but that in diabetic dogs with high serum ketone concentrations, serum β-hydroxybutyrate concentration was approximately 20% of the serum ketone concentration. Several values were greater than 100% owing to assay variability at low total ketone concentrations.

**Discussion**

Results of the present study suggested that in dogs with naturally occurring diabetes mellitus, serum lactate concentration was not significantly associated with serum ketone concentration, such that dogs with the highest serum ketone concentrations did not reliably have abnormally high serum lactate concentrations. In contrast, serum ketone concentration was highly dependent on serum insulin concentration, in that serum ketone concentrations > 1,000 μmol/L were identified.
only in dogs with serum insulin concentrations < 5 
µU/mL. Moreover, all dogs with serum ketone concen-
trations > 5,000 µmol/L had serum glucagon concen-
trations > 55 pg/mL, indicating that hypoinsulinemia 
was a necessary, but not sufficient, condition for hyper-
ketonemia. That there was only a weak linear associa-
tion between serum ketone concentration and serum 
glucose concentration suggested that insulin had 
a greater effect on ketone metabolism than on blood 
glucose concentration in dogs with naturally occurring 
diabetes mellitus. Furthermore, serum ketone concen-
tration was significantly associated with serum cortisol, 
norepinephrine, NEFA, and triglyceride concen-
trations, suggesting that these substances play a role in the 
pathophysiology of diabetes mellitus in dogs. Finally, 
serum β-hydroxybutyrate concentration, expressed as a 
percentage of serum ketone concentration, decreased as 
serum ketone concentration increased, a phenomenon 
not previously described in dogs.

Serum ketone concentration was significantly high-
er in the diabetic dogs than in the healthy dogs in the 
present study. However, there was some overlap in se-
rum ketone concentration between the 2 groups. Dogs 
in which diabetic ketoacidosis was diagnosed had the 
highest serum ketone concentrations; however, some 
dogs with similarly high serum ketone concentrations 
did not have markedly low venous blood pH values. 
Possible reasons for the disparity in venous blood pH 
values among dogs with high serum ketone concen-
trations include differences in respiratory compensation, 
differences in the use of serum ketones, differences in 
serum chloride concentration, and differences in the 
concentrations of other acids that contribute to acido-
sis. However, concentrations of other measured anions, 
such as lactate and creatinine, were not strongly asso-
ciated with pH or other acid-base parameters, making 
accumulation of other acids an unlikely cause of the 
differences in pH among dogs with high serum ketone 
concentrations.

Although values for venous blood pH and base ex-
cess did not differ significantly between healthy dogs 
and dogs with diabetes mellitus in the present study, 
other indicators of acid-base status, including anion gap 
and strong ion gap, did differ between groups, suggest-
ing that dogs with diabetes mellitus were more likely to 
have metabolic acidosis. Thus, our findings suggested 
that even those diabetic dogs classified as being meta-
bolically stable may have metabolic acidosis, although 
this might not be evident if only venous blood pH is 
measured. Strong ion difference was also significantly 
higher in diabetic than in healthy dogs. When serum 
chloride concentration decreases, the strong ion differ-
ce increases. Thus, a high strong ion difference typi-
cally reflects hypochloremic alkalosis. A combination 
of high anion gap and high strong ion difference would 
be suggestive of hypochloremic metabolic alkalosis su-
perimposed on unmeasured anion acidosis. The lack of 
difference in pH between groups in the present study 
exclude the evidence of metabolic acidosis in the dia-
betic dogs was attributable to the lower venous partial 
pressure of CO₂ in the diabetic dogs, which was consid-
ered evidence of respiratory compensation. The lack of 
difference in pH may also have reflected the fact that in 
the diabetic dogs, higher serum ketone concentrations 
were offset by lower serum chloride concentrations, 
such that pH was not changed. Although venous blood 
P H did not differ significantly between groups, there 
were significant linear associations between venous 
blood pH and serum ketone and lactate concentrations 
in the dogs with diabetes mellitus.

Dehydration and intrinsic renal failure can alter 
serum concentrations of organic anions, resulting in 
acidosis. Creatinine is typically present at such a low 
concentration in serum that it is unlikely to have any 
effect on acid-base status. In the present study, however, 
creatinine was used as a marker for unmeasured uremic 
anions, in that if serum creatinine concentration is not 
increased, it is unlikely that concentrations of other ure-
mic anions will be increased. There was no association 
between serum creatinine concentration and variables 
indicative of metabolic acidosis in the present study, sug-
uggesting that accumulation of organic anions secondary to 
diminished renal function was not a factor in the acidosis 
observed in dogs with diabetes mellitus.

Serum lactate concentration was significantly high-
er in the diabetic than in the healthy dogs in the present 
study. The high serum lactate concentration in the dia-
betic dogs could have been due to dehydration and de-
creased tissue perfusion or to decreased disposal of lac-
tate. Serum lactate concentration was not significantly 
associated with venous blood pH or any other indicator 
of acid-base status in dogs in the present study. This indi-
cated that although serum lactate concentration was 
high in the diabetic dogs, acidosis in these dogs was 
primarily a consequence of high serum ketone concen-
tration, not serum lactate concentration.

Serum ketone and glucose concentrations were 
significantly associated with serum sodium, potas-
sium, and chloride concentrations in diabetic dogs in 
the present study. Because strong ion difference, strong 
ion gap, and anion gap all are calculated on the basis 
of concentrations of these electrolytes, the effect of ke-
tones on acid-base status could be due to the effects of 
ketones on concentrations of these electrolytes. Serum 
ketones and glucose exert an osmotic effect, increas-
ing water in the vascular space, diluting plasma, and 
decreasing concentrations of these electrolytes. Lactate 
does not substantially influence osmolality because of 
its relatively low concentration and would not be ex-
pected to alter the concentrations of these electrolytes.

The finding that all diabetic dogs in the present 
study with serum ketone concentrations > 1,000 µmol/L 
had serum insulin concentrations < 5 µU/mL whereas 
none of the dogs with serum insulin concentrations > 20 
µU/mL had serum ketone concentrations > 200 µmol/L 
suggested that only small amounts of insulin were need-
ed to control ketosis in dogs and supported the practice 
of administering insulin to dogs with clinical ketosis or 
ketoacidosis. However, serum glucose concentration was 
only weakly associated with serum ketone concentration, 
providing an explanation for the clinical observation that 
ketonemia and ketoacidosis can be controlled without 
necessarily regulating serum glucose concentration. The 
concentration of insulin in serum, and not that of glu-
cose, appears to be important in determining a dog’s sus-
ceptibility to clinical ketonemia.
Glucagon is an important counter-regulatory hormone, and serum glucagon concentration is frequently high in humans with diabetic ketosis.\(^1\) Glucagon has important effects on glucose utilization, and unchecked glucagon activity in patients with diabetes mellitus is believed to be important in the pathophysiology of the disease.\(^3,17,18\) Consistent with observations in dogs with experimentally induced diabetic mellitus and in humans with naturally occurring diabetes mellitus,\(^19\) serum glucagon concentration was significantly higher in diabetic dogs than in healthy dogs in the present study. Furthermore, serum ketone concentration was significantly associated with both serum glucagon concentration and the glucagon-insulin ratio, suggesting that diabetic dogs developed ketosis not just because of low serum insulin concentration but also because of high serum glucagon concentration.\(^19–21\)

The glucagon-insulin ratio is abnormal in humans with diabetic ketoacidosis and other forms of ketoadisis, including alcohol-induced ketoadisis.\(^2,10,20\) Although the glucagon-insulin ratio was not significantly different between healthy and diabetic dogs in the present study, the range of values for the diabetic dogs was large. In addition, the glucagon-insulin ratio was linearly associated with serum ketone concentration in the diabetic dogs. This relationship implies that it is not just the serum concentration of insulin or glucagon that is important in the development of ketosis, but the relationship between the 2 hormones. An increase in the glucagon-insulin ratio is expected in dogs with diabetes mellitus because insulin concentration typically is low and glucagon concentration typically is high in affected dogs.\(^2,17,22\) This change decreases glucose usage while increasing the release of glucose, through glycolysis and gluconeogenesis, and ketones, through an increase in hepatic ketogenesis.\(^7\) Hyperglucagonemia has been associated with diabetes mellitus in humans\(^3\) and with development of ketosis and hyperglycemia in insulin-deprived humans.\(^1,17\)

Concentrations of cortisol and catecholamines are abnormal in some humans with diabetes mellitus, and although these abnormal concentrations are more likely a consequence rather than a cause of the disease, they might exacerbate ketonemia and hyperglycemia. Serum cortisol and plasma norepinephrine concentrations were significantly associated with serum ketone and glucose concentrations in the present study; whereas plasma epinephrine concentration was not. Norepinephrine increases ketone body production by increasing the rate of formation of NEFA, a substrate needed for ketosis, whereas epinephrine does not affect ketone body production in dogs with experimentally induced diabetes mellitus.\(^23\) However, whether the increases in norepinephrine and cortisol concentrations in these dogs were caused by the diabetes mellitus, were contributing to the disease, or both could not be determined in the present study.

Cortisol increases ketogenesis by increasing lipolysis, but insulin concentration usually is increased at the same time in response to cortisol’s effects on blood glucose concentration in dogs with normal beta-cell function.\(^3\) An increase in serum ketone concentration usually is not clinically apparent in dogs with normal beta-cell function because the fatty acids are diverted to triglyceride synthesis, most likely as a result of the concomitant increase in insulin concentration.\(^3\) This diversion of fatty acids to triglyceride synthesis may explain why hyperketonemia is not often associated with hyperadrenocorticism.\(^24\) However, in the diabetic dogs in the present study, insulin secretion could not increase, with the result that cortisol concentrations were associated with ketone concentrations.

The most potent regulators of ketone production are NEFA availability and the capacity of the liver to form ketones.\(^25\) An indicator of NEFA availability is its serum concentration, and serum NEFA concentration was significantly higher in diabetic dogs than in healthy dogs in the present study. An increase in lipolysis predisposes diabetic dogs to ketone formation, whereas insulin inhibits both lipolysis and NEFA oxidation, thereby decreasing ketone formation. When insulin concentration is decreased, ketone formation is enhanced so that the rate of production exceeds the rate of clearance, resulting in high serum ketone concentrations.

Serum triglyceride concentration was higher in diabetic dogs than in healthy dogs in the present study, which is consistent with findings of previous studies.\(^20,27\) The increase in serum triglyceride concentration in diabetic dogs is related to a decrease in lipoprotein lipase activation secondary to a decrease in insulin secretion.\(^27\) Hypertriglyceridemia also is a risk factor for development of diabetes mellitus and can make regulation of diabetes mellitus difficult because dogs with hypertriglyceridemia are prone to developing pancreatitis and other diseases in addition to diabetes mellitus.

Glucose and ketone concentrations were significantly higher in diabetic dogs than in healthy dogs in the present study. Although serum insulin concentration did not differ significantly between the diabetic and healthy dogs, serum insulin concentration in some of the diabetic dogs was higher than concentration in some of the healthy dogs. Possible explanations for the high serum insulin concentration in some diabetic dogs include administration of exogenous insulin and insulin resistance in the presence of preserved pancreatic beta-cell activity. Most of the diabetic dogs in the present study were being treated with exogenous insulin, and all had insulin-dependent diabetes mellitus. We tried to minimize the influence of exogenous insulin administration by timing the collection of blood samples such that at least 12 hours had passed since insulin administration, but some of the administered insulin could still have been detectable in the blood. However, serum insulin concentration was still higher in some dogs with recently diagnosed diabetes mellitus in which insulin administration had not yet been started than in some healthy dogs. Insulin concentration could have been high because of insulin resistance in these dogs. Importantly, diabetic dogs in the present study that had high serum insulin concentrations also had high serum NEFA concentrations, which is opposite of what one would expect if insulin were active.\(^2\) Insulin resistance is caused by many factors, including infection; estrus; obesity; and other endocrine and metabolic disorders, such as hyperadrenocorticism, acromegaly, and hypothyroidism.\(^28–31\) None of the dogs
in the present study had clinical signs consistent with hyperadrenocorticism, but ACTH stimulation testing and low-dose dexamethasone suppression testing were not performed. In addition, none of the dogs had clinical evidence of infectious diseases, and all of the female dogs in this study were spayed except for one with a high serum insulin concentration. Hypothyroidism could have increased insulin resistance, but tests of thyroid function were not performed. Although growth hormone concentrations were not measured in this study, acromegaly is extremely rare in dogs.

Although we did not perform pancreatic biopsies to differentiate type I from type II diabetes mellitus in the present study, all of the diabetic dogs were dependent on insulin at the time of diagnosis. Some of these dogs may have had some degree of insulin resistance, and all required exogenous insulin administration to regulate blood glucose concentration. The one exception was a sexually intact female in which the diabetes mellitus went into remission after the dog was spayed. It is unclear whether dogs develop insulin-dependent diabetes mellitus after previous non–insulin-dependent diabetes. Indeed, there is almost no empirical evidence that dogs develop diabetes mellitus secondary to insulin resistance, although anecdotal reports suggest that this does occur. Non–insulin-dependent diabetes mellitus in dogs is not recognized clinically, but glucose tolerance tests, which are needed to demonstrate insulin resistance, are rarely performed on dogs and were not performed on dogs in the present study.

In the present study, serum β-hydroxybutyrate concentration, expressed as a percentage of serum ketone concentration, decreased from approximately 60% to 20% as serum ketone concentration increased. This finding is the opposite of what occurs in human patients with diabetes mellitus, who typically have high β-hydroxybutyrate-to-acetoacetate ratios ranging from 3:1 to 20:1.33,34 Lactic acidosis may increase the β-hydroxybutyrate-to-acetoacetate ratio, although it has not been reported that the β-hydroxybutyrate-to-acetoacetate ratio changes in dogs with diabetic ketoacidosis, only that the concentration of β-hydroxybutyrate increases. In a previous study,35 β-hydroxybutyrate concentrations were higher in dogs with diabetic ketoacidosis than in dogs without ketosis and in dogs with ketosis but without acidosis; however, the β-hydroxybutyrate concentration was not reported as a percentage of serum ketone concentration. The range of β-hydroxybutyrate concentrations for all dogs in that study (10 to 2,020 µmol/L) was similar to the range for diabetic dogs in the present study (15 to 3,385 µmol/L). In the present study, β-hydroxybutyrate concentration increased while the β-hydroxybutyrate-to-acetoacetate ratio decreased. These findings indicated that production, metabolism, and excretion of ketone bodies in dogs can change in ways that result in greater increases in acetoacetate than β-hydroxybutyrate concentrations.

In conclusion, results of the present study suggest that dogs with diabetes mellitus, regardless of whether their condition is stable or unstable, have acid-base abnormalities consistent with metabolic acidosis. The acidosis in diabetic dogs is primarily attributable to hyperketonemia and not hyperlactatemia, making control of ketosis important in controlling acidosis.

The treatment of dogs with ketoacidosis consists of rehydration, insulin administration, and correction of serum electrolyte abnormalities. Because the change in acid-base status is due mainly to high serum ketone concentrations and because ketonemia occurs only in dogs with low serum insulin concentrations, the crucial aspect of treatment is insulin administration. Insulin increases utilization of ketone, which will decrease the serum ketone concentration and facilitate correction of acid-base disturbances in diabetic dogs.

References


