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STANDARD ARTICLE

Effect of oral or subcutaneous administration of cyanocobalamin in hypocobalaminemic cats with chronic gastrointestinal disease or exocrine pancreatic insufficiency

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Abstract

Background: No prospective study has evaluated the efficacy of oral supplementation with cobalamin in hypocobalaminemic cats.

Objectives: To investigate the efficacy of oral or SC supplementation with cyanocobalamin in normalizing serum cobalamin and methylmalonic acid (MMA) concentrations in hypocobalaminemic cats with chronic gastrointestinal disease (CGID) or exocrine pancreatic insufficiency (EPI).

Animals: Forty-eight client-owned hypocobalaminemic (<290 ng/L) cats with normal or abnormally high serum MMA concentrations.

Methods: This study was conducted based on the prospective randomized clinical trial method. Cats with CGID or EPI were randomly assigned to 2 groups that received either oral or SC supplementation with cobalamin (250 μg/cat) for 12 and 10 weeks, respectively, in addition to other medical and dietary interventions. Each cat was evaluated 3 times (baseline, 6-week postsupplementation, and 1-week postcompletion) by measuring serum cobalamin and MMA concentrations.

Results: In cats with CGID or EPI, cobalamin concentrations were normalized in all cats that received either oral or SC supplementation (mean 100% [95% CI: 80.6%-100%] in both groups in cats with CGID and 100% [67.6%-100%] in both groups in cats with EPI). Among 37 cats with elevated MMA concentrations at baseline (21 cats with CGID and 16 cats with EPI), MMA concentrations were normalized in most cats with CGID (70% in oral and 82% in SC group) or EPI (88% in both groups).

Conclusions and Clinical Importance: In hypocobalaminemic cats with CGID or EPI, in conjunction with other medical and dietary interventions, both oral and SC supplementation are effective at normalizing serum cobalamin and MMA concentrations.

Abbreviations: CGID, chronic gastrointestinal disease; EPI, exocrine pancreatic insufficiency; fPLI, feline pancreatic lipase immunoreactivity; fTLI, feline trypsin-like immunoreactivity; GI Lab, gastrointestinal laboratory; IF, intrinsic factor; MMA, methylmalonic acid; RI, reference interval; TVMDL, Texas A&M Veterinary Medical Diagnostic Laboratory; ULRI, upper limit of the reference interval.

KEYWORDS
alternative route, hypocobalaminemia, methylmalonic acid, small intestinal disease

1 | INTRODUCTION

Cobalamin (vitamin B12) is an essential cofactor for 2 intracellular cobalamin-dependent enzymes that play an important role in sustaining cell functions in mammals.[1-3] Dietary cobalamin binds with intrinsic factor (IF) to form a complex before being absorbed through specific receptors in the distal small intestine.[1] Because IF is exclusively produced by acinar cells in the exocrine pancreas in cats, exocrine pancreatic insufficiency (EPI) is a common cause of hypocobalaminemia in cats.[4-8] Chronic small intestinal disease, including low-grade intestinal T-cell lymphoma, has been identified as another common cause of hypocobalaminemia in cats.[9,10] Hepatic/hepatobiliary diseases or pancreatic diseases have been described in hypocobalaminemic cats; however, these diseases were often concurrently present with chronic intestinal diseases, making it difficult to determine that they were the sole cause of hypocobalaminemia in these cases.[9,10] In addition, congenital malfunction of cobalamin receptors is a rare cause of hypocobalaminemia in cats.[11]

Clinical signs in hypocobalaminemic cats are usually due to the primary disease causing hypocobalaminemia or from complications of hypocobalaminemia. Common clinical signs include decreased or increased appetite, lethargy, vomiting, diarrhea, weight loss, and much less commonly neurological signs, failure to thrive, anemia, or pancytopenia with bone marrow failure.[4,6,9,11-14]

Measurement of serum cobalamin concentration is commonly used for the evaluation of cobalamin status in cats. However, it is not a reliable maker for the evaluation of intracellular cobalamin status, and measuring serum methylmalonic acid (MMA) concentration is recommended for it.[15,16] Hypocobalaminemia is defined as having a serum cobalamin concentration below the lower limit of the reference interval (RI), and cobalamin deficiency is defined as having hypocobalaminemia and a concurrent elevation of serum MMA concentration above the upper limit of the RI (ULRI).[15-17]

Cobalamin supplementation is important in hypocobalaminemic cats because untreated cobalamin deficiency can hinder successful treatment for the underlying condition.[7,18] For cobalamin supplementation, the injectable route (SC or IM) has been the main method in cats; however, recent studies in dogs have shown that both oral and injectable supplementation with cobalamin were effective in normalizing serum cobalamin concentrations in hypocobalaminemic dogs caused by chronic gastrointestinal disease (CGID) or EPI.[19-23] Because there is only 1 retrospective study and an abstract available evaluating the efficacy of oral supplementation with cobalamin in hypocobalaminemic cats,[24,25] a randomized prospective clinical trial was needed to evaluate the efficacy of oral supplementation with cobalamin in hypocobalaminemic cats.

The aim of this study was to investigate the efficacy of oral or SC supplementation with cyanocobalamin in normalizing serum cobalamin and MMA concentrations in hypocobalaminemic cats (with or without abnormally high serum MMA) with CGID or EPI. We hypothesized that both oral and SC supplementation with cobalamin would be effective in normalizing serum cobalamin and MMA concentrations in hypocobalaminemic cats with CGID or EPI.

2 | MATERIALS AND METHODS

2.1 | Animals

Eligibility for enrollment of client-owned cats was determined based on the measurement of serum concentrations of cobalamin, folate, feline pancreatic lipase immunoreactivity (fPLI), and feline trypsin-like immunoreactivity (fTLI) through the Gastrointestinal Laboratory (GI Lab) at Texas A&M University. Criteria for initial screening included a history of chronic clinical signs of GI disease for more than 3 weeks' duration, hypocobalaminemia (<290 ng/L), an fPLI within the RI (≤3.5 μg/L), and an fTLI above 20 μg/L (RI: 12-82 μg/L) for CGID cats or an fTLI ≤8 μg/L for EPI cats. Cats with any history of cobalamin supplementation or diagnosed with other metabolic diseases (eg, hyperthyroidism) or extragastrointestinal disease were excluded based on previous (within 2 months) blood work results performed by each cats' primary care veterinarian. Eligibility of enrollment was confirmed based on the results of repeat blood work including CBC, serum chemistry profile, and a second GI panel (serum cobalamin, folate, fPLI, and fTLI) using the criteria described above. Instructions for a venous blood draw, including withholding food for at least 10 to 12 hours, were given to all participating veterinarians. Diet and medication for treatment CGID or EPI other than cobalamin supplementation during this study were decided by the primary care veterinarian. Additionally, a standardized diagnostic workup was not required for enrollment, and the decision regarding it was made by the primary care veterinarian. Before enrollment, an informed consent form was signed by each owner. The animal use protocol for this study was approved by the Animal Care and Use Committee at Texas A&M University (IACUC 2015 – 0286, IACUC 2018 – 0347, IACUC 2021 – 0283).

2.2 | Study design and cobalamin supplementation

This study was performed as a prospective randomized nonblinded clinical trial. Based on the fTLI results, enrolled cats were divided into 2 groups (CGID or EPI), and each cat was then randomly assigned to receive 1 of 3 supplementation options (oral supplementation of cobalamin, oral supplementation of cobalamin and folate, or SC supplementation of cobalamin) irrespective of baseline serum folate concentration. The random assignment for each cat was done by following the ordered number that was created using the block randomization method. Cats assigned to the

oral treatment groups (cobalamin only or cobalamin with folate) received daily oral chews containing either 250 µg of cyanocobalamin or 250 µg of cyanocobalamin and 50 µg of folate (Cobalequin), respectively, for a total of 12 weeks. Both chews were manufactured by Nutramax Laboratories, Inc. (Lancaster, South Carolina). Cats assigned to the injectable treatment group received weekly SC injections of 250 µg of cyanocobalamin (Vitamin B12 injection 1000 µg/mL, SPARHAWK Laboratories, Lenexa, Kansas; or Vitamin B12 injection 1000 µg/mL, VEDCO INC, Saint Joseph, Missouri) for 6 weeks, and an additional SC injection (250 µg of cyanocobalamin) 4 weeks after the last dose for a total of 7 SC injections. Administration of every SC injection was performed by a veterinary professional in a clinic setting. For each cat, 3 visits (baseline and 2 rechecks) were scheduled with their primary care veterinarian. The first recheck was performed 6 weeks after starting cobalamin supplementation (week 7), and the second recheck was performed 1 week after finishing cobalamin supplementation (week 13 for both oral supplementation groups and week 11 for the injectable supplementation group). During each visit, a history was taken, a physical exam was performed, and a fasted blood sample (after withholding food for at least 10-12 hours) was collected by venipuncture.

2.3 | Blood sample processing

About 5 mL of venous blood was collected from each cat for every blood draw with approximately 1 mL of whole blood placed into

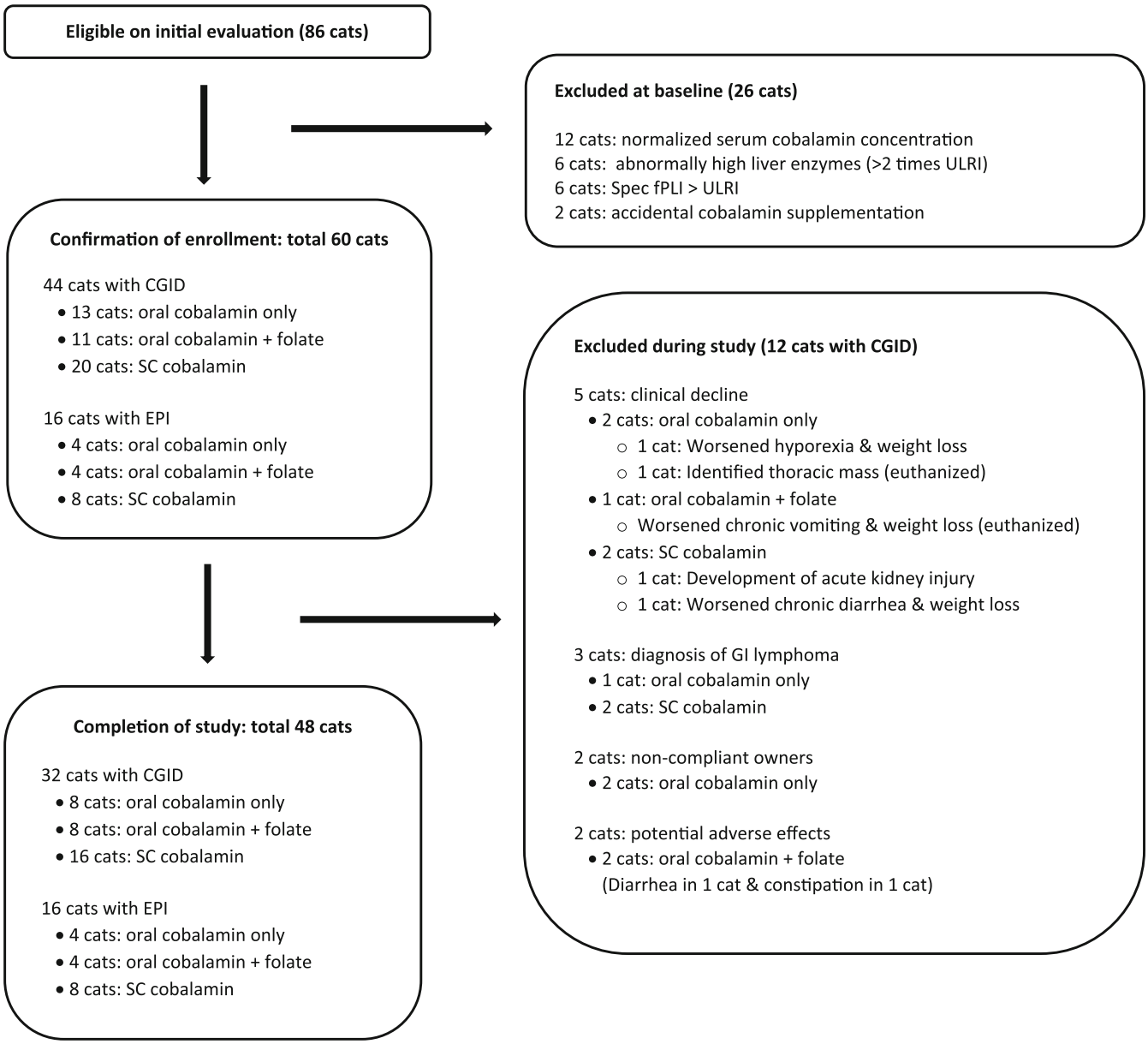

FIGURE 1 This flow chart illustrates the process of study participation from initial evaluation to completion including number of cats of each group, and reasons for exclusion at baseline and during the study. ULRI, upper limit of reference interval.

EDTA anticoagulant-containing blood tubes. The remaining whole blood was centrifuged, and serum was separated and aliquoted into plain red top tubes. The whole blood and serum samples (at least 1.5 mL) were shipped to the GI Lab overnight on an icepack. When shipment within the same business day was not possible, the blood tubes were kept in a refrigerator (3-5°C) overnight or over the weekend (up to 48 hours). Upon receiving the samples, serum samples were aliquoted into several plastic tubes. The EDTA containing blood tubes (for CBC) and one of the tubes with serum (for chemistry profile) were sent to the Texas A&M Veterinary Medical Diagnostic Laboratory (TVMDL). The remaining serum tubes were used for performing a GI panel and for measurement of MMA concentrations.

2.4 | Assays

Complete blood counts were performed using automated instrumentation (ADVIA 120 Hematology System, Siemens, Erlangen, Germany), and a board-certified clinical pathologist at the TVMDL reviewed the blood smear slides of all cats. An automatic analyzer (DxC 700 AU Chemistry Analyzer, Beckman Coulter, Inc., USA) at the TVMDL was used for chemistry profiles. An automated competitive binding chemiluminescence immunoassay system (IMMULITE 2000 XPi, Siemens, Erlangen, Germany) in the GI Lab was used for measuring serum cobalamin and folate concentrations. The RI for serum cobalamin concentration in cats is 290 to 1500 ng/L and lower and upper detection limits of the cobalamin assay are 150 and 1000 ng/L, respectively.[26] When serum cobalamin concentration exceeded 1000 ng/L, the remaining serum sample was diluted and rerun until a numerical value was obtained. The RI for serum folate in cats is 9.7 to 21.6 µg/L. The RI for serum MMA concentration in cats (determined by stable isotope dilution gas chromatography-mass spectrometry as previously described) is 139 to 898 nmol/L, and the RI for serum fTLI (measured by radioimmunoassay) is 12 to 82 µg/L.[15,27] A commercially available ELISA kit (Spec fPL, IDEXX Laboratories, Westbrook, Maine, USA) was used for evaluating serum fPLI, where the RI for fPLI is ≤3.5 µg/L.[28]

2.5 | Daily online questionnaire for owners

Owners were required to fill out a daily online questionnaire during the entire study for each enrolled cat. The questionnaire asked owners to comment on ease of supplement administration (for oral supplementation group only) and to note cat's appetite, episodes of vomiting or diarrhea, and any other adverse effects attributable to the cobalamin supplementation.

2.6 | Statistical analysis

Serum cobalamin, folate, and MMA concentrations were analyzed using a commercially available software package (GraphPad Prism version 9.2 for Windows, GraphPad Software, Boston, Massachusetts, USA, www.graphpad.com). Because all data were not normally distributed based on the Shapiro-Wilk normality test results, the comparison of data (serum cobalamin, folate, and MMA concentrations) between different time points (ie, baseline, first recheck, and second recheck) in each treatment group was performed using the Friedman test followed by Dunn's multiple comparison tests. As there was no significant change in serum folate identified in either oral treatment group (either cobalamin only or cobalamin with folate) or between 2 oral treatment groups, the data from both oral treatment groups were merged to achieve a higher statistical power. Consequently, 2 treatment groups (merged oral and SC) were evaluated for final statistical analyses. Statistical significance was set at $P < .05$.

3 | RESULTS

3.1 | Animals

Based on the initial evaluation, 86 client-owned cats were screened for enrollment. Among them, 38 cats were excluded for various reasons, and 48 cats (each from a different clinic) completed the

TABLE 1 Results (median and range) of body weight and body condition score at each time point in cats with chronic gastrointestinal disease (CGID) or exocrine pancreatic insufficiency (EPI) that received either oral or injectable supplementation with cobalamin.

Measurement	Cat group	Cobalamin supplementation	Baseline			First recheck (week 7)			Second recheck (week 13 or 11)		
			# Cats	Median	Range	# Cats	Median	Range	# Cats	Median	Range
Body weight (kg)	CGID	Oral (n = 16)	16	4.05	3-7.2	12	3.9	2.8-7.1	14	4.1	2.7-8.1
		Injectable (n = 16)	16	4	2.6-7.3	14	4.15	2.9-7	16	4.05	3.1-6.7
	EPI	Oral (n = 8)	8	3.7	2.3-7.1	7	4	3-6.9	8	4.05	3-6.7
		Injectable (n = 8)	8	3.3	2.5-5.4	7	4.3	2.8-5.2	8	4.6	2.8-5.2
Body condition score (1-9)	CGID	Oral (n = 16)	16	4	3-7	12	4	2-8	14	5	2-8
		Injectable (n = 16)	16	4	2-8	14	4	3-8	16	4	3-7
	EPI	Oral (n = 8)	8	4	2-7	7	5	3-6	8	4.5	3-6
		Injectable (n = 8)	8	3	2-5	7	4	2-6	8	5	2-6

Note: The baseline assessment was conducted before initiating cobalamin supplementation, and the second recheck (week 13 for oral supplementation and week 11 for injectable supplementation) was performed 1 week after completing the supplementation.

study, as shown in Figure 1. During the study, the owners of 2 cats chose to withdraw them due to owner-perceived adverse events coinciding with the initiation of oral supplementation with cobalamin (cobalamin + folate). One cat developed diarrhea after initiating supplementation, whereas another cat, with a history of constipation, experienced a relapse of constipation. Domestic shorthair (27 cats with CGID and 14 cats with EPI) was the most common breed in both groups, whereas other breeds included Domestic longhair cat (1 cat with CGID and 1 cat with EPI), Persian (1 cat with CGID), ragdoll (1 cat with CGID), and mixed breed (2 cats with CGID and 1 cat with EPI). The median ages of cats with CGID and EPI were 9 years old (range: 2.5-13 years) and 6 years old (range: 2-12 years), respectively. The sexual status of cats with CGID consisted of 1 intact male, 9 neutered males, and 12 spayed females. The sexual status of cats with EPI included 10 neutered males and 6 spayed females.

TABLE 2 Chief complaints, physical exam findings, and concurrent medical management at baseline and second recheck in cats with chronic gastrointestinal disease (CGID) or exocrine pancreatic insufficiency (EPI).

Chief complaints			
Cats with CGID (32 cats)		**Cats with EPI (16 cats)**	
Baseline (32 cats)	**Second recheck (30 cats)**	**Baseline (16 cats)**	**Second recheck (16 cats)**
Weight loss (18 cats, 56%) Chronic diarrhea (17 cats, 53%) Chronic vomiting (16 cats, 50%) Decreased appetite (4 cats, 13%) Lethargy (3 cats, 9%) More hiding than usual (1 cat, 3%) Weight loss + chronic diarrhea (6 cats, 19%) Weight loss + chronic vomiting (8 cats, 25%) Chronic diarrhea + chronic vomiting (7 cats, 22%) Weight loss + chronic diarrhea + chronic vomiting (2 cats, 6%)	No clinical signs (12 cats, 40%) Chronic diarrhea with partial improvement (6 cats, 20%) Chronic diarrhea without improvement (5 cats, 17%) Continued or not improved weight loss (6 cats, 20%) Intermittent vomiting (5 cats, 17%) Intermittently decreased appetite (4 cats, 13%)	Weight loss (14 cats, 88%) Chronic diarrhea (11 cats, 69%) Increased appetite (8 cats, 50%) Chronic intermittent vomiting (4 cats, 25%) Decreased appetite (2 cats, 13%) Increased drinking (1 cat, 6%) Weight loss + chronic diarrhea (6 cats, 38%) Weight loss + increased appetite (4 cats, 25%) chronic diarrhea + increased appetite (1 cat, 6%) Weight loss + chronic diarrhea + increased appetite (4 cats, 25%)	No clinical signs (12 cats, 75%) Chronic diarrhea with partial improvement (3 cats, 19%) Not improved weight loss (1 cat, 6%)
Physical exam findings			
Cats with CGID (32 cats)		**Cats with EPI (16 cats)**	
Baseline (32 cats)	**Second recheck (30 cats)**	**Baseline (16 cats)**	**Second recheck (16 cats)**
Generalized mild muscle wasting (6 cats, 19%) Mildly thickened SI on palpation (5 cats, 16%) Heart murmur (4 cats, 13%)	Generalized mild muscle wasting (1 cat, 3%) Generalized severe muscle wasting (1 cat, 3%) Mildly thickened SI on palpation (3 cats, 10%) Heart murmur (2 cats, 7%)	Mildly thickened SI on palpation (4 cats, 25%) Generalized mild muscle wasting (3 cats, 19%)	Mildly thickened SI on palpation (2 cats, 13%) Generalized mild muscle wasting (1 cat, 6%) Heart murmur (1 cat, 6%)
Concurrent medical management			
Cats with CGID (32 cats)		**Cats with EPI (16 cats)**	
Baseline (32 cats)	**Second recheck (30 cats)**	**Baseline (16 cats)**	**Second recheck (16 cats)**
Anti-inflammatory dose of prednisolone (3 cats, 9%) Metronidazole (3 cats, 9%) Maropitant (3 cats, 9%) Probiotics (3 cats, 9%) Tylosin (1 cat, 3%) Mirtazapine (1 cat, 3%) Fenbendazole (1 cat, 3%) Omeprazole (1 cat, 3%)	Anti-inflammatory dose of prednisolone (9 cats, 30%) Maropitant (3 cats, 10%) Probiotics (2 cats, 7%) Mirtazapine (2 cats, 7%)	Pancreatic enzyme replacement therapy (8 cats, 50%) Metronidazole (3 cats, 19%) Anti-inflammatory dose of prednisolone (2 cats, 13%) Probiotics (2 cats, 13%) Maropitant (2 cats, 13%)	Pancreatic enzyme replacement therapy (14 cats, 88%) Anti-inflammatory dose of prednisolone (2 cats, 13%) Probiotics (2 cats, 13%) Tylosin (1 cat, 6%) Maropitant (1 cat, 6%) Lactulose (1 cat, 6%) Cisapride (1 cat, 6%)

Note: The baseline assessment was conducted before initiating cobalamin supplementation, and the second recheck was performed 1 week after completing the supplementation.
Abbreviation: SI, small intestine.

TABLE 3 Abnormalities of complete blood counts in cats with chronic gastrointestinal disease (CGID) or exocrine pancreatic insufficiency (EPI).

CGID group (total 32 cats)

				Baseline (n = 30)				First recheck (n = 27)				Second recheck (n = 30)			
Variable	Reference interval	Unit	High/Low	# cats	% cats	Median	Results or range	# cats	% cats	Median	Results or range	# cats	% cats	Median	Results or range
Leukocytes	5.5-19.5	$10^3/\mu L$	Leukocytosis	2	7	25.15	22.2-28.1	1	4	N/A	33.7	1	3	N/A	24.3
			Leukopenia	2	7	4.65	4.6-4.7	1	4	N/A	4.1	1	3	N/A	3.8
Eosinophils	0.1-1.5	$10^3/\mu L$	Eosinophilia	4	13	2.1	1.9-2.2	3	11	2	2-5.5	3	10	4.8	3.4-6.8
Lymphocytes	1.5-7.0	$10^3/\mu L$	Lymphocytosis	1	3	N/A	7.4	-	-	-	-	2	7	N/A	7.6
			Lymphopenia	6	20	0.8	0.1-1.4	3	11	0.5	0.4-1.3	5	17	0.5	0.3-1
Neutrophils	2.5-12.5	$10^3/\mu L$	Neutrophilia	2	7	19.85	18.9-20.8	4	15	15.4	13-28.4	3	10	13.3	12.6-20
			Neutropenia	2	7	1.45	0.8-2.1	1	4	N/A	1.5	3	10	1.26	1.1-1.8
Monocytes	0.1-0.85	$10^3/\mu L$	Monocytosis	1	3	N/A	2.3	4	15	1	0.9-1.1	3	10	1.3	1-1.5
Hematocrit (Hct)	24-45	%	Increased Hct	-	-	-	-	-	-	-	-	1	3	N/A	46.6
Thrombocytes	300-800	$10^3/\mu L$	Thrombocytosis	-	-	-	-	-	-	-	-	1	3	N/A	1455
			Thrombocytopenia with clumps	3	11	134	97-136	3	11	229	222-256	1	4	N/A	192

EPI group (total 16 cats)

				Baseline (n = 16)				First recheck (n = 14)				Second recheck (n = 16)			
Variable	Reference interval	Unit	High/Low	# cats	% cats	Median	Results or range	# cats	% cats	Median	Results or range	# cats	% cats	Median	Results or range
Leukocytes	5.5-19.5	$10^3/\mu L$	Leukocytosis	-	-	-	-	-	-	-	-	2	13	21.5	20.2-22.8
Eosinophils	0.1-1.5	$10^3/\mu L$	Eosinophilia	1	6	N/A	2.4	1	7	N/A	2	-	-	-	-
Lymphocytes	1.5-7.0	$10^3/\mu L$	Lymphocytosis	-	-	-	-	1	7	N/A	7.4	2	13	8.05	7.5-8.6
			Lymphopenia	3	19	1	1-1.3	-	-	-	-	1	6	N/A	0.7
Neutrophils	2.5-12.5	$10^3/\mu L$	Neutrophilia	1	6	N/A	12.8	1	7	N/A	15.3	2	13	16.8	13.5-20.1
			Neutropenia	-	-	-	-	-	-	-	-	1	6	N/A	1.15
Monocytes	0.1-0.85	$10^3/\mu L$	Monocytosis	-	-	-	-	1	7	N/A	0.9	-	-	-	-
Thrombocytes	300-800	$10^3/\mu L$	Thrombocytopenia with clumps	5	31	207	108-266	-	-	-	-	3	19	224	61-251

Note: The baseline assessment was conducted before initiating cobalamin supplementation, and the second recheck (week 13 for oral supplementation and week 11 for injectable supplementation) was performed 1 week after completing the supplementation.
Abbreviation: N/A, not applicable.

TABLE 4 Abnormalities of chemistry profiles in cats with chronic gastrointestinal disease (CGID) or exocrine pancreatic insufficiency (EPI).

CGID group (total 32 cats)

				Baseline (n = 32)				First recheck (n = 28)				Second recheck (n = 28)			
Variable	Reference interval	Unit	High/low	# cats	% cats	Median	Results or range	# cats	% cats	Median	Results or range	# cats	% cats	Median	Results or range
Albumin	3.2-4.7	g/dL	↓ Albumin	11	34	2.7	2.1-3.1	5	18	2.8	2.3-2.9	7	25	2.8	2.3-3
ALT	27-127	U/L	↑ ALT	2	6	175.5	157-194	2	7	196.5	135-258	2	7	167	130-204
BUN	17-35	mg/dL	↑ BUN	1	3	N/A	36	1	4	N/A	42	1	4	N/A	41
			↓ BUN	-	-	-	-	-	-	-	-	2	7	14	N/A
Calcium	8.2-11.5	mg/dL	↓ Calcium	1	3	N/A	8.1	1	4	N/A	8.0	-	-	-	-
Cholesterol	73-265	mg/dL	↓ Cholesterol	2	6	36.5	29-44	3	11	66	25-70	2	7	57.5	51-64
Globulin	2.8-4.8	g/dL	↑ Globulin	-	-	-	-	1	4	N/A	5.5	1	4	N/A	5.9
			↓ Globulin	2	6	2.4	2.3-2.5	-	-	-	-	-	-	-	-
Glucose	63-140	mg/dL	↑ Glucose	1	3	N/A	143	1	4	N/A	144	3	11	160	146-213
Phosphorus	2.7-6.5	mg/dL	↑ Phosphorus	-	-	-	-	-	-	-	-	1	4	N/A	7.3
			↓ Phosphorus	1	3	N/A	2.5	-	-	-	-	-	-	-	-
Total protein	6.5-8.9	g/dL	↑ Total protein	-	-	-	-	-	-	-	-	1	4	N/A	9.6
			↓ Total protein	8	25	5.8	4.4-6.4	4	14	5.8	5.5-6.4	5	18	5.7	5.3-6.2

EPI group (total 16 cats)

				Baseline (n = 16)				First recheck (n = 15)				Second recheck (n = 16)			
Variable	Reference interval	Unit	High/Low	# cats	% cats	Median	Results or range	# cats	% cats	Median	Results or range	# cats	% cats	Median	Results or range
Albumin	3.2-4.7	g/dL	↓ Albumin	4	25	2.8	1.9-3.1	2	13	2.6	2.1-3	2	13	2.5	2-3
ALT	27-127	U/L	↑ ALT	4	25	146	140-165	2	13	163.5	140-187	2	13	170	141-199
BUN	17-35	mg/dL	↑ BUN	2	13	42	36-48	1	7	N/A	54	1	6	N/A	53
Calcium	8.2-11.5	mg/dL	↓ Calcium	1	6	N/A	7.7	-	-	-	-	-	-	-	-
Cholesterol	124-335	mg/dL	↑ Cholesterol	1	6	N/A	369	1	7	N/A	347	2	13	359	274-444
			↓ Cholesterol	1	6	N/A	58	-	-	-	-	-	-	-	-
Glucose	63-140	mg/dL	↑ Glucose	3	19	169	158-189	1	7	N/A	198	4	25	166	143-284
Total protein	6.5-8.9	g/dL	↓ Total protein	6	38	5.9	5.3-6.4	1	7	N/A	5.4	2	13	5.85	5.6-6.1

Note: The baseline assessment was conducted before initiating cobalamin supplementation, and the second recheck (week 13 for oral supplementation and week 11 for injectable supplementation) was performed 1 week after completing the supplementation.
Abbreviations: ALT, alanine transaminase; BUN, blood urea nitrogen; N/A, not applicable.

TABLE 5 Results (median and range) of serum cobalamin and methylmalonic acid concentrations at each time point in cats with chronic gastrointestinal disease (CGID) or exocrine pancreatic insufficiency (EPI) that received either oral or injectable supplementation with cobalamin.

Measurement (reference interval)	Cat group	Cobalamin supplementation	Baseline		First recheck (week 7)				Second recheck (week 13 or week 11)			
			Median	Range	Median	Range	Δ Median	Δ Range	Median	Range	Δ Median	Δ Range
Cobalamin (290-1500 ng/L)	CGID	Oral (n = 16)	149	149 to 244	7083	321 to 14 510	6929	161 to 14 361	3638	509 to 55 139	3456	360 to 54 990
		Injectable (n = 16)	149	149 to 196	2790	1001 to 8869	2641	852 to 8720	1824	969 to 6424	1675	820 to 6228
	EPI	Oral (n = 8)	149	149 to 283	3938	702 to 13 418	3789	553 to 13 269	1522	352 to 16 873	1373	203 to 16 724
		Injectable (n = 8)	149	149 to 287	3666	1001 to 4749	3448	852 to 4600	2453	1001 to 4072	2304	852 to 3785
Methylmalonic acid (139-898 nmol/L)	CGID	Oral (n = 16)	3034	406 to 27 390	340	201 to 1679	−2402	−205 to −25 711	331	169 to 26 889	−1678	23 337 to −27 062
		Injectable (n = 16)	2239	381 to 28 175	457	181 to 3234	−1505	1401 to −27 259	468	191 to 1480	−1398	318 to −27 686
	EPI	Oral (n = 8)	27 000	1190 to 30 342	327	142 to 2182	−25 596	−908 to −29 996	424	222 to 1264	−26 333	−356 to −30 120
		Injectable (n = 8)	22 014	1022 to 27 878	367	174 to 727	−21 415	−567 to −27 636	234	170 to 1325	−21 609	−827 to −27, 445

Note: Δ indicates the difference between each recheck and baseline. The baseline assessment was conducted before initiating cobalamin supplementation, and the second recheck (week 13 for oral supplementation and week 11 for injectable supplementation) was performed 1 week after completing the supplementation.

3.2 | Pertinent history and physical exam findings

History and physical examination were performed at each time point for each cat. The body weight and body condition score at each time point are summarized in Table 1. Additionally, the detailed information available on chief complaints, physical exam findings, and concurrent medical management at baseline and at the second recheck is summarized in Table 2.

3.3 | Complete blood counts and serum chemistry profiles

For each cat, CBC and serum chemistry profiles were completed at 3 time points, and the identified abnormalities are (Tables 3 and 4). No noticeable change in abnormalities was identified over time in the results of CBC and serum chemistry profiles. When CBC results were not available at baseline (2 cats with CGID) due to broken blood tubes during transit, a CBC recently performed by the primary veterinarian (within 2 months before enrollment) was evaluated for enrollment decision.

3.4 | Serum cobalamin concentrations

For all cats that completed the study, serum cobalamin concentrations were available for all 3 time points. At the rechecks, there were 3 cats that did not have sufficient serum to dilute and reanalyze in order to get an exact value for serum cobalamin concentrations; as such, the results (>1000 ng/L) were truncated and reported as 1001 ng/L. These 3 cats included 1 cat with CGID in the SC treatment group at the first recheck and 2 cats with EPI (1 cat with oral and 1 cat with SC treatment group) at both the first and second rechecks. Serum cobalamin concentrations were normalized in all cats with either CGID or EPI that received either oral or SC supplementation (mean 100% [95% CI: 80.6%-100%] in both groups in cats with CGID and 100% [67.6%-100%] in both groups in cats with EPI). In Table 5, serum cobalamin concentrations (median and range) at each time point and the difference (Δ) of serum cobalamin concentrations between each recheck and baseline are reported. In cats with CGID or EPI, both oral and injectable supplementation with cobalamin induced significant increases in serum cobalamin concentrations between baseline and both rechecks (Figures 2 and 3).

3.5 | Serum folate concentrations

Serum folate concentrations at 3 time points were available for all cats that completed the study. At baseline, 3 cats had serum folate concentrations below the lower limit of the RI, as shown in Figures 4 and 5. No significant difference of serum folate concentrations was identified between time points for either the oral or

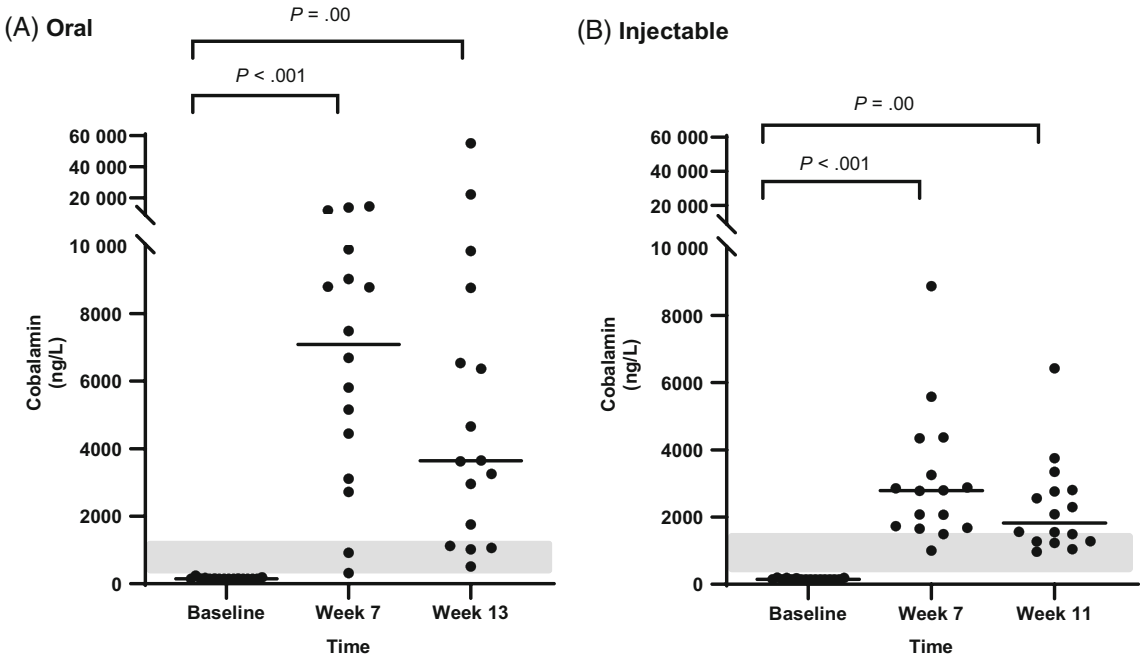

FIGURE 2 Serum cobalamin concentrations (ng/L) at different time points (baseline, first recheck, and second recheck) in cats with CGID that received either oral (A, n = 16) or injectable (B, n = 16) supplementation with cobalamin. The baseline assessment was conducted before initiating cobalamin supplementation, and the second recheck (week 13 for oral supplementation and week 11 for injectable supplementation) was performed 1 week after completing the supplementation. The RI for serum cobalamin concentration (290-1500 ng/L) is shown as a shaded gray area, and the bar shows the median of each dataset.

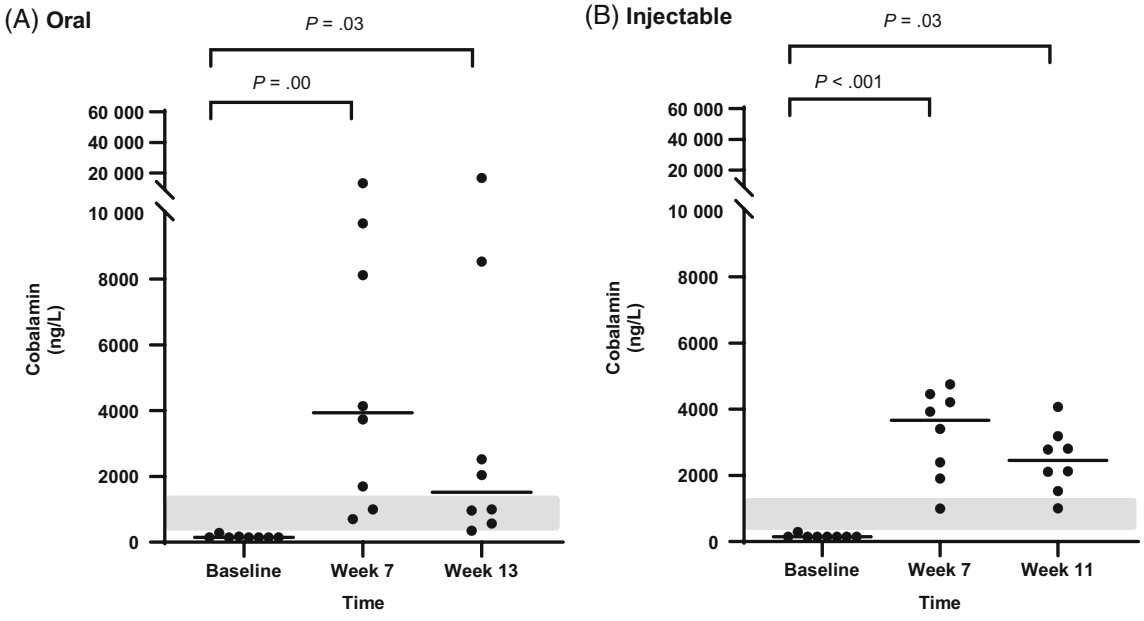

FIGURE 3 Serum cobalamin concentrations (ng/L) at different time points (baseline, first recheck, and second recheck) in cats with EPI that received either oral (A, n = 8) or injectable (B, n = 8) supplementation with cobalamin. The baseline assessment was conducted before initiating cobalamin supplementation, and the second recheck (week 13 for oral supplementation and week 11 for injectable supplementation) was performed 1 week after completing the supplementation. The RI for serum cobalamin concentration (290-1500 ng/L) is shown as a shaded gray area, and the bar shows the median of each dataset.

injectable treatment groups in the CGID cats (Figure 4). In cats with EPI, the oral treatment group showed no significant difference in serum folate concentrations between time points. However, the injectable treatment group had significantly higher serum folate concentrations at the first recheck compared with baseline (Figure 5).

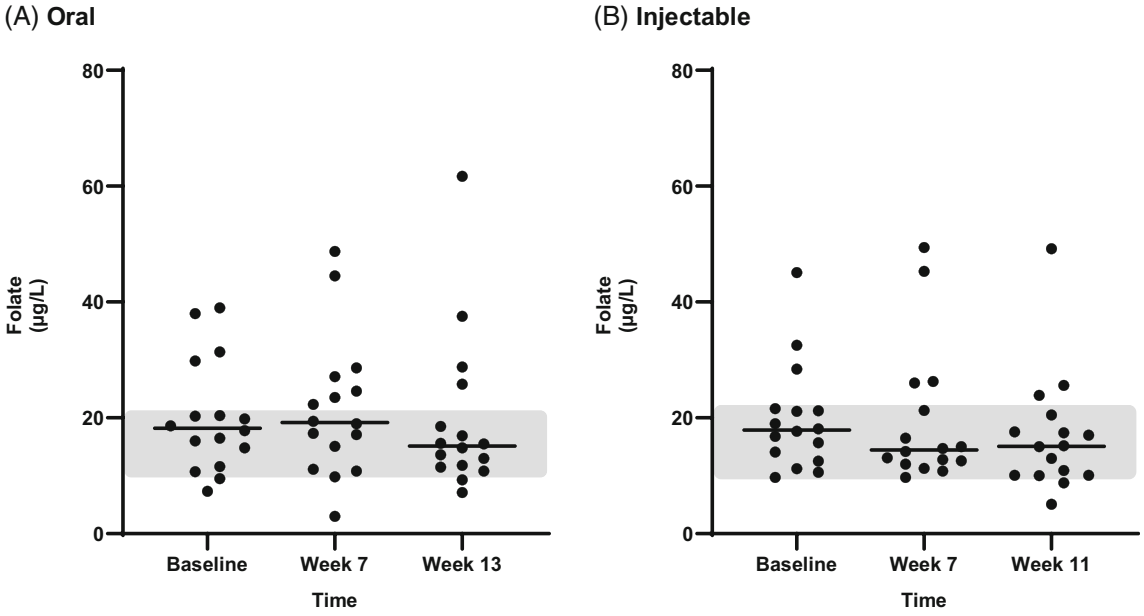

FIGURE 4 Serum folate concentrations (µg/L) at different time points (baseline, first recheck, and second recheck) in cats with CGID that received either oral (A, n = 16) or injectable (B, n = 16) supplementation with cobalamin. The baseline assessment was conducted before initiating cobalamin supplementation, and the second recheck (week 13 for oral supplementation and week 11 for injectable supplementation) was performed 1 week after completing the supplementation. The RI for serum folate concentration (9.7-21.6 µg/L) is shown as a shaded gray area, and the bar shows the median of each dataset.

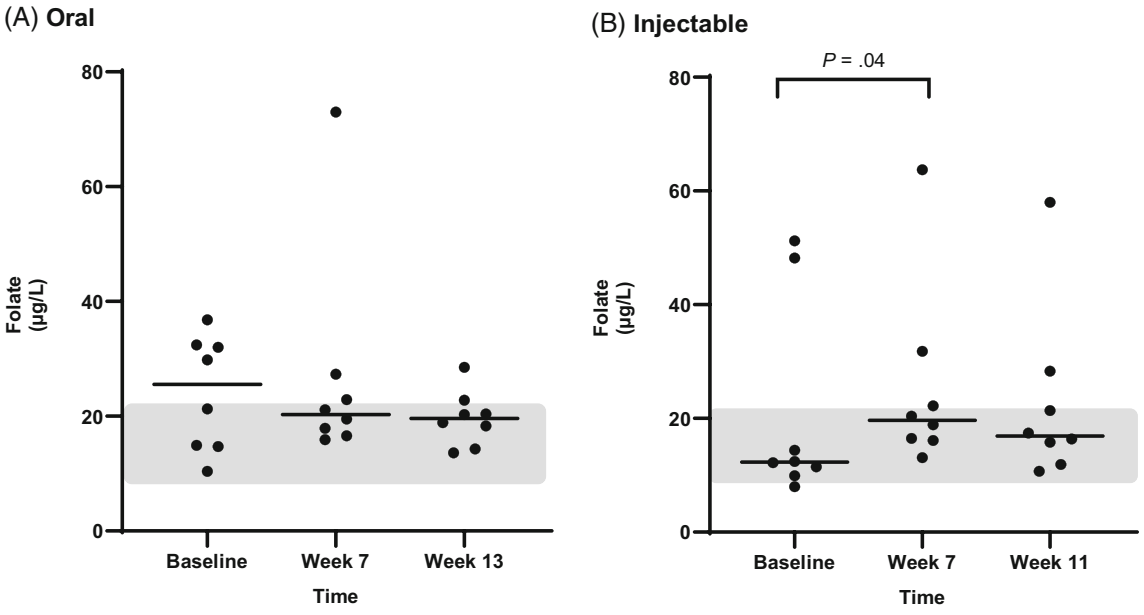

FIGURE 5 Serum folate concentrations (µg/L) at different time points (baseline, first recheck, and second recheck) in cats with EPI that received either oral (A, n = 8) or injectable (B, n = 8) supplementation with cobalamin. The baseline assessment was conducted before initiating cobalamin supplementation, and the second recheck (week 13 for oral supplementation and week 11 for injectable supplementation) was performed 1 week after completing the supplementation. The RI for serum folate concentration (9.7-21.6 µg/L) is shown as a shaded gray area, and the bar shows the median of each dataset.

3.6 | Serum MMA concentrations

For all cats that completed the study, serum MMA concentrations were available for all 3 time points. At baseline, all 16 cats (100%) with EPI and 21/32 cats (66%) with CGID (10/16 cats in the oral and 11/16 cats in the SC group) had elevated serum MMA concentrations indicating cellular cobalamin deficiency. After completion of supplementation, among these 37 cats, serum MMA concentrations were

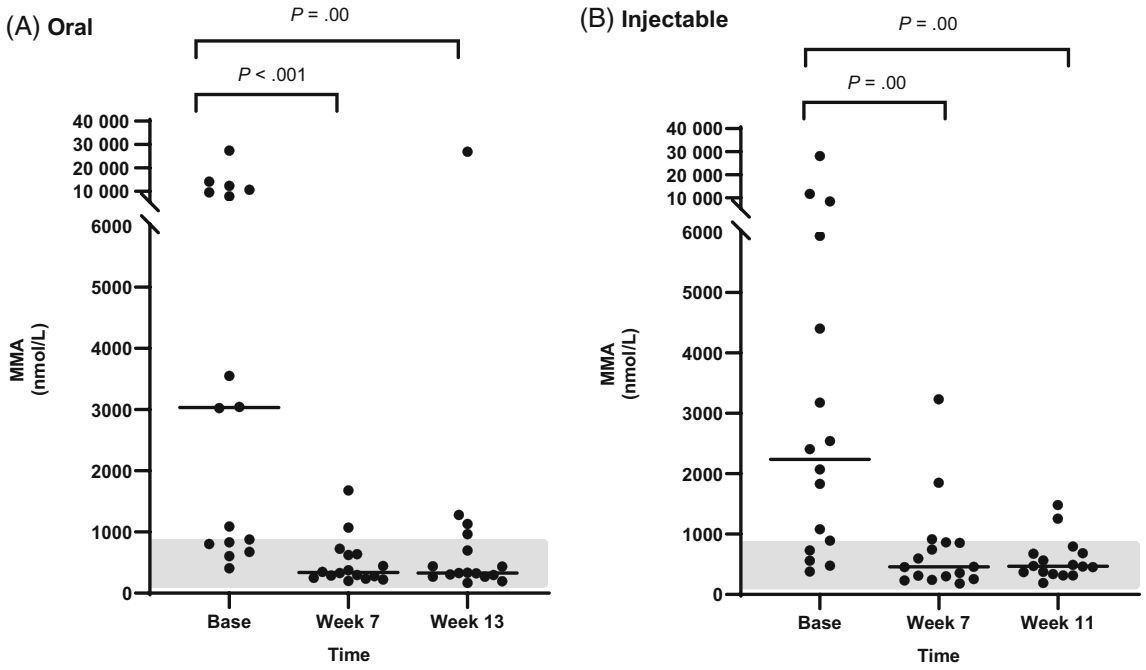

FIGURE 6 Serum MMA concentrations (nmol/L) at different time points (baseline, first recheck, and second recheck) in cats with CGID that received either oral (A, n = 16) or injectable (B, n = 16) supplementation with cobalamin. The baseline assessment was conducted before initiating cobalamin supplementation, and the second recheck (week 13 for oral supplementation and week 11 for injectable supplementation) was performed 1 week after completing the supplementation. The RI for serum MMA concentration (139-898 nmol/L) is shown as a shaded gray area, and the bar shows the median of each dataset.

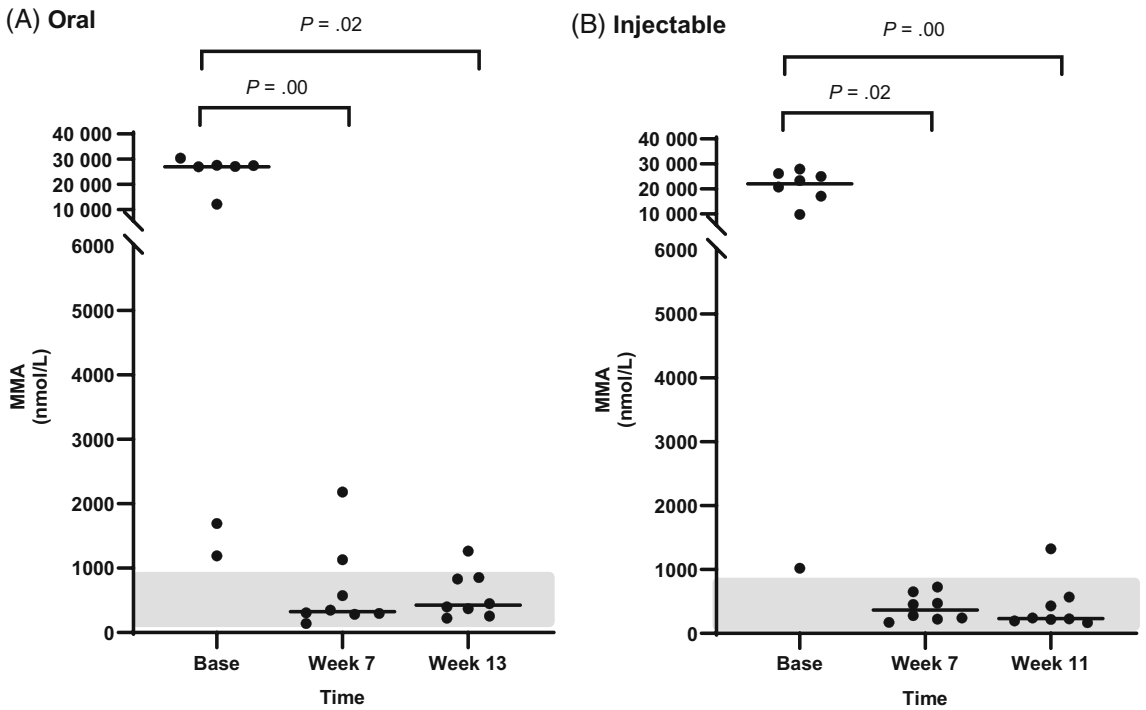

FIGURE 7 Serum MMA concentrations (nmol/L) at different time points (baseline, first recheck, and second recheck) in cats with EPI that received either oral (A, n = 8) or injectable (B, n = 8) supplementation with cobalamin. The baseline assessment was conducted before initiating cobalamin supplementation, and the second recheck (week 13 for oral supplementation and week 11 for injectable supplementation) was performed 1 week after completing the supplementation. The RI for serum MMA concentration (139-898 nmol/L) is shown as a shaded gray area, and the bar shows the median of each dataset.

TABLE 6 Results (median and range) of body weight and body condition score at each time point in cats with chronic gastrointestinal disease (CGID) or exocrine pancreatic insufficiency (EPI) that received either oral or injectable supplementation with cobalamin.

Measurement (reference interval)	Cat group	Cobalamin supplementation	Baseline		First recheck (week 7)		Second recheck (week 13 or 11)	
			Median	Range	Median	Range	Median	Range
fPLI (≤3.5 μg/L)	CGID	Oral (n = 16)	1.7	0.9-3.2	2.3	1.1-14.4	1.7	1-50
		Injectable (n = 16)	2	0.7-3.1	1.85	0.8-3.7	2.15	1.2-5.5
	EPI	Oral (n = 8)	1.5	0.6-2.6	1.45	0.6-4	1.05	0.6-5.6
		Injectable (n = 8)	1.4	0.7-2.6	1.05	0.9-8.3	1.4	0.6-9.7
fTLI (12-82 μg/L)	CGID	Oral (n = 16)	43.8	20.1-92.1	43.4	20.1-98	46.9	21.7-151.6
		Injectable (n = 16)	49.2	26-90.6	40.8	19.7-141.4	41.2	25.5-101.4
	EPI	Oral (n = 8)	2	0.1-7.1	1.1	0.1-16	0.6	0.1-25.9
		Injectable (n = 8)	3.9	1.8-7.8	3.1	1.7-8.2	4.6	2.1-10.3

Note: The baseline assessment was conducted before initiating cobalamin supplementation, and the second recheck (week 13 for oral supplementation and week 11 for injectable supplementation) was performed 1 week after completing the supplementation.

TABLE 7 Summary of daily online questionnaire responses in both treatment groups.

		Oral treatment		Injectable treatment	
Cat group		CGID (14 cats)	EPI (8 cats)	CGID (16 cats)	EPI (8 cats)
Ease of administration (yes)		13 cats (93%)	8 (100%)	N/A	N/A
Vomiting	None	2 cats (14%)	2 cats (25%)	2 cats (12%)	2 cats (25%)
	1-5 days total	9 cats (65%)	4 cats (50%)	7 cats (44%)	3 cats (37.5%)
	6-10 days total	2 cats (14%)	2 cats (25%)	3 cats (19%)	3 cats (37.5%)
	>10 days total	1 cat (7%)	0	4 cats (25%)	0
Diarrhea	1-5 days total	7 cats (50%)	8 cats (100%)	10 cats (62%)	8 (100%)
	Intermittent	5 cats (36%)	0	3 cats (19%)	0
	Persistent	2 cats (14%)	0	3 cats (19%)	0
Appetite	Same to increased	6 cats (43%)	0	8 cats (50%)	0
	Same	8 cats (57%)	8 (100%)	7 cats (44%)	8 (100%)
	Decreased to same	0	0	1 cat (6%)	0

normalized in most cats with CGID (7/10, 70% [39.7%-89.2%] in the oral group and 9/11, 82% [52.3%-96.7%] in the SC group) or EPI (88% [52.9%-99.4%] in both oral and SC groups). Table 5 shows serum MMA concentrations (median and range) at each time point and the difference (Δ) of serum MMA concentrations between each recheck and baseline. In cats with CGID or EPI, both oral and injectable supplementation with cobalamin caused a significant decrease of serum MMA concentrations between baseline and both rechecks (Figures 6 and 7).

3.7 | Serum fPLI and fTLI concentrations

Serum fPLI and fTLI concentrations for all 3 time points were available for all cats that completed the study, and the results (median and range) are described in Table 6. Serum fPLI was within RI at baseline in all cats with CGID or EPI. Serum fTLI was above 20 μg/L in all cats with CGID and was decreased, consistent with EPI, in all cats with EPI at baseline. Two cats with EPI in the oral treatment group had serum fTLI concentrations within the RI at either recheck.

3.8 | Online daily questionnaire for owners

The summarized results of the questionnaire are shown in Table 7.

4 | DISCUSSION

The results from this prospective, randomized clinical trial show that when used in conjunction with other medical and dietary interventions, both oral and SC supplementation with cobalamin are effective at increasing serum cobalamin concentrations in hypocobalaminemic cats with either CGID or EPI. Many of these cats (21/32 cats with CGID and all 16 cats with EPI) had elevated serum MMA concentrations in addition to hypocobalaminemia. Accordingly, this study also

indicates that both oral and SC supplementation with cobalamin, when combined with other medical and dietary interventions, effectively decrease serum MMA concentrations in cats with hypocobalaminemia or cobalamin deficiency caused by CGID or EPI.

The efficacy of SC or IM supplementation with either cyanocobalamin or hydroxocobalamin in normalizing or improving serum cobalamin or both serum cobalamin and MMA concentrations in hypocobalaminemic cats with CGID or EPI is described in several studies.[18,29,30] Our study used cyanocobalamin rather than other forms of cobalamin, such as hydroxocobalamin, for SC supplementation to align with the cyanocobalamin administered orally. Additionally, our study was designed before the efficacy of hydroxocobalamin given IM in cats was described. However, there are only 2 studies that have evaluated the efficacy of oral supplementation with cobalamin in hypocobalaminemic cats. The first study, which is only available as an abstract, evaluated the efficacy of oral supplementation with cobalamin in 14 geriatric cats (>12 years of age) with idiopathic fat malabsorption that did not, however, have EPI.[25] In this study, oral supplementation with cobalamin was given to all cats by adding the mixture (1 mg of commercially available cobalamin solution and 1 mL of a liquid flavor enhancer) to the food once daily for 2 months. Only 4/14 cats had hypocobalaminemia (<290 ng/L) and the other 10 cats had normal serum cobalamin concentrations right before starting the supplementation. Serum cobalamin concentrations were above the ULRI after 1 week of daily administration in all 14 cats (1892-13 109 ng/L) and remained above the ULRI during the supplementation in 12 cats. The second, retrospective study evaluated the efficacy of oral supplementation with cobalamin in 25 cats with GI diseases, including 2 cats with EPI (only 12/25 cats had a measurement of fTLI for evaluation of EPI).[24] Oral supplementation (250 μg of cyanocobalamin tablets) was given once daily to all 25 hypocobalaminemic cats (<290 ng/L) or low normal serum cobalamin concentrations (within the lowest 10% of the RI [290-1000 ng/L]), and the serum cobalamin concentration was reevaluated at recheck (between 28 and 94 days). This study demonstrated that serum cobalamin concentration was significantly increased and normalized in all cats at recheck. However, this study had several limitations due to its retrospective nature. First, reevaluation of serum cobalamin concentration after initiating supplementation was done only once at various time points. Also, serum MMA concentration was measured only in 2 cats. Lastly, as only 2 cats with confirmed EPI were included and about a half of the cats (13/25) were not evaluated for EPI, it was difficult to differentiate cats with different types of underlying conditions.

Our current study is the first prospective study evaluating the efficacy of oral supplementation with cobalamin in hypocobalaminemic cats with CGID or EPI. The results of this current study are similar to those of the previously mentioned 2 studies showing the efficacy of oral supplementation with cobalamin in increasing serum cobalamin concentrations in hypocobalaminemic cats with chronic GI signs.[24,25] In contrast to the previous studies, this study had 2 distinct groups of cats (ie, CGID or EPI) allowing evaluation of each group after cobalamin supplementation. Also, this study measured serum MMA concentrations in all cats, allowing the evaluation of cobalamin status on a cellular level.

The results of this study in cats with CGID showed that more cats in the oral treatment group (11 cats in the first recheck and 6 cats in the second recheck) than in the injectable treatment group (2 cats in the first recheck and 1 cat in second recheck) had very high serum cobalamin concentrations (> 5000 ng/L). In cats with EPI, the serum cobalamin concentrations in some cats (3 cats in the first recheck and 2 cats in the second recheck) in the oral treatment group were very high (> 5000 ng/L); however, none of the cats in the injectable treatment group had serum cobalamin concentrations >5000 ng/L. This might be due to the wider range of serum cobalamin concentrations achieved with oral supplementation compared to injectable supplementation as previously shown in dogs.[19] These very high serum concentrations were also identified in some cats in the previous 2 studies that evaluated the efficacy of oral supplementation with cobalamin in cats.[24,25] An interesting finding is that none of the dogs that received either oral or injectable supplementation with cobalamin in previous studies had been reported to have such very high serum cobalamin concentrations after supplementation, indicating a potential difference between dogs and cats.[19,20,23]

Also, in this current study, all cats (except for 1 cat with EPI that received oral supplementation with cobalamin) had serum cobalamin concentrations greater than 400 ng/L at the second recheck. Consequently, no further supplementation was required based on the current recommendations from the GI Lab at Texas A&M University. All cats in the previous retrospective study evaluating oral supplementation with cobalamin had serum cobalamin concentrations greater than 400 ng/L at the recheck, as well.[24] In contrast, in the previous study with hypocobalaminemic dogs, 6/27 dogs with CGID (3 dogs in the oral and 3 dogs in the injectable treatment group, respectively) and 5/19 dogs with EPI (5 dogs in the injectable treatment group) had serum cobalamin concentration less than 400 ng/L at the second recheck, suggesting a need for continued supplementation with cobalamin.[19] This difference between cats and dogs might be related to the use of relatively higher dosages of supplemented cobalamin in cats compared to dogs, dissimilar cobalamin receptor expressions and subsequently different cobalamin absorption capacity in the small intestine, or disparate cobalamin metabolism after absorption between the species.

Our study evaluated serum MMA concentrations in all cats at all 3 time points, allowing us to evaluate the cobalamin status on a cellular level before and after finishing oral or injectable supplementation with cobalamin. This is the first study that evaluated serum MMA concentrations after oral supplementation with cobalamin in hypocobalaminemic cats. In cats with CGID, a significant decrease of serum MMA concentrations was identified with both oral and injectable supplementation with cobalamin indicating that both routes of supplementation were effective in significantly improving or normalizing the cobalamin status on a cellular level. This finding is consistent with the results of previous studies in hypocobalaminemic dogs with CGID, showing significantly decreased serum MMA concentrations after oral or injectable supplementation with cobalamin.[19,21] Interestingly, 6/32

cats (19%) with CGID (4 cats and 2 cats in the oral and injectable treatment group, respectively) had excessively high serum MMA concentrations (>10 000 nmol/L) at baseline. Similarly, some cats in the previous studies evaluating injectable supplementation with cobalamin in hypocobalaminemic cats with CGID reported excessively high serum MMA concentrations at baseline.[29,30] This finding might indicate a difference between cats and dogs or that the cats evaluated had more severe cobalamin deficiency compared to the dogs examined in previous studies. Among the 32 cats with CGID in this study, 21 cats (66%, 10 cats and 11 cats in the oral and injectable treatment group, respectively) had serum MMA concentrations higher than the ULRI, indicating cobalamin deficiency at baseline, and all these 21 cats had normalized or improved serum MMA concentrations at either recheck. The remaining 11 cats (34%, 6 cats and 5 cats in the oral and injectable treatment group, respectively) that had serum MMA concentrations within the RI at baseline also had decreased serum MMA concentrations at either recheck, indicating improved intracellular cobalamin status after supplementation.

In cats with EPI, a significant decrease of serum MMA concentrations was also found with both oral and injectable supplementation with cobalamin showing that when used in conjunction with other medical and dietary interventions, both routes of supplementation are effective at improving or normalizing cobalamin status on a cellular level. Interestingly, 12/16 cats (75%) with EPI (6 cats in both the oral and the injectable treatment groups, respectively) had excessively high serum MMA concentrations (>10 000 nmol/L) at baseline in our study. The reason why the hypocobalaminemic cats with EPI have more severe cobalamin deficiency than hypocobalaminemic cats with CGID or hypocobalaminemic dogs with CGID or EPI is not clear; however, this might be related to differences in disease severity. EPI in cats is still less well known than in dogs, which may delay proper diagnosis and thus lead to more severe cobalamin deficiency. Of special note, all 16 cats (100%) with EPI in this study had serum MMA concentrations above the ULRI at baseline, indicating cellular cobalamin deficiency, compared to only 21/32 cats (66%) with CGID at baseline. After either oral or injectable supplementation with cobalamin, serum MMA concentrations were significantly improved or normalized in all 16 cats with EPI. This finding is different from the results in hypocobalaminemic dogs with EPI in the previous study, showing a significant decrease of serum MMA concentrations only in the oral treatment group, but not in the injectable treatment group.[19] This difference between the 2 studies might be related to differences in cobalamin metabolism between cats and dogs or to the relatively higher dosage of supplemented cobalamin given to cats compared to dogs. In the previous study with dogs with EPI, only half of the dogs had serum MMA concentrations above the ULRI, suggesting a cobalamin deficiency at baseline.[19] Indeed, 1 previous study described cobalamin supplementation as one of the predictors for having a positive clinical response to the treatment in cats with EPI.[7] In addition, similarly to dogs, oral supplementation with cobalamin might be a convenient alternative for owners who do not want to bring their cats to the veterinary clinic for repeated injections of cobalamin or who do not feel comfortable giving injections at home. Also, most cats with EPI receive daily pancreatic enzyme replacement therapy by mouth, and adding oral supplementation with cobalamin might be an easier option for some owners than giving injections. Interestingly, 2 cats with EPI in the oral treatment group had serum fTLI concentrations within the RI at either recheck (one cat at the first recheck and the other at the second recheck). This finding might be related to fluctuations of fTLI due to ongoing concurrent pancreatic inflammation and destruction of acinar cells.

Our findings might indicate that shorter duration or less frequent supplementation with cobalamin than the currently recommended protocol might be sufficient to normalize serum cobalamin and MMA concentrations in most hypocobalaminemic cats. A recent study evaluating the efficacy of hydroxocobalamin given intramuscularly (total 4 injections given every 2 weeks) in cats with cobalamin deficiency showed that this protocol was efficacious in normalizing serum cobalamin and MMA concentrations in most cats.[30] Unfortunately, little information is currently available regarding the ideal duration of oral supplementation with cobalamin or when to repeat measurement of serum cobalamin concentrations after discontinuing supplementation in cats. The circulating half-life of cobalamin after 1 SC injection with 1 mg of cyanocobalamin was reported to be shorter in sick cats with GI disease than in healthy cats in one study (5 days vs 12.75 days); however, only 2 cats with inflammatory bowel disease and 4 healthy cats were evaluated in that study.[9] Our study showed a decreasing trend of serum cobalamin concentrations at the second recheck (evaluated 1 week after discontinuing the cobalamin supplementation) compared to the first recheck. However, this current study's design did not allow for long-term evaluation of serum cobalamin concentrations after discontinuation of supplementation. The results of 2 previous studies using intramuscular supplementation with either cyano- or hydroxocobalamin described the similar pattern showing a decreasing trend of serum cobalamin concentrations and an increasing trend of serum MMA concentrations at the rechecks up to 4 to 10 weeks postcompletion of supplementation.[29,30] Until further research is completed, repeated measurements of serum cobalamin concentrations after discontinuation of supplementation might be beneficial to evaluate the need for continued cobalamin supplementation.

Even though several studies, including this current study, have shown the efficacy of oral supplementation with cobalamin in improving/normalizing serum cobalamin and MMA concentrations in hypocobalaminemic cats and dogs, the exact mechanism of cobalamin absorption in the compromised GI tract or in the absence of IF in cats or dogs with EPI is unclear.[19,20,22-25]

Several limitations should be mentioned for this study. First, due to the limited range of diagnostic tests performed for each cat, specific disease entity causing chronic GI signs could not be definitively determined. For example, some cats might have had pancreatitis as pancreatitis cannot be completely ruled out based on a fPLI test result within the RI. Additionally, 8 cats had elevated serum fPLI concentrations at either recheck despite having a serum fPLI concentration within the RI at baseline; however, only 2/8 cats had a serum fPLI concentration >8.8 µg/L, which is a newly suggested decision

threshold for diagnosing pancreatitis in cats.[31] Second, because various diets were fed to the enrolled cats during the study, different cobalamin content in various diets might have affected serum cobalamin concentrations. Despite diet-related hypocobalaminemia seems to be rare even in cats fed with vegetarian diets,[32] a further study evaluating the effect of diet on serum cobalamin concentrations in cats is warranted. Third, because various therapies were given to the enrolled cats for the treatment of their underlying diseases (CGID or EPI), this might have independently influenced the control of underlying disease status and subsequent cobalamin absorption in the GI tract and cobalamin concentrations in serum. Finally, at the end of the study, the remaining number of oral cobalamin chews was not counted, leaving a possibility of having noncompliant owners who did not follow the study instruction for daily oral administration of oral cobalamin chews.

In conclusion, when used in conjunction with other medical and dietary interventions using the dosing strategies described above, both oral and SC supplementation with cobalamin showed efficacy for normalizing cobalamin status in blood and on a cellular level in hypocobalaminemic cats with CGID or EPI. Consequently, oral supplementation with cobalamin can be used as an alternative option for supplementation in hypocobalaminemic cats with CGID or EPI.

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CONFLICT OF INTEREST DECLARATION

All authors are employed by the Gastrointestinal Laboratory at Texas A&M University, which offers measurement of serum cobalamin and methylmalonic acid concentrations on a fee for service basis. Dr. Steiner acts as a consultant for Nutramax Laboratories, Inc., and Dr. Steiner, Dr. Suchodolski, and Dr. Lidbury also act as paid speakers for Nutramax Laboratories, Inc.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

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

The animal use protocol for this study was approved by the Animal Care and Use Committee at Texas A&M University (IACUC 2015 – 0286, IACUC 2018 – 0347, IACUC 2021 – 0283).

HUMAN ETHICS APPROVAL DECLARATION

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

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