The prevalence of hypocobalaminemia in dogs with chronic gastrointestinal disease ranges from 6% to 19%.1,2 Cobalamin deficiency may develop during chronic and severe small intestinal disease as a result of damage of mucosal receptors for the intrinsic factor–cobalamin complex, with subsequent reduced cobalamin absorption and, once cobalamin stores are depleted, resultant cobalamin deficiency.3

Cobalamin deficiency in dogs is traditionally assessed by measurement of the concentration of cobalamin in serum. This method provides only a 1-time assessment of the patient’s current serum cobalamin status, and it cannot be used to evaluate a potential cobalamin deficiency on a cellular level. An established method of evaluating the cellular cobalamin status is measurement of serum MMA concentrations.4,5 Serum concentrations of MMA increase when the tissue lacks adenosyl-cobalamin, a necessary cofactor for the conversion of methylmalonyl-CoA to succinyl-CoA. Without cobalamin, the metabolism of methylmalonyl-CoA is shifted to an alternate pathway, which yields MMA.6 Therefore, increased serum MMA concentrations are considered a marker of cellular cobalamin deficiency.5,7

Serum MMA concentrations may also be increased in patients with renal insufficiency and are correlated

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**Objective**—To determine the prevalence of hypocobalaminemia or methylmalonic acidemia (or both) in dogs with chronic gastrointestinal disease.

**Sample**—Serum samples from 56 dogs with chronic gastrointestinal disease and 43 control dogs.

**Procedures**—Serum cobalamin and methylmalonic acid (MMA) concentrations were measured in all samples and compared between groups. A correlation between serum cobalamin and MMA concentrations and the canine chronic enteropathy clinical activity index was evaluated via the Spearman rank correlation.

**Results**—20 of 56 (36%) dogs with gastrointestinal disease had hypocobalaminemia. Serum cobalamin concentrations were significantly lower in dogs with gastrointestinal disease than in control dogs. Five of 56 (9%) dogs with chronic gastrointestinal disease and 5 of 20 (25%) hypocobalaminemic dogs had increased MMA concentrations. There was a significant negative correlation (Spearman $r = -0.450$) between serum cobalamin and MMA concentrations in dogs with gastrointestinal disease. No correlation was found between the canine chronic enteropathy clinical activity index and serum cobalamin or MMA concentrations.

**Conclusions and Clinical Relevance**—These data indicated the prevalence of hypocobalaminemia in dogs with chronic gastrointestinal disease was 20 of 56 (36%). Five of 20 (25%) hypocobalaminemic dogs had increased serum MMA concentrations, which indicated that although hypocobalaminemia was common in these dogs, it did not always appear to be associated with a deficiency of cobalamin on a cellular level. Hypocobalaminemia is a risk factor for negative outcome in dogs with chronic gastrointestinal disease and should be considered in every patient with corresponding clinical signs. (Am J Vet Res 2013;74:84–89)
with serum creatinine concentrations in humans.6,8 However, increases in serum MMA concentrations are generally only seen with serum creatinine concentrations > 2.5 mg/dL, and even then, changes are considered minor in magnitude (up to 500 nmol/L). In a recent study, serum MMA and creatinine concentrations were evaluated in > 500 canine serum samples, but there was no correlation between the concentrations of these 2 substances. Thus, it appears unlikely that minor variations in serum creatinine concentrations would substantially impact serum MMA concentrations. However, to rule out potential impacts of renal insufficiency on serum MMA concentrations, it may be advisable to evaluate serum creatinine concentrations whenever serum MMA concentrations are determined.

Evaluation of cobalamin and MMA concentrations in > 500 canine serum samples submitted to the Gastrointestinal Laboratory at Texas A&M University revealed that 70 of 153 (46%) dogs with hypocobalaminema had evidence of tissue cobalamin deficiency, as determined on the basis of an increase in serum MMA concentration. Furthermore, a number of dogs with a serum concentration for canine tryptsin-like immunoreactivity > 5.7 µg/L were excluded. If gastrointestinal histopathology reports were available, results were reviewed to exclude dogs with gastrointestinal neoplasia.

Serum samples were also obtained from 43 healthy, client-owned control dogs. In the time of blood sample collection, a brief physical examination was performed by a veterinarian, and all of these dogs appeared healthy. Additionally, owners of the dogs were asked to complete a questionnaire to help exclude dogs with underlying diseases. The study was approved by the Clinical Research Review Committee of the College of Veterinary Medicine at Texas A&M University.

Measurement of serum creatinine concentrations—Routine serum creatinine analyses were performed on all samples. Analyses were performed via an automated chemistry analyzer system with an enzymatic assay method. The reference interval for the serum creatinine concentration was 0.5 to 1.4 mg/dL.

Measurement of serum cobalamin and MMA concentrations—Serum cobalamin concentrations were measured with a competitive binding chemiluminescence assay. The laboratory’s reference interval for this assay was 251 to 908 ng/L. The lower and upper detection limits for the cobalamin assay were 150 and 1,000 ng/L, respectively.

Serum MMA concentrations were measured via a stable isotope dilution GC-MS method, as described elsewhere. Briefly, MMA was extracted from serum samples by use of an on-column liquid-liquid extraction method, which was followed by silylation with N-methyl-N-tert-butyldimethylsilyltrifluoroacetamide for subsequent GC-MS analysis. The GC-MS separation and analysis were performed with a 100% dimethylpolysiloxane column on a gas chromatograph and mass selective detector as described elsewhere. Assay performance was verified via use of standards prepared with pure MMA in serial dilution from 16,000 to 63 nmol/L. The reference interval for the MMA assay was 413 to 1,193 nmol/L, and the lower and upper detection limits were 63 and 16,000 nmol/L, respectively. Serum MMA concentrations were diluted and assayed again to allow measurement within the linear range of the assay. Concentrations for diluted samples were then back-calculated to obtain the actual sample concentration. Concentrations of MMA were quantified via the area under the curve for MMA and the internal standard (ie, deuterated MMA).

Statistical analysis—Data were analyzed with a commercial statistical program. All data sets were analyzed for normal distribution via a D’Agostino and Pearson omnibus normality test. The mean ± SD or the median and ranges of the data sets were calculated, as appropriate. Serum cobalamin and MMA concentrations for dogs with gastrointestinal disease were compared with concentrations for the group of 43 healthy control dogs.
dogs via a Mann-Whitney U test. A correlation between serum cobalamin and MMA concentrations, as well as between both serum substances and the CCECAI, in dogs with chronic gastrointestinal disease was evaluated with the Spearman rank correlation. A Spearman rank correlation was also calculated for serum MMA and serum creatinine concentrations. A possible association between serum MMA concentrations and age as well as duration of clinical signs was evaluated by use of a Spearman rank correlation as well as a Mann-Whitney U test. Significance for all tests was set at a value of \( P < 0.05 \).

**Results**

**Dogs with gastrointestinal disease**—A total of 56 of the 69 dogs with gastrointestinal disease evaluated were eligible for enrollment. Of the 56 dogs, 29 were females (3 sexually intact and 26 spayed) and 27 were males (4 sexually intact and 23 castrated). The dogs ranged from 0.4 to 13.0 years of age (mean ± SD, 6.0 ± 3.3 years). There was not a significant \( (P = 0.974) \) difference in age between female and male dogs.

Forty-six dogs were purebreds. Breeds represented included Labrador Retriever \((n = 4)\), German Shepherd Dog \((3)\), Boxer \((2)\), Cocker Spaniel \((2)\), English Bulldog \((2)\), Golden Retriever \((2)\), American Pit Bull Terrier \((2)\), Pointer \((2)\), Standard Poodle \((2)\), Yorkshire Terrier \((2)\), Australian Shepherd \((1)\), Basenji \((1)\), Belgian Malinois \((1)\), Bichon Frise \((1)\), Border Collie \((1)\), Boston Terrier \((1)\), Cairn Terrier \((1)\), Chesapeake Bay Retriever \((1)\), Chinese Shar Pei \((1)\), English Mastiff \((1)\), French Bulldog \((1)\), Irish Wolfhound \((1)\), Jack Russell Terrier \((1)\), Miniature Poodle \((1)\), Miniature Schnauzer \((1)\), Newfoundland \((1)\), Pomeranian \((1)\), Rhodesian Ridgeback \((1)\), Soft-Coated Wheaten Terrier \((1)\), Shiba Inu \((1)\), Shih Tzu \((1)\), Springer Spaniel \((1)\), and Toy Poodle \((1)\). The remaining 10 dogs were of mixed breeds, with a mean ± SD body weight of 14.4 ± 10.7 kg (range, 2.6 to 31.5 kg).

The median CCECAI for the 56 dogs with gastrointestinal disease was 6 (range, 1.0 to 15.0).

**Healthy control dogs**—The 43 healthy control dogs comprised 23 spayed females and 20 males (4 sexually intact and 16 castrated). The control dogs ranged from 0.7 to 11.0 years of age (median, 3.0 years). There was not a significant \( (P = 0.411) \) difference in age between female and male dogs. However, dogs with gastrointestinal disease were significantly \( (P < 0.001) \) older (median, 6.1 years; range, 0.4 to 13 years) than the healthy control dogs (median, 3.0 years; range, 0.7 to 11.0 years).

Of the 43 control dogs, 29 were purebreds. Breeds represented were German Shepherd Dog \((n = 3)\), Australian Shepherd \((2)\), Beagle \((2)\), Border Collie \((2)\), Doberman Pinscher \((2)\), American Pit Bull Terrier \((1)\), American Bulldog \((1)\), Bloodhound \((1)\), Australian Cattle Dog \((1)\), Boston Terrier \((1)\), Chesapeake Bay Retriever \((1)\), Welsh Corgi \((1)\), Dachshund \((1)\), English Springer Spaniel \((1)\), Foxhound \((1)\), Great Dane \((1)\), Jack Russell Terrier \((1)\), Labrador Retriever \((1)\), English Mastiff \((1)\), Neapolitan Mastiff \((1)\), Rat Terrier \((1)\), Rottweiler \((1)\), and Staffordshire Bull Terrier \((1)\). The remaining 14 dogs were of mixed breeds, with a mean ± SD body weight of 24.6 ± 7.2 kg (range, 12.0 to 34.2 kg).

**Serum creatinine concentrations**—The mean ± SD serum creatinine concentration for the 56 dogs with gastrointestinal disease was 0.9 ± 0.3 mg/dL. Two of the 56 dogs had serum creatinine concentrations (1.7 and 1.8 mg/dL, respectively) that were higher than the upper limit of the reference interval. One of these 2 dogs had a high serum MMA concentration (3,543 nmol/L) and an undetectable serum cobalamin concentration (<150 ng/L). There was not a significant \( (P = 0.555) \) correlation between serum MMA and serum creatinine concentrations.

**Serum cobalamin and MMA concentrations**—Serum cobalamin concentrations in the 56 dogs with chronic gastrointestinal disease (median, 344 ng/L; range, 150 to 1,000 ng/L) were significantly \( (P = 0.001) \) lower than concentrations in the 43 healthy control dogs (median, 515 ng/L; range, 333 to 835 ng/L; Figure 1). Twenty of 56 (36%) dogs with gastrointestinal disease had cobalamin concentrations less than the lower limit of the reference interval (<251 ng/L), and 7 (13%) dogs had undetectable serum cobalamin concentrations (<150 ng/L).

The median serum MMA concentration in dogs with chronic gastrointestinal disease was 741 nmol/L (range, 447 to 205,399 nmol/L), and serum MMA concentrations in dogs with chronic gastrointestinal disease were significantly \( (P = 0.036) \) higher than concentrations in the 43 healthy dogs (median, 649 nmol/L; range, 228 to 1,233 nmol/L; Figure 2). Five of 56 (9%) dogs with gastrointestinal disease had MMA concentrations higher than the upper limit of the reference interval (>1,193 nmol/L). All 5 of these dogs had undetectable serum cobalamin concentrations. Therefore, 5 of 20 (25%) hypocobalaminemic dogs had high serum MMA concentrations.

When data for the 2 dogs with increased serum creatinine concentrations were removed from the analysis, serum MMA concentrations did not differ significantly \( (P = 0.562) \) between dogs with gastrointestinal disease
ly twice as high as has been previously reported for chronic gastrointestinal disease, which is approximate cobalaminemia of 36% in this group of dogs with disease. Concentrations in dogs with chronic gastrointestinal disease. To our knowledge, this increase in serum MMA concentration, in dogs with cobalamin deficiency on a cellular level, as evidenced by an gate the prevalence of hypocobalaminemia and cobalamin concentrations or the CCECAI and serum MMA concentrations. No significant correlation was detected between duration of clinical signs (median, 6 months; range, 0.75 to 96 months) and serum MMA concentrations. Duration of clinical signs did not differ significantly (P = 0.175) between dogs that had high serum MMA concentrations (median, 18 months; range, 4 to 84 months) and dogs with MMA concentrations within the reference interval (median, 6 months; range, 0.75 to 96 months). Similarly, there was no association between age (median, 6.1 years; range, 0.4 to 13 years) and serum MMA concentrations. Age did not differ significantly (P = 0.687) between dogs with high serum MMA concentrations (median, 7.5 years; range, 1.8 to 10 years) and dogs with MMA concentrations within the reference interval (median, 6.0 years; range, 0.4 to 13 years).

Discussion

The purpose of the present study was to investigate the prevalence of hypocobalaminemia and cobalamin deficiency on a cellular level, as evidenced by an increase in serum MMA concentration, in dogs with chronic gastrointestinal disease. To our knowledge, this is the first prospective study to determine serum MMA concentrations in dogs with chronic gastrointestinal disease.

Analysis of the data revealed a prevalence of hypocobalaminemia of 36% in this group of dogs with chronic gastrointestinal disease, which is approximately twice as high as has been previously reported for dogs with chronic gastrointestinal disease. Of the 20 hypocobalaminemic dogs, 5 (25%) also had a high serum MMA concentration, which suggested cellular cobalamin deficiency. This number is lower than the value reported for dogs with hypochobalaminemia in another study conducted by our research group. From the data in the present study, it is apparent that the difference in serum MMA concentrations between dogs with chronic gastrointestinal disease and healthy control dogs is minor. In fact, significant differences were no longer detected once data for the 2 dogs with a slightly high serum creatinine concentration were removed from the analysis. We chose to include the data for these 2 dogs because analysis of data from a previous study as well as the present study failed to indicate a correlation between serum creatinine and MMA concentrations in dogs, which is in contrast to humans, in which such a correlation has been reported. In the previous study, dogs with a serum creatinine concentration as high as 3 to 4 mg/dL had serum MMA concentrations within the reference interval, which led us to believe that dogs would probably have to be in major renal failure before that medical condition would have a potential impact on serum MMA concentrations. Thus, the serum creatinine concentrations in the 2 dogs reported here (1.7 and 1.8 mg/dL) likely did not contribute substantially to their serum MMA concentrations.

In the aforementioned study, 70 of 153 (46%) dogs with a serum cobalamin concentration < 251 ng/L had high serum MMA concentrations, whereas the prevalence of hypochobalaminemia in the present study (25%) was only approximately half that value. This discrepancy was likely attributable to differences in the populations that were used for these 2 studies. In the earlier study, surplus serum samples that had been submitted to our laboratory were used, and no clinical data were available for the dogs included in that study. In the present study, dogs with conditions other than chronic gastrointestinal disease (eg, EPI or intestinal neoplasia) were excluded from analysis. Exocrine pancreatic insufficiency is one of the main causes of cobalamin deficiency in dogs, with approximately 80% of EPI-affected dogs developing a deficiency. Intestinal neoplasia is also more likely to cause cobalamin malabsorption, and intestinal lymphoma has been associated with cobalamin deficiency in cats. For example, in the present study, all 4 dogs that were excluded from analysis because of intestinal neoplastic disease had hypochobalaminemia or methylmalonic acidemia (or both). Therefore, it is conceivable that the described differences in the populations between the 2 studies are partially responsible for the variation in the results observed.

The reason that so many of the dogs with chronic gastrointestinal disease in this study had serum MMA concentrations within the reference interval despite low serum cobalamin concentrations, is unclear. It is possible that the disease process in many of these dogs was not of sufficient chronicity to deplete cellular stores of cobalamin, which would be considered to be a prerequisite to the production of MMA. An attempt was made to analyze the association between duration of clinical signs and serum MMA concentrations in the present study. No significant correlation between these 2 variables was detected. Although there was a longer

**Figure 2—Serum MMA concentrations for 43 healthy control dogs and 96 dogs with chronic GI disease. The median for each group of dogs is indicated (solid line), and the upper limit of the reference interval for the serum MMA concentration (1.193 nmol/L) is also indicated (dotted line).**
duration of clinical signs in dogs with high serum MMA concentrations (median of 18 months, compared with 6 months in dogs with serum MMA concentrations within the reference interval), it was difficult to statistically evaluate these data because of the low number of dogs in the group with high serum MMA concentrations (n = 5); therefore, no final conclusions should be drawn. The half-life of cobalamin in healthy dogs is approximately 2 months (48 to 70 days), which is substantially less than in humans, in which the half-life of cobalamin is approximately 1 year.12-13 These time frames are only applicable to healthy individuals. Investigators in 1 study12 found that cobalamin in cats with inflammatory bowel disease (5 days) had a shortened half-life when compared with the half-life of cobalamin in healthy cats (12.8 days). It is likely that the half-life of cobalamin is similarly decreased in dogs with gastrointestinal disease as a result of reduced absorption, while enterohepatic circulation with physiologic excretion of cobalamin in bile is ongoing.

Data on the temporal relationship between the onset of hypocobalaminemia and development of methylmalonic acidemia in dogs are scarce. Experiments in dogs with selective cobalamin malabsorption attributable to an inherited defect in the expression of proteins in the intrinsic factor–cobalamin receptor complex (cubam [cubilin and amnionless]) have revealed that serum MMA concentrations may be increased as early as 2 weeks after the onset of hypocobalaminemia. However, it is uncertain whether these findings also hold true for dogs with cobalamin malabsorption attributable to chronic gastrointestinal disease. Dogs with the inherited defect are not able to absorb cobalamin, whereas this is likely not the case in most dogs with gastrointestinal disease, where the onset of cobalamin malabsorption may be more gradual. Thus, further studies to investigate the temporal relationship in dogs with chronic gastrointestinal disease may be warranted.

The CCECAI was of moderate severity (median, 6) in the dogs with gastrointestinal disease, with scores ranging from 1 to 15. There was no correlation between the CCECAI and serum cobalamin or MMA concentrations, which indicated that hypocobalaminemia and methylmalonic acidemia are not always associated with severity of clinical disease. This can be explained by the fact that cobalamin deficiency in dogs with chronic gastrointestinal disease is caused by malabsorption of cobalamin attributable to damage to the mucosal receptors for the intrinsic factor–cobalamin complex in the distal portion of the small intestine. Therefore, a patient with severe disease of the distal portion of the small intestine is more likely to develop cobalamin deficiency than is a patient with disease affecting other sites of the intestinal tract. In the present study, it was not always possible to determine the portion of the intestine that was primarily affected, but after exclusion of dogs with EPI, it can be assumed that dogs with low serum cobalamin concentrations had at least some degree of ileal disease. Consequently, dogs with a high CCECAI but with serum cobalamin or MMA concentrations within the reference interval may have had disease of a higher severity in other portions of the gastrointestinal tract. Also, a potential limitation of the study was that we cannot exclude with certainty the possibility that some of the dogs received cobalamin at some point prior to enrollment into the study, even though we requested this information from the attending veterinarians prior to sample submission and dogs were excluded from analysis if cobalamin was indicated as a treatment. In many cases, dogs were enrolled into the study through a referral clinic, but the dogs had previously been examined by their primary care veterinarian. In those dogs, a small chance remained that cobalamin administration may have been used but was not reported. This could have led to some discordance between the serum cobalamin concentrations and the clinical scores.

On the basis of the data in the present study, we conclude that hypocobalaminemia is commonly detected in dogs with chronic gastrointestinal disease, but it does not always appear to be associated with a deficiency of cobalamin on a cellular level. Nevertheless, the high prevalence of hypocobalaminemia in these dogs and the fact that hypocobalaminemia is a risk factor for negative outcome in dogs with chronic gastrointestinal disease2 lead us to believe that serum cobalamin concentrations should be measured in all dogs with clinical signs of chronic gastrointestinal disease. Treatment with cobalamin is strongly advised for all patients with a serum cobalamin concentration below the reference interval and is also suggested for patients with serum cobalamin concentrations within the low part of the reference interval (< 350 ng/L) if there are clinical signs of gastrointestinal disease, regardless of whether serum MMA concentrations are high. The reason for this recommendation is that cobalamin deficiency can cause gastrointestinal tract abnormalities (eg, mucosal inflammation and villous atrophy), which can lead to malabsorption of nutrients, including cobalamin.3 Thus, unless the cobalamin deficiency is rectified, patients may not respond to treatment for the underlying disease process. Further studies are warranted to investigate the temporal relationship between the onset of hypocobalaminemia and cobalamin deficiency on a cellular level in dogs with chronic gastrointestinal disease.

a. Sirrus clinical chemistry analyzer, Stanbio Laboratory, Boerne, Tex.
b. Direct creatinine LiquiColor test, Stanbio Laboratory, Boerne, Tex.
d. Chem Elut cartridges, 1 mL, unbuffered, Agilent Technologies, Santa Clara, Calif.
f. DB-1ms, Agilent Technologies, Santa Clara, Calif.
g. 6890N gas chromatograph, Agilent Technologies, Santa Clara, Calif.
h. 5975C mass selective detector, Agilent Technologies, Santa Clara, Calif.
i. MMA, Sigma-Aldrich, St Louis, Mo.
j. Methyl-d3-malonic acid, CDN Isotopes, Pointe-Claire, QC, Canada.
k. GraphPad Prism, version 5.00, GraphPad Software Inc, San Diego, Calif.
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