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Daily oral cyanocobalamin supplementation in Beagles with hereditary cobalamin malabsorption (Imerslund-Gräsbeck syndrome) maintains normal clinical and cellular cobalamin status

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Abstract: BACKGROUND: Efficacy of PO cobalamin (Cbl) supplementation in dogs with hereditary Cbl malabsorption (Imerslund-Gräsbeck syndrome, IGS) is unknown. OBJECTIVES: To evaluate PO Cbl supplementation in Beagles with IGS previously treated parenterally. We hypothesized that 1 mg cyano-Cbl daily PO would maintain clinical and metabolic remission. ANIMALS: Three client-owned Beagles with IGS and 48 healthy control dogs. METHODS: Prospective study. Daily PO cyanocobalamin (cyano-Cbl; 1 mg) supplementation was monitored for 13 (2 dogs) and 8 months (1 dog). Health status was assessed by owner observations. Methylmalonic acid (MMA)-to-creatinine concentrations were measured using an ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-TMS) method on urine samples collected monthly. Concurrent measurements of serum MMA concentration (n = 7; UPLC-TMS) were available for 1 dog. RESULTS: All dogs remained in excellent health during PO supplementation. Urine MMA remained consistently low in 2 dogs (median, 2.5 mmol/mol creatinine; range, 1.2-9; healthy dogs [n = 30], median, 2.9 mmol/mol creatinine; range, 1.3-76.5). Urine MMA ranged from 38.9-84.9 mmol/mol creatinine during the first 6 months in 1 dog already known to excrete comparable amounts when supplemented parenterally. Brief antibiotic treatment for an unrelated condition after 6 months resulted in low urine MMA (median, 2.8 mmol/mol creatinine; range, 1.9-4.8) for the next 7 months. All concurrent serum MMA concentrations (median, 651 nmol/L; range, 399-919) before and after month 6 were within the established reference interval (393-1476 nmol/L; n = 48). CONCLUSIONS AND CLINICAL IMPORTANCE: One milligram of cyano-Cbl daily PO appears efficacious for maintaining normal clinical status and normal cellular markers of Cbl metabolism in Beagles with IGS.

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STANDARD ARTICLE

Daily oral cyanocobalamin supplementation in Beagles with hereditary cobalamin malabsorption (Imerslund-Gräsbeck syndrome) maintains normal clinical and cellular cobalamin status

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Background: Efficacy of PO cobalamin (Cbl) supplementation in dogs with hereditary Cbl malabsorption (Imerslund-Gräsbeck syndrome, IGS) is unknown.

Objectives: To evaluate PO Cbl supplementation in Beagles with IGS previously treated parenterally. We hypothesized that 1 mg cyano-Cbl daily PO would maintain clinical and metabolic remission.

Animals: Three client-owned Beagles with IGS and 48 healthy control dogs.

Methods: Prospective study. Daily PO cyanocobalamin (cyano-Cbl; 1 mg) supplementation was monitored for 13 (2 dogs) and 8 months (1 dog). Health status was assessed by owner observations. Methylmalonic acid (MMA)-to-creatinine concentrations were measured using an ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-TMS) method on urine samples collected monthly. Concurrent measurements of serum MMA concentration (n = 7; UPLC-TMS) were available for 1 dog.

Results: All dogs remained in excellent health during PO supplementation. Urine MMA remained consistently low in 2 dogs (median, 2.5 mmol/mol creatinine; range, 1.2-9; healthy dogs [n = 30], median, 2.9 mmol/mol creatinine; range, 1.3-76.5). Urine MMA ranged from 38.9-84.9 mmol/mol creatinine during the first 6 months in 1 dog already known to excrete comparable amounts when supplemented parenterally. Brief antibiotic treatment for an unrelated condition after 6 months resulted in low urine MMA (median, 2.8 mmol/mol creatinine; range, 1.9-4.8) for the next 7 months. All concurrent serum MMA concentrations (median, 651 nmol/L; range, 399-919) before and after month 6 were within the established reference interval (393-1476 nmol/L; n = 48).

Conclusions and Clinical Importance: One milligram of cyano-Cbl daily PO appears efficacious for maintaining normal clinical status and normal cellular markers of Cbl metabolism in Beagles with IGS.

KEYWORDS

dogs, methylmalonic acid, serum, urine, vitamin B12

Abbreviations: Cbl, cobalamin; IGS, Imerslund-Gräsbeck syndrome; MMA, methylmalonic acid; IF, intrinsic factor; cubam, functional receptor complex composed of AMN and CUBN subunits; CUBN, cubilin; AMN, amnionless; GC-MS, gas chromatography-mass spectrometry; RI, reference interval; cyano-Cbl, cyanocobalamin; hydroxo-Cbl, hydroxocobalamin; UPLC-TMS, ultra-performance liquid chromatography-tandem mass spectrometry; CV, coefficients of variation.

1 | INTRODUCTION

Metabolic derivatives of cobalamin (Cbl), or vitamin B12, act as essential cofactors of methylmalonyl-CoA mutase, which convert methylmalonyl-CoA to succinyl-CoA, and methionine synthase, which is required for the remethylation of homocysteine. A decrease in

activity of these intracellular enzymes results in increases in methylmalonic acid (MMA) and homocysteine concentrations.[1] Dogs are dependent on intestinal uptake of dietary Cbl bound to intrinsic factor (IF). The Cbl-IF complex binds to functional receptor complex composed of amnionless (AMN) and cubilin (CUBN) subunits (cubam), a heteromeric, multiligand, endocytic receptor, consisting of 2 proteins, AMN and CUBN.[2] In people, mutations in either the AMN or CUBN gene lead to selective Cbl malabsorption known as Imerslund-Gräsbeck syndrome (IGS).[3,4] In dogs, selective intestinal Cbl malabsorption, comparable to IGS in humans, has been described in Australian Shepherds,[5,6] Beagles,[7–13] Border Collies[14–17], and Giant Schnauzers.[18] It is an autosomal recessive trait caused by 2 distinct AMN mutations in Giant Schnauzers[18] and Australian Shepherds, [6] and our group and others recently identified 2 independent CUBN mutations in Border Collies[19,20] and Beagles.[10,21] The condition is lethal in affected dogs unless detected and treated appropriately.[9–11,16,18] Supplementation with adequate amounts of Cbl leads to rapid and complete clinical remission when Cbl is administered sufficiently early in the course of disease, and the long-term prognosis is excellent with adequate lifelong treatment.[6,8–10,12,13]

To date, all reported treatments in dogs have consisted of parenteral supplementation to circumvent the underlying cause of the problem (ie, deficient intestinal uptake of the vitamin). However, sporadic reports in human medicine indicate that PO supplementation also leads to normalization of intracellular markers of Cbl deficiency,[22,23] and it was shown that approximately 1% of free Cbl is absorbed along the entire intestine by passive diffusion without the need for an intact cubam receptor.[24] Although this route of absorption has not been demonstrated in dogs, PO Cbl supplementation recently was determined to effectively increase serum Cbl concentrations in dogs with chronic enteropathies and low serum Cbl concentrations.[25,26] Our group also has had positive experiences with PO supplementation in older Beagles and Border Collies with IGS that could not tolerate repeated IM injections. These dogs remained clinically healthy and had urine MMA results within reference intervals (RIs) when evaluated sporadically. We recently studied the long-term efficacy of monthly and bimonthly (every 2 months) IM Cbl injections including regular assessment of MMA as a marker of cellular Cbl availability in 6 juvenile Beagles with IGS.[13] All dogs thrived and were clinically healthy, but the prospect of a lifelong need for IM injections prompted owners of 3 dogs to try PO Cbl supplementation. We were able to conduct follow-up assessments of these dogs, which included regular monitoring of markers of intracellular Cbl availability. We hypothesized that a daily supraphysiologic PO dose of 1 mg cyano-Cbl would maintain clinical and metabolic remission in dogs with genetically confirmed IGS.

2 | MATERIALS AND METHODS

2.1 | Animals

The clinical and metabolic health of 3 client-owned purebred Beagles with IGS was monitored for 13 (n = 2) and 8 (n = 1) months. All dogs had been diagnosed with IGS using a commercial genetic test (www.laboklin.de) for the CUBN mutation and initially had received monthly, followed by bimonthly, IM injections of 1 mg Cbl (VITAMIN B-12 Depot Rotexmedica Injektionslösung) for a period of 15-21 months before being switched to PO supplementation.[13] The age of the dogs at the time of transition to PO supplementation was 31 (dog 1, male, neutered) and 29 (n = 2) months. The 2 younger dogs were littermates (male [dog 2] and female [dog 3]). The owners and primary care veterinarians considered all dogs to be in excellent health at the time of transition to PO Cbl supplementation.

2.2 | Study design

2.2.1 | Beagles with IGS

Voided urine samples for baseline analysis of MMA concentration were collected 4 weeks after the last bimonthly Cbl injection (=time point 0 [t0]) and at monthly intervals (t1, t2, t3, etc.) thereafter. Starting the day after t0, all dogs were given 1 tablet containing 1 mg cyano-Cbl (B12 Ankermann Wörwag Pharma) PO along with a small treat, once daily. In dog 1, the concentrations of serum MMA (t1, and t5-t10) and plasma homocysteine (t1-t3, and t5-t10) were also available in addition to urine MMA analyses. Additional blood analyses in dog 1 were done at the owners' request and not because of health concerns. Serum MMA concentration also was measured once at t7 in dog 3. The participating primary care veterinarians were asked to centrifuge blood samples for the determination of plasma homocysteine concentration within 30 minutes. All samples were stored at −20°C for analysis later. The dog owners remained in close contact (telephone, email) with the first author of this study. Because serum Cbl concentrations have been shown to be of limited value for the assessment of Cbl status in supplemented dogs with IGS,[13,18] the decision to include this variable along with urinary MMA concentration was ultimately left to the discretion of the primary care veterinarian and owner. When included, serum samples for Cbl determination were collected before PO administration of Cbl that day. All samples were shipped to our clinic on dry ice using a commercial carrier.

2.3 | Healthy control dogs

In our previous study on parenteral Cbl supplementation, the RI for urinary MMA concentration was based on urine samples from 28 clinically healthy dogs.[13] In the present study, the results of 2 more healthy dogs with unremarkable laboratory (CBC, serum biochemistry, urinalysis) results and serum Cbl concentrations within RIs were added for a total of 30 clinically healthy dogs. Serum samples from 20 healthy dogs were used for establishing a preliminary RI for serum MMA concentrations in our earlier study,[13] and we were able to strengthen this preliminary RI by adding serum MMA results from an additional 28 healthy dogs with unremarkable clinical and laboratory (see above) evaluations (n = 48).

2.4 | Analyses

Either available serum Cbl concentrations were measured at a commercial veterinary laboratory (www.laboklin.de; n = 1) or samples were frozen and shipped together with urine samples to our laboratory (n = 2). The commercial veterinary laboratory used a

chemiluminescence assay for Cbl determination, and the RI was 221-590 pmol/L (B12 ECLIA on Cobas 8000 e602). An automated competitive binding chemiluminescence assay (Immulite 2000, vitamin B12; Siemens Healthcare Diagnostics) and an RI of 146-721 pmol/L[27] were used in our laboratory.

Urine and serum MMA concentrations were analyzed at the Division of Clinical Chemistry of the University Children's Hospital Zurich according to accredited methods.[28] In brief, the samples were supplemented with an internal standard, precipitated, and analysis was done by ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-TMS) on an Ultimate 3000 XRS UHPLC system (Dionex; Thermo Scientific) with a SCIEX5500 mass spectrometer (SCIEX, Framingham, Massachusetts) using multiple reaction monitoring. The lower limit of quantification for this method was 25 nmol/L in serum and 651 nmol/L in urine. Results for urine MMA were expressed as mmol MMA per mol creatinine. Creatinine concentrations were determined using a kinetic Jaffe method on a DxC600 clinical chemistry analyzer (Beckman Coulter International S.A., Nyon, Switzerland) using commercial reagents. The inter-assay coefficients of variation (CV) of the analyses were 5.8% for MMA in serum and urine and 2.7% for creatinine in urine.

Plasma homocysteine concentration was measured in the same laboratory using a commercial Axis-Shield enzymatic homocysteine assay (Axis-Shield Diagnostics Ltd., Dundee, Scotland) on a DxC600 chemistry analyzer (Beckman Coulter International S.A.) with an inter-assay CV of 3.2%.

2.5 | Statistical analysis

Data were tested for normal distribution using D'Agostino and Pearson omnibus tests. Urine MMA results of healthy dogs are presented as range and median values. The RI for serum MMA concentration was calculated using the 5th and 95th percentiles.

3 | RESULTS

3.1 | Healthy control dogs

Urinary MMA concentrations were not normally distributed in 30 healthy control dogs and ranged from 1.3 to 76.5 mmol/mol creatinine (median, 2.9 mmol/mol creatinine). Serum MMA concentrations in 48 healthy dogs ranged from 311 to 1740 nmol/L (median, 585 nmol/L). The RI was established as 393 nmol/L (90% confidence interval [CI], 325-400 nmol/L) to 1476 nmol/L (90% CI, 1136-2216 nmol/L) .

3.2 | Response to daily oral cyano-Cbl supplementation

3.2.1 | Clinical signs

Dogs received daily PO cyano-Cbl supplementation for a median of 9 (range, 8-13) months. All dogs were considered free of clinical signs and described as active and alert by owners and veterinarians while receiving daily PO cyano-Cbl supplementation. Two dogs (dogs 1 and 3) were considered to be athletic based on appearance and activity level. Dog 1 (Figure 1, red squares) was treated for a bacterial skin infection with a 10-day course of cephalexin (Rilexine; Virbac S.A., Carros, France) and amoxicillin (Kesium, Biokema SA, Crissier, Switzerland) starting 2 days after urine sampling at t6. No other drugs were given during the study period except routine administration of anthelmintics. The results of periodic hematologic evaluations undertaken by the primary care veterinarians were unremarkable in all dogs.

3.3 | Serum Cbl concentration

Sporadically evaluated serum Cbl concentrations were within RIs. In dog 1 (Figure 1, red squares), serum Cbl concentration was measured at t6 (512 pmol/L). In dog 2 (Figure 1, purple triangles), serum Cbl concentration was measured at t7 (758 pmol/L), and in dog 3 (Figure 1, orange triangles), serum Cbl concentration was 633 pmol/L at t3.

3.4 | Urinary MMA concentrations

Thirty-four urinary MMA results were available from dogs receiving PO cyano-Cbl supplementation. All urine MMA concentrations from dogs 2 and 3 remained in the low-normal range (median, 2.5 mmol/mol creatinine; range, 1.2-9 mmol/mol creatinine). This result was comparable to previously recorded urine MMA concentrations in dogs receiving monthly and bimonthly Cbl injections.[13] Dog 1 continued to excrete higher amounts of MMA in urine for an additional 6 months, which was also similar to previous results in this dog when treated with IM injections (Figure 1). However, at t7 when the prescribed course of antibiotics had been finished, urine MMA concentrations had decreased into the range of dogs 2 and 3 and remained in that range (1.9-4.8 mmol/mol creatinine) for the next 7 months (Figure 1).

3.5 | Serum MMA concentrations

In dog 1, serum MMA concentrations were measured at t1 and from t5 to t10. All 7 results were within the RI and ranged from 399 to 919 nmol/L (median, 651 nmol/L; RI, 393-1476 nmol/L). Serum MMA concentration also was measured in dog 3 at t7 and was 642 nmol/L (RI, 393-1476 nmol/L).

3.6 | Plasma homocysteine concentrations

In dog 1, plasma homocysteine concentrations at t1-t3 were 21.5, 18.8, and 27.4 μmol/L, respectively, and they ranged from 15 to 23.8 μmol/L (median, 17.7 μmol/L) between t5 and t10.

4 | DISCUSSION

The results of our study indicate that daily PO administration of 1 mg cyano-Cbl represents an efficacious regimen for maintaining normal health status as well as unremarkable cellular Cbl status based on urine and serum MMA concentrations in Beagles with IGS. Compared to our previous study evaluating parenteral Cbl supplementation in IGS dogs,[13] we herein report a more robust serum MMA RI (393-1476 nmol/L) based on 48 healthy dogs, which compares

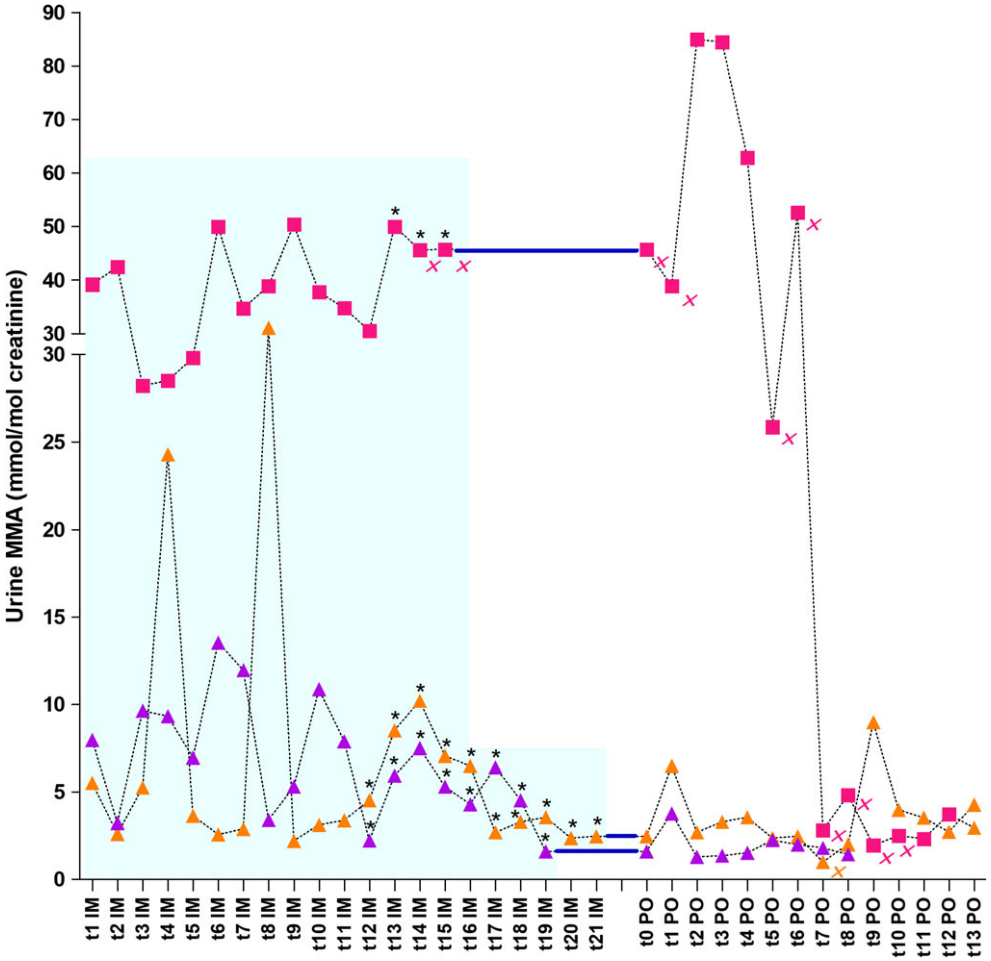

FIGURE 1 Light-blue shaded area: Urinary methylmalonic acid (MMA) concentrations (mmol/mol creatinine) from voided urine samples in 3 dogs with IGS during monthly, and bimonthly (*) supplementation with 1 mg OH-Cbl, administered IM (*J Vet Intern Med* 2018;32 (3):1033-1040). Collection of voided urine samples was continued at monthly intervals for the evaluation of PO Cbl supplementation. Baseline urine MMA concentrations at t0 PO again represent the preceding urine MMA concentration recorded 4 weeks after the last Cbl injection (identical values connected with dark-blue solid line). Starting the day after t0 PO, all dogs were given 1 tablet containing 1 mg cyano-Cbl orally, once daily. Additional x signs represent the concurrent documentation of normal serum MMA concentration (RI: 393-1476 nmol/L). Please note that dog 1 (pink squares) was treated for an unrelated bacterial skin infection with a 10-day course of cephalexin and amoxicillin starting 2 days after urine sampling at t6

favorably to what has been published recently using similar methodology in 43 healthy dogs (414.7-1192.5 nmol/L).[29]

Daily PO cyano-Cbl supplementation recently has been shown to effectively increase serum Cbl concentrations in dogs with enteropathy-associated low-normal and subnormal serum Cbl concentrations.[25,26] It was speculated that dogs have an alternative pathway for Cbl absorption, similar to that demonstrated in studies involving radioactively labeled Cbl in humans.[24] However, it was not possible with that study design to determine whether dogs with chronic enteropathy had a total or only partial lack of active cubam receptor-mediated absorption of Cbl and to establish the extent of passive absorption by diffusion that led to normalization of serum Cbl concentration. A factor that may have aided Cbl absorption via active receptors was that all dogs with chronic enteropathy received treatment for their underlying intestinal disease.[25,26] The results of our study using IGS-affected dogs with a known functional defect of cubam but otherwise healthy intestinal tract indicate for the first time that passive intestinal Cbl absorption alone leads to a normal clinical and cellular Cbl status in dogs. This is especially true for the Beagle breed because a recent study showed that CUBN expression was undetectable in tissue samples from an affected dog,[10] whereas in Border Collies with IGS, expression of small amounts of residual CUBN retaining IF-Cbl binding recently was reported.[20] However, this laboratory finding does not appear to be associated with effective Cbl absorption because all available publications concerning Border Collies with IGS have reported the presence of severe Cbl deficiency.[14–17]

Cobalamin deficiency has been associated with changes in permeability, absorptive function, and histologic appearance of the intestinal mucosa in humans, and mucosal structure and function normalized in these patients after parenteral Cbl treatment.[30] Similar effects on the intestinal tract frequently have been discussed in dogs with Cbl deficiency. In the present study, all dogs entered the PO supplementation phase after having been supplemented parenterally for an extended period of time (15, 19, and 21 months). We therefore can confidently rule out possible negative effects of preexisting Cbl deficiency on intestinal absorptive capacity.

We used a dose of 1 mg cyano-Cbl daily in dogs that weighed approximately 11 kg, which differed from previous studies in which the dose varied from 0.25 to 1 mg cyano-Cbl, depending on body weight.[25,26] The dogs in our study were known to suffer from complete lack of Cbl absorption in contrast to dogs with enteropathy-associated hypocobalaminemia, and therefore we chose to use a whole tablet (1 mg) per day rather than dividing a tablet. Moreover, experience with a number of older Beagles and Border Collies with IGS from our clinic caseload has shown that this dose works well. In addition, our approach precluded issues with accurate dosing because the manufacturer's recommendation was not to divide coated tablets. Lastly, adverse effects related to high PO doses of Cbl have not been reported. We generally prefer hydroxo-Cbl for parenteral Cbl supplementation in our hospital because it is the natural form of Cbl and considered the treatment of choice for children with IGS.[4,31–33] Moreover, early studies in dogs showed that hydroxo-Cbl produces more prolonged increases in serum Cbl concentrations when injected IM than injection of equal amounts of cyano-Cbl.[34] In addition, compared to cyano-Cbl, it has been shown that hydroxo-Cbl has increased liver uptake and less rapid urinary excretion in humans.[35] For these reasons, we had also used hydroxo-Cbl in our previous study on the efficacy of parenteral Cbl supplementation in Beagles with IGS.[13] However, at the time of the present study, cyano-Cbl was the only available PO Cbl product in Switzerland.

Although hundreds of IGS cases have been published worldwide in human medicine, only 3 articles[36–38] have examined the efficacy of PO Cbl supplementation and only 1 article specifically assessed the effect of PO treatment on cellular Cbl status in people.[36] Biweekly PO treatment with 1 mg cyano-Cbl appeared to be a successful maintenance regimen in 14 children with IGS based on clinical signs, hematologic evaluations, and serum Cbl concentrations,[37] whereas 1 mg cyano-Cbl daily for 7 days every month maintained normal clinical health in a teenager with IGS who was monitored for >5 years.[38] However, some abnormalities were identified when cellular Cbl status was assessed in a more recent study. Biweekly PO treatment with 1 mg cyano-Cbl for >5 years resulted in unremarkable hematologic results but did not correct all other markers of Cbl deficiency.[36] Twenty-five percent of patients with congenital Cbl deficiency still had increased concentrations of MMA (n = 4) and homocysteine (n = 1) but unexpectedly had Cbl concentrations within RI, which resulted in discussion of the adequacy of hematologic variables and Cbl concentrations as measures of Cbl status.[36] We also were interested in exploring less intense dosing schedules for our patients, but when owners were asked whether they would consider every-other-day administration of PO Cbl, they declined. Reasons for a lack of interest included the low cost of the PO Cbl product and the impracticability of every-other-day dosing.

We did not measure serum Cbl concentrations routinely because long-term experience has shown that low or even undetectable serum Cbl concentrations often are encountered in clinically healthy Border Collies and Beagles that have received parenteral treatment for IGS.[13,16] This finding also has been reported in clinically healthy Border Collies, Beagles, and Giant Schnauzers that were receiving long-term Cbl supplementation for IGS.[7,13,15,16,18] The results of our previous study on parenteral Cbl supplementation supported this finding with the majority of serum Cbl concentrations (22 of 24 samples) being below the RI (11) or even undetectable (11) in the face of low MMA concentrations.[13] We speculated that the half-life of Cbl bound intracellularly as a coenzyme is longer than the residence time of Cbl in the circulation, which would mean that Cbl continues to be active in tissues and Cbl-dependent metabolism remains normal even when serum concentrations are low. Interestingly, the few available serum Cbl concentrations in this study were all within RI, similar to what has been published recently.[25,26]

Urine MMA results from dog 1 stood out insofar as they initially remained higher than those of dogs 2 and 3. Urine MMA concentrations at t1-t6 were in agreement with those of a previous study when Dog 1 was treated with monthly and bimonthly parenteral Cbl supplementation (Figure 1).[13] In contrast, concurrent evaluation of serum MMA concentrations (t1, t5-t10) showed that all results were within the RI (Figure 1), a finding that had also been previously noted in this dog during parenteral (bimonthly) Cbl supplementation.[13] At that time, we had speculated that bacterial biosynthesis of MMA contributed to the mild increases in urine MMA excretion in dog 1 because several species of bacteria can synthesize MMA and all urine samples were voided samples.[39,40] With regard to this hypothesis, it is important to note that in dog 1, antibiotic treatment for a bacterial skin infection resulted in an abrupt and persistent decrease in urine MMA concentration. The urine MMA concentration decreased after the prescribed course of antibiotics at t7 and remained in the range recorded for the other dogs receiving PO and parenteral supplementation[13] for the next 6 months. Our hypothesis cannot be validated without urine culture results. However, it seems likely that the higher urine MMA concentrations measured in dog 1 in the present and previous studies were caused by bacterial MMA synthesis, especially when one considers that there were no other plausible reasons for a sudden decrease.

Similar to serum MMA concentrations, results of concurrently measured plasma homocysteine concentration in dog 1 indicated adequate Cbl supplementation when compared to published RIs.[41] We had previously been working with a homocysteine RI of 4.3-18.4 μmol/L that was established using HPLC and fluorometric detection in 35 dogs.[27] Because of laboratory and methodology changes, we currently do not have an in-house homocysteine RI. A homocysteine RI of 5-22.1 μmol/L recently was established using a gas chromatography-mass spectrometry (GC-MS) method in 35 healthy dogs.[41] Most homocysteine concentrations in dog 1 were within this RI, whereas 2 of 9 measurements, over the 13-month period of PO supplementation were minimally higher. In this regard, we cannot fully eliminate pre-analytical factors, such as the time between blood sampling and centrifugation and separation of plasma, as a cause of increased homocysteine concentrations. In humans with IGS, normalization of homocysteine concentrations could be achieved with biweekly PO cyano-Cbl (1 mg) treatment.[36] In dogs with IGS, very little is known about homocysteine as a marker for disease monitoring. Although hyperhomocysteinemia has been reported in dogs with IGS with markedly depleted cellular Cbl stores before treatment,[9,16] normalization of homocysteine concentrations has only been reported once in a Beagle that was treated with IM (0.25 mg) Cbl supplementation biweekly[9] and once in a Giant Schnauzer

4 weeks after IM (1 mg) Cbl supplementation.[18] Clearly, the value of homocysteine as a therapeutic marker in dogs with IGS requires further study.

The main limitation of our study is the small number of dogs; however, we were dependent on the willingness of pet owners to cooperate. We are working with several Border Collies and Beagles with IGS that are doing very well clinically with daily PO Cbl supplementation but unfortunately do not have a complete data set to publish these cases. The situation is similar in humans when considering the ratio of treatment studies[36,38] to disease prevalence of IGS.

In conclusion, a maintenance dose of 1 mg cyano-Cbl administered PO appears efficacious for maintaining normal clinical status and cellular markers of Cbl metabolism in dogs with IGS caused by a mutation in the CUBN gene. Our findings further corroborate the hypothesis that an alternative absorptive pathway of Cbl absorption exists in dogs. We assume that comparable results can be achieved when treating other breeds of dogs with IGS caused by different mutations, but this warrants further study.

CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Authors declare no IACUC or other approval was needed.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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