

A Comprehensive Pathological Survey of Duodenal Biopsies from Dogs with Diet-Responsive Chronic Enteropathy

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Background: The detailed pathological phenotype of diet-responsive chronic enteropathy (CE) and its modulation with dietary therapy remain poorly characterized.

Hypothesis/Objectives: Key mucosal lesions of diet-responsive CE resolve with dietary therapy.

Methods: This was a prospective observational study of 20 dogs with diet-responsive CE. Endoscopic duodenal biopsies collected before and 6 weeks after the start of a dietary trial were assessed by means of qualitative and quantitative histopathological, immunohistochemical, and ultrastructural criteria. Control duodenal biopsies were obtained from 10 healthy Beagle dogs on 1 occasion.

Results: Compared with control dogs, the CE dogs had higher villus stunting scores and higher overall WSAVA scores, a lower villus height-to-width ratio, and higher lamina propria density of eosinophils. The CE dogs also had ultrastructural lesions of the mitochondria and brush border. In common with other studies in which the disease and control populations are not matched for breed, age, sex, and environment, these comparisons should be interpreted with caution. Comparing biopsies collected at presentation and 6 weeks after starting the dietary trial, mean lamina propria mononuclear cell score and lamina propria densities of eosinophils and mononuclear cells decreased. Dietary therapy also improved ultrastructural lesions of the mitochondria and brush border, eliciting a decrease in intermicrovillar space and an increase in microvillus height.

Conclusions and Clinical Importance: In dogs with diet-responsive CE, the remission of clinical signs with dietary therapy is associated with subtle decreases in lamina propria density of eosinophils and mononuclear cells, and resolution of ultrastructural lesions of the enterocyte.

Key words: Eosinophil; Inflammatory bowel disease; Microscopy; Permeability; Ultrastructure; WSAVA standards.

Idiopathic inflammatory bowel disease (IBD) is a complex disorder that is thought to involve defective epithelial barrier function and mucosal tolerance,

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Abbreviations:

CCECAI	canine chronic enteropathy clinical activity index
CE	chronic enteropathy
CI	confidence interval
CRP	C-reactive protein
G	3-O-D-methylglucose
GSD	German Shepherd Dog
IEL	intraepithelial lymphocyte
IQR	interquartile range
L	lactulose
QMHA	Queen Mother Hospital for Animals
R	rhamnose
RVC	Royal Veterinary College
X	D-xylose

coupled with imbalances in the intestinal microbial flora.^{1,2} Although IBD is a common diagnosis in dogs with chronic gastrointestinal signs, pathogenesis of the disorder in this species is poorly understood.³ Clinical response to dietary manipulation or antibiotic treatment in some dogs has prompted the emergence of descriptors such as “diet-responsive enteropathy”⁴ and “antibiotic-responsive diarrhea”,⁵ reflecting the relative importance of dietary factors and dysbiosis to the pathogenesis of the disease in specific dogs. Some gastroenterologists reserve the term “IBD” for dogs requiring immunosuppressive treatment to achieve clinical remission, when dietary and antibiotic trials have failed. Regardless of the clinical terms used to describe this spectrum of disorders, they have all previously been characterized by the presence of variable degrees of intestinal inflammation and may be identified by the

generic label “chronic enteropathy” (CE).^{6–9} However, increasing recognition of the uncertainties of histopathological identification of mucosal inflammation^{3,10} and the apparent discordance between clinical presentation and severity of inflammation¹¹ have prompted the generation of a standardized histopathological scoring system accommodating both inflammatory and morphological observations under the auspices of the World Small Animal Veterinary Association (WSAVA).¹² One of the conundrums of the field has been the apparent lack of resolution of duodenal histopathological lesions, examined either by qualitative or quantitative criteria, with clinical remission of CE.^{13–15} We have re-visited this phenomenon in this study by means of a comprehensive survey of inflammatory and morphological duodenal lesions in a cohort of dogs with diet-responsive CE, assessed by means of the WSAVA scoring system and a number of quantitative criteria at both the light and electron microscopic level.

Materials and Methods

Clinical Cases and Controls

Twenty-nine dogs with gastrointestinal signs of at least 4 weeks' duration were recruited into this study by the Small Animal Internal Medicine Service of the Queen Mother Hospital for Animals (QMHA) at the Royal Veterinary College (RVC) between March 2007 and July 2008; all dogs were at least 8.0 kg in body weight at the time of presentation. The study was approved by the RVC Ethics and Welfare Committee, and written informed consent was sought before recruitment of all cases. Of the 29 dogs initially recruited, 20 dogs completed the 6-week study and are described in this manuscript, whereas the remaining 9 were withdrawn because of deterioration in clinical signs requiring immunosuppressive treatment (2), discovery or development of comorbidities (immune-mediated disease, 2; neoplastic pulmonary lesions, 1; cardiac arrhythmia, 1), intractability of the patient (1), and failure of the owner to present the dog for follow-up evaluation (2). All dogs underwent a thorough diagnostic evaluation (including a CBC, serum biochemical profile, routine urinalysis of a voided sample, fecal flotation, abdominal ultrasound examination by a board-certified radiologist, assays for serum trypsin-like immunoreactivity [TLI] and canine pancreatic-specific lipase immunoreactivity [cPLI], serum folate and cobalamin concentrations, and an ACTH stimulation test) to rule out the underlying structural, metabolic, endocrine, neoplastic, infectious, and autoimmune disease. The administration of corticosteroids within 4 weeks of presentation, the presence of panhypoproteinemia, or the necessity for extended hospitalization or immunosuppressive treatment, prompted by the presence of systemic ill health or rapid deterioration of clinical signs, all were considered exclusion criteria. Videoendoscopy of the stomach, duodenum, and colon was performed in every dog after withholding food for 36–48 hours and preparation with an osmotic laxative and 2 warm-water enemas. At the time of procurement of biopsies for this study, ileoscopy was not routinely performed in every dog by all study endoscopists in the QMHA. Using standard nonserrated forceps, at least 10 endoscopic biopsy samples deemed to be of adequate quality (ie, clearly visible villi with discernible depth of mucosa) were harvested from the cranial duodenal flexure and at least 4 biopsy samples were collected from each of 4 regions of the stomach (pyloric antrum, body, incisura angularis, and fundus or cardia) and each of the 3 segments of

the colon (ascending, transverse, and descending). This study reports the results of the duodenal biopsies. Of these, at least 8 were placed into 10% v/v formalin-saline and at least 2 were placed into glutaraldehyde/paraformaldehyde fixative for electron microscopy (see below). All biopsy samples were processed within 48 hours after collection. Control tissues comprised endoscopic biopsies collected from the cranial duodenal flexure of 10 healthy Beagle dogs immediately after euthanasia, undertaken for reasons unrelated to this study. The health of these dogs, including fecal consistency, had been monitored daily, and necropsy examinations performed while harvesting the duodenal biopsies were unremarkable.

Study Design

This was a prospective observational study of dogs with diet-responsive CE. Dogs were evaluated at presentation (week 0) and then 2 and 6 weeks after starting a “hypoallergenic” diet based on hydrolyzed soy protein.^a Serum C-reactive protein (CRP) concentration was measured and a combined test of intestinal permeability and absorptive function was performed at each visit. Gastroduodenoscopy was performed at weeks 0 and 6, after all other diagnostic tests had been completed. Clinical signs were assessed by means of the canine chronic enteropathy clinical activity index (CCECAI)¹⁴ on all visits. Dogs were discharged from the study at week 6, but were subsequently monitored by means of telephone consultations for periods of at least 6 months.

Intestinal Permeability and Absorptive Function

On the day after presentation (week 0) or the day of presentation (weeks 2 and 6), a solution containing 10 g/L lactulose (L), 10 g/L D-xylose (X), 10 g/L rhamnose (R), 10 g/L 3-O-D-methylglucose (G), and 40 g/L sucrose was administered to each dog by means of an orogastric tube, on each occasion after an overnight fast. Dogs weighing <10 kg received 100 mL of the sugar solution, those weighing from 10 to 20 kg received 200 mL, and those weighing >20 kg received 400 mL. Dogs were allowed free time to urinate in a run before administration of the sugar solution. Midstream (voided) urine samples were collected and an aliquot of 10 mL was stored with sodium azide at a final concentration of 0.1% w/v at –20°C until the time of analysis to quantitate baseline concentrations of the sugars, which were all zero or trivial. Dogs were kept in their kennels for 6 hours after administration of the sugar solution. A midstream urine sample again was collected at 6 hours, from which an aliquot of 10 mL was stored with 0.1% w/v sodium azide at –20°C until analysis. Ultrasound-guided cystocentesis was performed in dogs that did not urinate. If a dog urinated in the kennel during the 6 hours after administration of the sugar solution, the test was aborted. Urinary L/R and X/G ratios were determined by means of gas chromatography-mass spectrometry at the Gastrointestinal Laboratory at Texas A&M University using an established protocol.

Serum C-Reactive Protein

Serum C-reactive protein concentrations were determined by means of a commercial, previously validated ELISA,^b performed according to the manufacturer's recommendations.¹⁶

Endoscopic Biopsy Evaluation

Light Microscopy. Slides of duodenal biopsies were examined at low power^c to identify and label all longitudinally sectioned, well-orientated villi that were associated with, or adjacent to,

cryptal tissue and subvillous lamina propria. According to the criteria of Willard et al, none of the biopsies examined in this study was considered “inadequate” in quality.¹⁷ A random number generator (www.random.org) was used to select 5 “eligible” villi per dog per visit. By consensus of the study participants, this number was chosen as a workable compromise between wishing to capture as much information as possible from the available biopsy material and the time required for analysis. Cryptal tissue associated with these villi was assessed in parallel; if the chosen villi had no or insufficient crypts, the nearest adjacent cryptal tissue on the slide was examined. Evaluation of the chosen villi and cryptal tissue was both qualitative and quantitative, the former by means of the WSAVA Standards for the Diagnosis of Gastrointestinal Inflammation in Endoscopic Biopsy Samples.¹² We undertook qualitative scoring on the basis of the described lesions, in which severity was scored as 0 = normal, 1 = mild, 2 = moderate, and 3 = marked for each respective criterion; in certain instances, lesions were deemed to fall between whole scores, prompting the use of half scores. All initial histopathological assessments for clinical purposes were undertaken by a board-certified anatomical pathologist (KS). After pilot studies undertaken by AM and CD under the supervision of the senior author (OAG) and consultation with KS to ensure consistency of approach, all qualitative and quantitative data recorded in this study were collected by OAG by independent assessment of all biopsies from all dogs to eliminate interobserver variability.

Morphometric Criteria. Quantitative variables in the assessment of duodenal biopsies included (1) villous height—taken as the distance from the base of the villus to its tip, bisecting the villus along its length and taking the sum of individual measurements if the villi were not straight in orientation; (2) villous width—taken as the maximum distance from 1 side of the villus to the other, measuring perpendicular to the midline of the villus; (3) numbers of intraepithelial lymphocytes and goblet cells per 100 enterocytes, counting enterocytes, lymphocytes, and goblet cells on 1 side of the villus or the other, determined randomly by flipping a coin; and (4) lamina propria areal density of mononuclear cells, eosinophils, and neutrophils—defining the region of interest as the middle one-third of the villus, excluding the central lacteal, muscle fibers, and blood vessels, and counting mononuclear cells indirectly by dividing the total nuclear area by the mean area of 10 randomly chosen representative nuclei, and eosinophils and neutrophils directly by identifying them on the section. A specific macro was designed to enable areal densities to be calculated by image analysis software⁴; results were expressed as number of cells per 10,000 μm^2 of lamina propria.

Immunohistochemistry. Five well-orientated villi associated with cryptal tissue were randomly chosen for immunohistochemical assessments, in a similar manner to those for routine hematoxylin and eosin staining. When possible, villi were matched on the CD3 and IgA slides; if villi could not be paired in this way, a random number generator (www.random.org) was used to select unmatched villi in place of the corresponding villi. A routine protocol for immunohistochemical staining was applied (see Supporting Information). Areal densities of CD3⁺ and IgA⁺ cells were determined in a similar fashion to those for the hematoxylin and eosin-stained sections, defining the middle third of the villus and excluding lacteals, muscle fibers, and blood vessels. A specific macro was developed to enable areal densities to be calculated based on cytoplasmic staining of positive cells, expressing the results as numbers of cells per 10,000 μm^2 of lamina propria.

Ultrastructural Lesions. Biopsies were processed for electron microscopy in routine fashion, with fixation in a 2% glutaraldehyde and 2% paraformaldehyde solution in sodium cacodylate buffer, before sequential staining in 1% osmium tetroxide and 2% uranyl acetate. After dehydration, infiltration with resin,

trimming of blocks, preparation of semithin and ultrathin sections, and counterstaining with lead citrate, the sections were assessed both qualitatively and quantitatively by transmission electron microscopy.⁵ A random number generator (www.random.org) was used to select 5 well-orientated villi. Randomly picking 1 side of the villus or the other by flipping a coin, 5 adjacent enterocytes from the middle one-third of each villus were chosen for assessment, measuring 10 microvilli per enterocyte. Owing to the large volume of data collected in these analyses (approximately 33,000 data points), measurements were made on endoscopic biopsies harvested from a random sample of 5 of the 20 study dogs. The assessment protocol was developed in house by OAG specifically for this study and is described in the Supporting Information. S Miller undertook a pilot study under the supervision of OAG and S Miah, while AK-T collected all of the ultrastructural data in this study, verified by independent measurements on 2 of the same cases by OAG. Qualitative observations included an assessment of (1) mitochondrial lesions, (2) microvillar vesiculation, and (3) cytoplasmic vesiculation (scoring the lesions from 1 [mild] to 3 [severe], 0 being normal); quantitative observations included (1) microvillous height and diameter, (2) intermicrovillar space, and (3) tight junction width.

Statistical Analyses

The clinical, histopathological, immunohistochemical, and ultrastructural data at presentation and during the study were summarized as mean (\pm standard deviation [SD]) and median (interquartile range [IQR]). Normality was assessed by visual inspection of histograms of the data. In the case of the quantitative histopathological measurements of intraepithelial lymphocytes per 100 enterocytes, goblet cells per 100 enterocytes, and CD3⁺ and IgA⁺ cells per 10,000 μm^2 , logarithmic transformation was undertaken before further statistical analysis.

A linear mixed-effects model was adopted to analyze quantitative clinical variables with time (visit number), accounting for repeated measures from the same dog. The quantitative histopathological and immunohistochemical data were analyzed in the same fashion, both at presentation and with visit number. A generalized linear mixed-effects model was adopted to compare qualitative histopathological data at presentation and with visit number, accounting for repeated measures from the same dog and recoding all of the scores as 0 or 0.5+ for the majority of variables, owing to the low number of higher scored observations. In the case of “villous stunting”, “mucosal fibrosis”, and “lamina propria mononuclear cells”, sufficient higher scored observations were made to allow recoding in 3 (rather than 2) groups, namely 1 = scores 0 and 0.5, 2 = scores 1 and 1.5, and 3 = scores 2, 2.5, and 3.

A linear mixed-effects model also was adopted to compare quantitative ultrastructural data at presentation and with visit number. Generalized mixed-effects models were used for the corresponding qualitative ultrastructural data at presentation and during the study. Qualitative scores for the 10 microvillous repeats for each of the enterocytes were identical, and thus only 1 microvillus was used for each enterocyte. “Dog”, “villus within dog”, and “enterocyte within villus within dog” were considered as random effects in all models. Likelihood ratio tests were used to assess significance of effects between nested models. When appropriate, *posthoc* analysis was undertaken by Tukey contrasts, and estimated overall difference between groups or visits was reported as mean \pm standard error of the mean (SEM) or, for all of the qualitative variables other than World Small Animal Veterinary Association score, odds ratios, and 95% confidence intervals (95% CI). All analyses were carried out by a statistical software package.¹

Results

Clinical Cases and Controls

Twenty dogs completed the study, comprising 5 Labrador Retrievers (25% of the study cohort), 2 Staffordshire Bull Terriers (10%), 2 Boxers (10%) and a single German Shepherd Dog (GSD), West Highland White Terrier, Border Terrier, Cocker Spaniel, Greyhound, Lurcher, Rough Collie, Standard Poodle, GSD-cross, English Bulldog-cross, and Rottweiler \times Newfoundland (each 5% of the study cohort). Of a total of 1,586 dogs presenting to the Internal Medicine Service of the QMHA over the same time period, there were 41 Labrador Retrievers (2.6% prevalence), 45 Staffordshire Bull Terriers (2.8%), and 73 Boxers (4.6%). Three study dogs were intact males, 6 neutered males, 1 intact female, and 10 neutered females. Median age at presentation was 3.1 (IQR, 2.1) years, with a range of 0.8 to 10.3 years, whereas mean (\pm SD) body weight at presentation was 26.5 ± 11.4 kg, with a range of 8.68 to 50.5 kg. Clinical signs included inappetence (6/20); vomiting (14/20), sometimes with hematemesis (4/20); small (3/20), large (1/20), or mixed (13/20) bowel diarrhea; weight loss (3/20); melena (3/20); and periodic borborygmus and flatulence (2/20), associated with overt abdominal pain in 1 case. Clinical signs had been observed for periods ranging from 1 month to the lifetime of the dog. A number of therapies had been administered before presentation, all without inducing clinical remission, including a variety of antibiotics (metronidazole [6/20], cefalexin [1/20], amoxicillin/clavulanate [4/20], enrofloxacin [2/20], marbofloxacin [1/20], erythromycin [1/20], and spiramycin [1/20]), H₂ blockers (4/20), sucralfate (4/20), omeprazole (1/20), metoclopramide (1/20), sulfasalazine (1/20), ursodiol (1/20), a probiotic (1/20), kaolin (1/20), and a pancreatic enzyme supplement (1/20). None of the dogs had been exposed to the hypoallergenic diet^a used in the study before recruitment, but all had undergone some form of nonspecific dietary manipulation before presentation without clinical improvement. Median CCECAI at presentation was 6.5 (4.0), ranging from 3 to 15.

Median serum cPLI concentration at presentation was 30.0 (53.3) μ g/L, ranging from 30 to 362 μ g/L. Two dogs (both Boxers) had results above the reference limit of 200 μ g/L. However, both results were lower than the commonly accepted threshold of 400 μ g/L for the diagnosis of pancreatitis, and both dogs had unremarkable abdominal ultrasound examinations and showed clinical responses to dietary treatment. Median serum vitamin B12 concentration was 368 (208) ng/L, ranging from 150 to 1,200 ng/L. Only a single dog had a serum vitamin B12 concentration below the reference limit of 200 ng/L. Nevertheless, this animal showed a convincing clinical response to dietary treatment. Median serum TLI concentration was 9.0 (5.9) μ g/L, ranging from 5.1 to 26.1 μ g/L. Two dogs had serum TLI concentrations just below the reference limit of 6.0 μ g/L, but all serum TLI

concentrations were above 2.5 μ g/L and these 2 dogs showed clinical responses to dietary treatment.

Histopathological, Immunohistochemical, and Ultrastructural Findings on Presentation

Sixteen of the dogs with CE had mild lymphoplasmacytic inflammation of the duodenum, in 8 cases associated with mild eosinophilic infiltrates and in 1 case associated with mild, mixed (eosinophilic/neutrophilic) infiltrates. One dog had mild-to-moderate lymphoplasmacytic inflammation associated with mild, mixed infiltrates, and 1 dog had moderate-to-marked plasmacytic inflammation. Additional observations included mild villous atrophy and increased lymphocytic exocytosis across the villous epithelium. The group of dogs with CE showed more villous stunting and higher overall WSAVA scores than the healthy control dogs, but all other individual qualitative lesion scores showed no significant differences between the groups (Table 1). Of the quantitative variables, villous height-to-width ratio was lower and the density of eosinophils in the lamina propria was higher in the dogs with CE than in control dogs (Table 2). There were no significant differences in villous height, counts of IELs or goblet cells, and densities of mononuclear cells, neutrophils, CD3⁺ cells, or IgA⁺ cells between the groups (Table 2). Ultrastructural lesions were identified in the dogs with CE, which showed increased severity of mitochondrial lesions and cytoplasmic vacuolation, increased space between microvilli, and increased tight junction width at presentation when compared with the healthy control dogs (Table 3; Fig 1).

Clinical Findings with Dietary Treatment

The owners of all dogs perceived an improvement in clinical signs after beginning the hypoallergenic diet.^a Furthermore, all owners chose to continue feeding their dogs the hypoallergenic diet for at least 6 months from inception of the study, during which time the dogs remained asymptomatic or experienced only trivial clinical signs.

Mean body weight increased from weeks 0 to 6 ($P = .007$) by a 0.87 ± 0.29 kg (Fig 2A). Mean CCECAI decreased from weeks 0 to 2 ($P < .0001$), but then showed no significant change from weeks 2 to 6 ($P = .44$; Fig 2A). Estimated overall decrease in CCECAI was 4.55 ± 0.72 from weeks 0 to 2 and 5.43 ± 0.73 from weeks 0 to 6. Similarly, mean serum CRP concentration decreased from weeks 0 to 2 ($P = .02$), but then showed no significant change from weeks 2 to 6 ($P = .98$; Fig 2A). However, of 55 serum samples, only 1 had a CRP concentration above the upper limit of the reference interval of 7.6 mg/L—an outlying result of 29.7 mg/L in a single dog on week 0. Estimated overall decrease in serum CRP concentration was 3.56 ± 1.33 mg/L from weeks 0 to 2 and 3.79 ± 1.33 mg/L from weeks 0 to 6.

Table 1. Histopathological lesion scores at presentation.

Variable	Group	Mean	SD	Median	IQR	Min	Max	Estimated Odds Ratio (95% CI)	P Value
Villous stunting	Control	0.80	0.64	1.0	0.63	0	2	3.56 (1.43–8.89)	.007
	CE	1.43	0.90	1.5	1.5	0	3		
IELs	Control	0.04	0.13	0	0	0	0.5	5.89 (0.69–50.1)	.07
	CE	0.18	0.37	0	0	0	2.0		
Epithelial injury	Control	0.32	0.34	0.5	0.5	0	1	0.46 (0.10–2.10)	.33
	CE	0.26	0.36	0	0.5	0	1.5		
Crypt distension	Control	0	0	0	0	0	0	NA	NA
	CE	0.14	0.43	0	0	0	2		
Lacteal dilatation	Control	0.02	0.09	0	0	0	0.5	1.11 (0.12–10.4)	.92
	CE	0.04	0.21	0	0	0	1.5		
Mucosal fibrosis	Control	0.54	0.61	0.5	1	0	1.5	2.39 (0.50–11.4)	.27
	CE	0.81	0.68	0.75	1.5	0	3		
Mononuclear cells	Control	0.59	0.45	0.5	1	0	1.5	0.80 (0.27–2.36)	.68
	CE	0.52	0.56	0.5	1	0	2.5		
Eosinophils	Control	0	0	0	0	0	0	NA	NA
	CE	0.11	0.36	0	0	0	2		
Neutrophils	Control	0.07	0.18	0	0	0	0.5	1.04 (0.28–3.90)	.95
	CE	0.07	0.18	0	0	0	0.5		
Overall WSAVA score	Control	2.38	1.09	2.25	1.5	0.5	5.0	1.17 ± 0.47 ^a	.015
	CE	3.55	1.66	3.50	2	0	8.5		

SD, standard deviation; IQR, interquartile range; CI, confidence interval; CE, chronic enteropathy; IEL, intraepithelial lymphocyte; NA, not applicable: statistical tests unreliable because all values in the control group were 0. The estimated odds ratio compares CE with control data.

^aA linear effects model was used to analyze data for overall WSAVA score, for which the corresponding metric of difference is the “estimated overall difference ± standard error of the mean” rather than an odds ratio.

Table 2. Morphometric and immunohistochemical data at presentation.

Variable	Group	Mean	SD	Median	IQR	Min	Max	Estimated Overall Difference (± SEM)	P Value
Villous height (µm)	Control	557	89	592	120	325	669	55.1 ± 34.9	.11
	CE	502	178	485	263	188	965		
Villous width (µm)	Control	201	40	192	38	129	299	20.3 ± 10.6	.06
	CE	222	39	221	54	132	346		
Villous height: width ratio	Control	2.85	0.61	2.78	0.97	1.70	3.98	−0.50 ± 0.26	.05
	CE	2.35	1.05	2.10	1.19	0.77	7.31		
IELs per 100 enterocytes	Control	8.57	3.03	8.71	5.30	2.35	13.1	0.13 ± 0.15 ^a	.39
	CE	10.7	5.80	9.46	7.22	1.22	30.1		
Goblet cells per 100 enterocytes	Control	4.41	2.27	4.15	3.74	1.22	8.57	0.08 ± 0.20 ^a	.72
	CE	4.43	2.92	4.11	3.36	0.00	14.1		
Mononuclear cells per 10,000 µm ²	Control	140.7	12.7	138.9	18.2	118.8	168.5	10.5 ± 8.4	.21
	CE	151.3	30.5	147.3	48.5	100.1	218.4		
Eosinophils per 10,000 µm ²	Control	0.15	0.29	0.00	0.07	0.00	1.05	0.83 ± 0.33	.012
	CE	0.98	1.02	0.84	0.83	0.00	7.53		
Neutrophils per 10,000 µm ²	Control	0.07	0.17	0.00	0.00	0.00	0.60	0.01 ± 0.04	.87
	CE	0.08	0.20	0.00	0.00	0.00	0.98		
CD3 ⁺ cells per 10,000 µm ²	Control	19.3	7.18	19.0	9.35	6.70	37.6	0.31 ± 0.25 ^a	.21
	CE	31.8	24.2	23.5	23.9	4.3	123.2		
IgA ⁺ cells per 10,000 µm ²	Control	36.0	16.3	30.2	19.5	14.0	84.1	0.09 ± 0.25 ^a	.76
	CE	39.0	27.2	31.5	30.0	5.8	126.4		

SEM, standard error of the mean; IQR, interquartile range; CE, chronic enteropathy; IEL, intraepithelial lymphocyte. The estimated overall difference compares CE with control data.

^aEstimated overall difference (± SEM) is on a logarithmic scale.

Mean urinary L/R ratio showed no significant differences between hospital visits ($P = .86$; Fig 2B). Mean urinary X/G ratio increased from weeks 2 to 6 (0.77 ± 0.31 ; $P = .03$), but there was no significant difference in X/G ratio between weeks 0 and 2 ($P = .11$) or weeks 0 and 6 ($P = .87$; Fig 2B).

Histopathological, Immunohistochemical, and Ultrastructural Findings with Dietary Treatment

Of all the qualitative variables assessed by the WSAVA Standards, only the mean mononuclear cell score in the lamina propria showed a significant

Table 3. Ultrastructural data at presentation.

Variable	Group	Mean	SD	Median	IQR	Min	Max	Estimated Odds Ratio (95% CI)	Estimated Overall Difference (\pm SEM)	<i>P</i> Value																																																																																																								
Mitochondrial lesions	Control	0.17	0.49	0	0	0	3	24.2 (4.34–135)	0.00 \pm 0.20	.0006																																																																																																								
	CE	0.84	1.12	0	2	0	3				Microvillar vesiculation	Control	0.07	0.36	0	0	0	3	3.69 (0.0013–10,440)	41.3 \pm 13.9	.48	CE	0.27	0.82	0	0	0	3	Cytoplasmic vacuolation	Control	0.11	0.36	0	0	0	2	31.3 (4.57–214.6)	2.87 \pm 1.46	.0002	CE	0.82	1.09	0	2	0	3	Microvillous height (μ m)	Control	1.81	0.46	1.77	0.66	0.13	4.17	8.43 \pm 5.11	.10	.007	CE	1.82	0.67	1.70	0.88	0.29	3.98	Microvillous width (nm)	Control	93.9	20.3	90.0	30.0	10.0	150.0	2.87 \pm 1.46	.49		CE	102.4	19.4	100.0	20.0	10.0	170.0	Intermicrovillar space (nm)	Control	26.9	30.6	20.0	20.0	3.0	380	41.3 \pm 13.9	.007		CE	68.2	112.4	40.0	60.0	10.0	1,320	Tight junction width (nm)	Control	10.5	3.70	9.0	4.0	0	34.0	2.87 \pm 1.46	.49		CE	13.4	14.5
Microvillar vesiculation	Control	0.07	0.36	0	0	0	3	3.69 (0.0013–10,440)	41.3 \pm 13.9	.48																																																																																																								
	CE	0.27	0.82	0	0	0	3				Cytoplasmic vacuolation	Control	0.11	0.36	0	0	0	2	31.3 (4.57–214.6)	2.87 \pm 1.46	.0002	CE	0.82	1.09	0	2	0	3	Microvillous height (μ m)	Control	1.81	0.46	1.77	0.66	0.13	4.17	8.43 \pm 5.11	.10	.007	CE	1.82	0.67	1.70	0.88	0.29	3.98	Microvillous width (nm)	Control	93.9	20.3	90.0	30.0	10.0	150.0	2.87 \pm 1.46	.49		CE	102.4	19.4	100.0	20.0	10.0	170.0	Intermicrovillar space (nm)	Control	26.9	30.6	20.0	20.0	3.0	380	41.3 \pm 13.9	.007		CE	68.2	112.4	40.0	60.0	10.0	1,320	Tight junction width (nm)	Control	10.5	3.70	9.0	4.0	0	34.0	2.87 \pm 1.46	.49		CE	13.4	14.5	10.0	3.0	5.0	201														
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SEM, standard error of the mean; IQR, interquartile range; CE, chronic enteropathy; IEL, intraepithelial lymphocyte. The estimated odds ratio (for qualitative data) and estimated overall difference (for quantitative data), respectively, compare CE with control data.

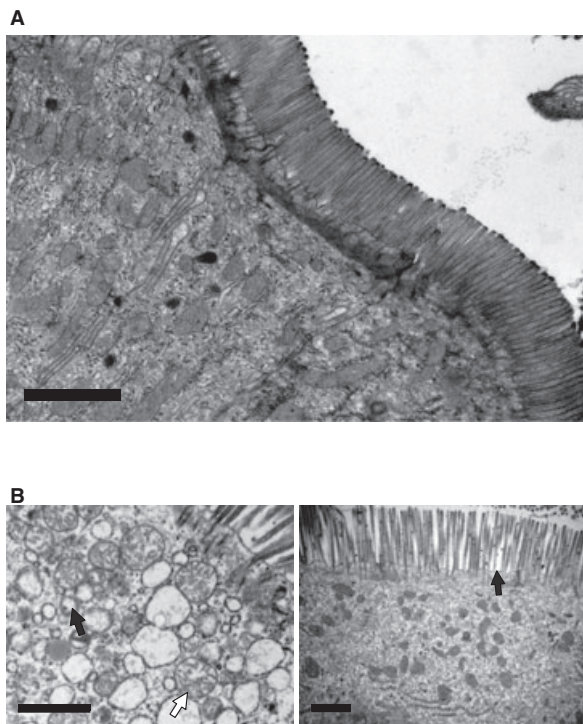


Fig 1. Ultrastructural lesions in dogs with diet-responsive chronic enteropathy. Part (A) shows a transmission electron micrograph of several enterocytes within an endoscopic duodenal biopsy from a healthy Beagle dog, showing no signs of mitochondrial cristolysis, cytoplasmic vacuolation, or microvillar vesiculation (Bar = 2 μ m). By contrast, the left-hand panel in part (B) illustrates extensive cytoplasmic vacuolation (black arrow) and mitochondrial swelling and cristolysis (white arrow), whereas the right-hand panel shows increased intermicrovillar spacing (black arrow), in an endoscopic biopsy harvested from a dog with chronic enteropathy before dietary treatment (Bar = 1 μ m).

decrease from weeks 0 to 6 ($P = .01$), with an estimated odds ratio of 0.45 (95% CI, 0.23–0.85; Fig 3A). All of the other variables showed no

significant differences between visits (Supporting Information).

The mean density of eosinophils in the lamina propria decreased from weeks 0 to 6 ($P = .02$) by 0.26 ± 0.11 per 10,000 μm^2 (Fig 3B). Similarly, the mean density of mononuclear cells in the lamina propria decreased between visits ($P = .04$) by 7.77 ± 3.69 per 10,000 μm^2 (Fig 3B). However, the mean density of neutrophils in the lamina propria showed no change with time (mean \pm SD, 0.08 ± 0.20 [week 0] versus 0.06 ± 0.19 [week 6] per 10,000 μm^2 ; $P = .54$), and when specific populations of mononuclear cells were examined by immunohistochemical staining, there were no differences in mean density of either CD3⁺ cells ($P = .27$) or IgA⁺ cells ($P = .52$; Fig 3C). Similarly, mean numbers of IELs ($P = .06$) and goblet cells ($P = .42$) per 100 enterocytes, and villous height ($P = .76$), width ($P = .17$) and height: width ratio ($P = .61$), all showed no significant change between visits (Supporting Information). The number of IELs per 100 enterocytes increased in 9/20 dogs and decreased in 11/20 dogs, prompting us to view the near-significant result for this variable with caution.

A number of ultrastructural lesions also showed improvement with dietary treatment. Of the qualitative variables, the mitochondrial lesion score decreased from weeks 0 to 6 ($P = .03$), with an estimated odds ratio of scores of >0 over this time of 0.33 (95% CI, 0.13–0.88; Fig 4A). In contrast, neither cytoplasmic vacuolation ($P = .07$) nor microvillar vesiculation ($P = .43$) scores showed any significant overall difference between visits. Nevertheless, 4/5 dogs showed a decrease in cytoplasmic vacuolation score, but the comparison between visits was not significant, suggesting the possibility of a type II error in the analysis of this variable (Supporting Information). Of the quantitative variables, mean intermicrovillar space decreased from weeks 0 to 6 ($P = .005$) by 31.7 ± 11.1 nm (Fig 4B,C). By comparison, mean microvillous height increased from weeks 0 to 6 ($P = .02$) by

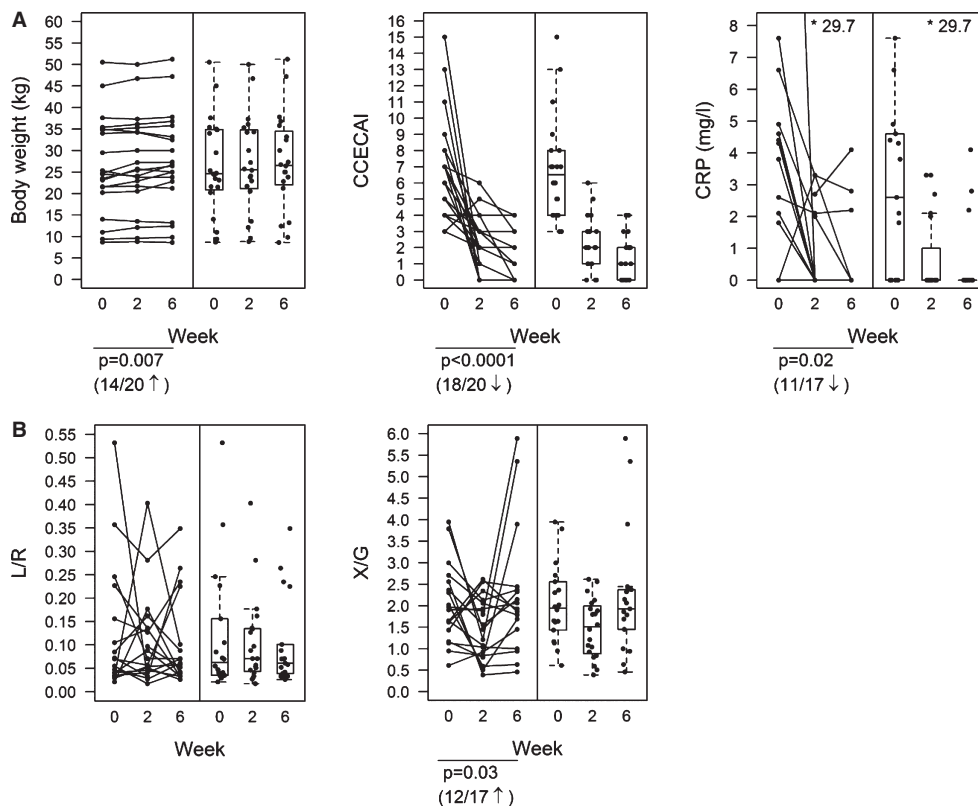


Fig 2. Clinical findings in dogs with chronic enteropathy during a 6-week period of dietary treatment. Data from the 20 study dogs are shown at presentation (week 0) and 2 weeks and 6 weeks after starting a hypoallergenic diet. Each panel shows a dot plot, with measurements from individual dogs joined by a line to highlight changes, and an associated box-and-whisker plot showing summary data with overlain individual data points. The boxes show the respective 25th and 75th percentiles, the horizontal lines median values, and the whiskers, the lowest and highest data points still within 1.5 times the interquartile range of the respective lower and upper quartiles; a single outlying value is represented by means of an asterisk. All P values $\leq .05$ are shown under the respective plots, whereas the numbers of observations from individual dogs concordant with the prevailing changes in mean values (increase/decrease) are shown in parentheses. (Not all dogs had all measurements taken at every visit.) Part (A) shows an increase in mean body weight and decrease in mean canine chronic enteropathy clinical activity index (CCECAI) and serum C-reactive protein (CRP) concentration from weeks 0 to 2. Intestinal permeability measured by the urinary lactulose/rhamnose (L/R) ratio showed no overall change ($P = .86$), whereas mean intestinal absorptive capacity measured by the urinary xylose/3-O-methylglucose ratio increased from weeks 2 to 6 (B).

$0.39 \pm 0.17 \mu\text{m}$ (Fig 4B,C). In contrast to these variables, neither microvillous width ($P = .59$) nor tight junction width ($P = .11$) showed any significant change from weeks 0 to 6 (Supporting Information).

Discussion

To the authors' knowledge, this is the first study to characterize in parallel the functional, histopathological, immunohistochemical, and ultrastructural duodenal lesions associated with diet-responsive CE. A number of lesions at both the light and electron microscopic level were observed, some of which resolved with a 6-week period of dietary treatment and others of which persisted. In our assessment of the biopsies, we complemented the WSAVA scoring system with a comprehensive panel of morphometric measurements, but also developed a novel scoring system for ultrastructural lesions that may be applied in future studies of this nature. In common with other studies, a sex predisposition was not apparent in our cases,^{14,18–20}

and their average age was similar to that of the previously published work.^{7,8,14,18–21} Bias introduced by owners or clinicians in determining which dogs to recruit into this study precluded definitive breed comparisons between cases and the general hospital population, but Labrador Retrievers, Staffordshire Bull Terriers, and Boxers were more common in the study cohort, consistent with their apparent predisposition to CE in previous studies.^{7,8,14,18,20–22}

Clinical signs of the study dogs generally were similar to those reported in the literature,^{7,8,13,15,18,22,23} but we were surprised to observe melena in 3 dogs, a sign that usually is associated with more severe disease predisposing to ulceration. Furthermore, although the median CCECAI of 6.5 suggested the presence of moderate disease, values ranged up to 15, demonstrating that diet-responsive CE may be severe in its manifestations in certain dogs and underlining the fact that such patients should not be necessarily denied an initial dietary trial before antibiotic and immunosuppressive drugs are considered.

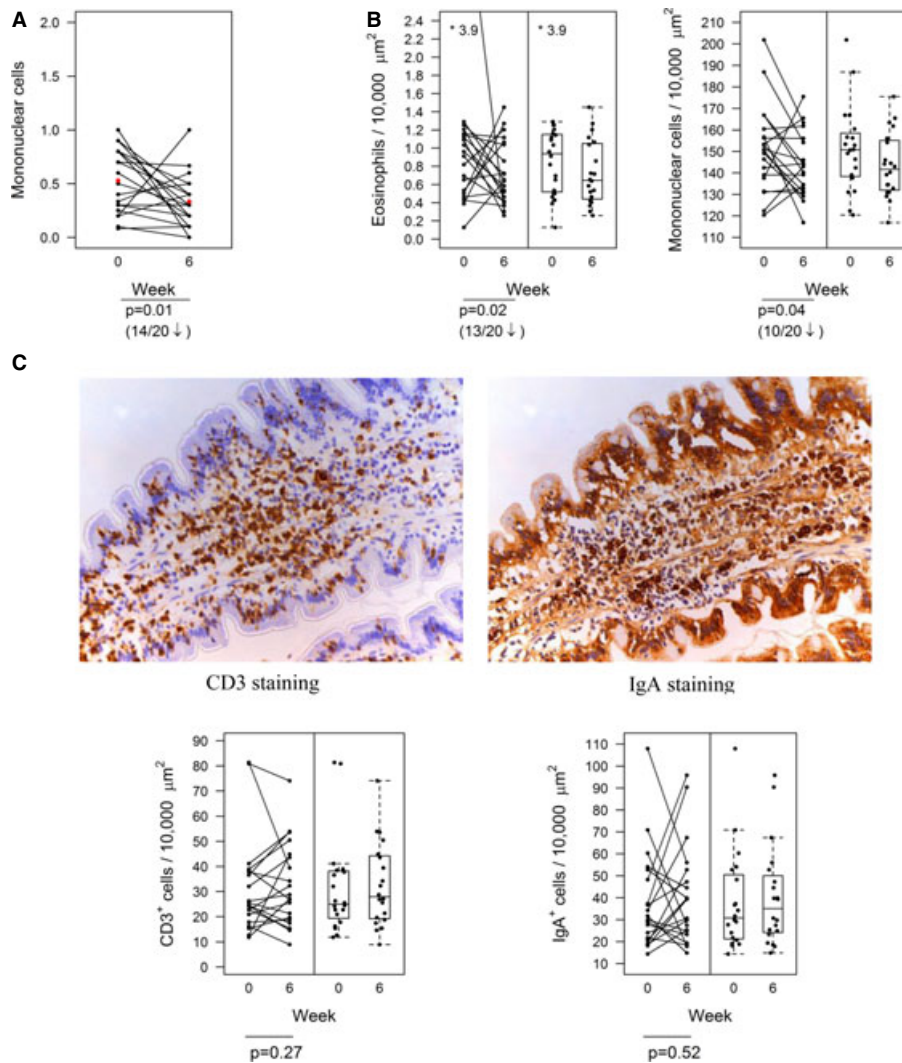


Fig 3. Histological and immunohistochemical observations in dogs with chronic enteropathy before and after a 6-week period of dietary treatment. Data from the 20 study dogs are shown at presentation (week 0) and 6 weeks after starting a hypoallergenic diet. Each panel shows a dot plot, with measurements from individual dogs joined by a line to highlight changes, and—for quantitative variables—an associated box-and-whisker plot showing summary data with overlain individual data points; red symbols represent overall mean values per visit. All *P* values are shown under the respective plots, whereas the numbers of observations from individual dogs concordant with the prevailing changes in mean values (increase/decrease) are shown in parentheses when $P \leq .10$. While mononuclear cell score showed a significant decrease from weeks 0 to 6 (A), all of the other qualitative variables showed no significant change with time. Mean lamina propria areal densities of both eosinophils and mononuclear cells decreased between visits (B), but neither CD3⁺ nor IgA⁺ areal density showed significant change with time (C). Examples of CD3 and IgA staining from a dog with chronic enteropathy before dietary treatment are shown ($\times 40$ objective magnification). (See Figure 2 legend for explanation of boxes and whiskers.)

Although the WSAVA scoring system represents an important advance in the histopathological assessment of intestinal biopsies, superseding a number of disparate grading schemes reported in the older literature,^{13,14,18,19,21,22,24} it has not been a panacea for the historical problem of inconsistency of lesion identification and grading among different pathologists. Factors such as tissue processing²⁵ and quality of biopsies¹⁷ have a major impact on lesion interpretation, even when using the WSAVA template. Furthermore, the WSAVA scoring system does not currently incorporate an assessment of epithelial goblet cells, which may play an important role in the pathogenesis of certain forms

of CE,^{3,26} and has not been validated as a means of quantitating overall lesion severity, being designed primarily as a pictorial template. To address these shortcomings, all endoscopic procedures were undertaken according to a standard operating protocol adhering to best practices to ensure optimal biopsy quality; all tissue processing was carried out by the same, experienced laboratory; all of the biopsies were rigorously examined by the senior author (OAG) to eliminate interobserver variability; and all of the biopsies were subject to additional morphometric assessments. These included villous height and width to gauge villous atrophy, numbers of intraepithelial lymphocytes and

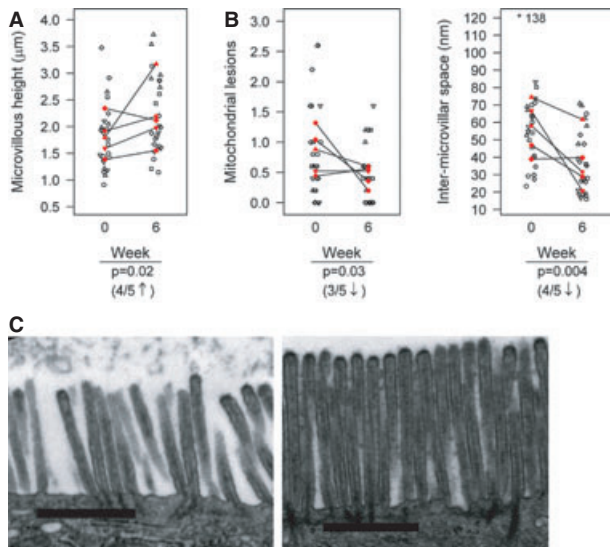


Fig 4. Ultrastructural observations in dogs with chronic enteropathy before and after a 6-week period of dietary treatment. Unfilled symbols represent mean values for 5 adjacent enterocytes for each of 5 longitudinally orientated villi per dog. Owing to the large volume of data collected in these analyses, measurements were made on a random sample of 5 of the 20 study dogs. Red symbols represent overall mean values per dog, joined by a line between the time of presentation (week 0) and 6 weeks after starting a hypoallergenic diet to highlight changes. All *P* values are shown under the respective plots, whereas the numbers of observations from individual dogs concordant with the prevailing change in mean values (increase/decrease) are shown in parentheses. Mitochondrial lesion score decreased significantly from weeks 0 to 6 (A), whereas mean intermicrovillar space decreased and mean microvillous height increased between visits (B). Part (C) shows an example of the repair of brush border lesions with dietary treatment: the left-hand panel illustrates mild-to-moderate microvillous atrophy and increased space between adjacent microvilli, whereas the right-hand panel illustrates resolution of these lesions with dietary treatment (Bar = 1 µm).

goblet cells to gauge epithelial inflammation and response to injury, and density of mononuclear cells, eosinophils, and neutrophils to gauge lamina propria inflammation. For the purposes of qualitative analysis of lesion severity, we assigned scores of 0 for normal tissue and 1, 2, and 3 for mild, moderate, and marked lesions, respectively. Future efforts to validate the WSAVA scoring system as a measure of overall lesion severity should consider the merits of differentially weighting individual criteria. An additional variable that may confound the interpretation of results is the potentially multifocal nature of duodenal lesions. However, we obtained at least 10 biopsies in a standardized manner from each dog and the process of choosing villi for assessment was randomized and consistent.

At the time of presentation, the clinical cases had more severe mean villous stunting and higher overall WSAVA scores than the control dogs. Furthermore, the cases had a lower mean villous height : width ratio,

corroborating the impression of villous stunting, and a higher mean density of eosinophils in the lamina propria. The lack of a difference in the qualitative eosinophil score likely reflects the insensitivity of visual assessment as a means to detect subtle differences in numbers of a minority population of cells within the lamina propria. Indeed, the estimated overall difference in eosinophil density between cases and controls was only approximately 1 cell per 100,000 µm², calling into question whether this statistical difference was actually of clinical relevance. Nevertheless, both partial villous atrophy and an increased number of lamina propria eosinophils have been associated with intestinal inflammation. The former is thought to be a consequence of accelerated epithelial proliferation associated with an increase in the crypt proliferative compartment and a decrease in the villous absorptive compartment,²⁷ and the latter a consequence of a hypersensitivity response to luminal antigens, presumably in this case predominantly of dietary origin.^{28–30}

As well as demonstrating differences at the level of the light microscope, our case and control groups showed striking differences in both qualitative and quantitative ultrastructural variables. Although electron microscopy is not a mainstream diagnostic tool in medicine, it remains an important imaging modality for the diagnosis of microvillous inclusion disease³¹ and certain nephropathies in humans,^{32,33} and ciliary dyskinesia in the dog.^{34,35} Moreover, it is a robust research tool to examine the ultrastructure of the cell. Ultrastructural assessment of intestinal epithelial injury generally involves examination of mitochondrial size and integrity; cytoplasmic vacuolation; microvillous abundance, height, diameter, and vesiculation; and tight junction integrity.³⁶ We developed a novel ultrastructural scoring and measurement protocol that incorporates all of these observations. Occurring as a consequence of cellular insults of an inflammatory nature, lesions in the CE dogs included mitochondrial cristolysis, reflecting a process of mitochondrial injury beginning with the ingress of ions and water that ultimately leads to uncoupling of oxidative phosphorylation^{37,38}; cytoplasmic vacuolation, reflecting the accumulation of ions and water in injury-induced vesicles^{39,40}; increased space between microvilli, reflecting the shedding and loss of microvilli⁴¹; and increased tight junction width, reflecting the disruption of junctional integrity and expansion of the intercellular space.^{42,43}

An important caveat to the conclusions derived from both the light and electron microscopic studies was the lack of breed-matching of the cases and controls, reflecting the challenges of sourcing healthy control dogs in veterinary clinical research in general. Laboratory Beagles do not represent an ideal control group, both because they are known to be associated with intrinsic differences in enteric bacterial populations, intestinal permeability, and mucosal cytokine expression patterns from other breeds,^{44,45} and because they live in an exposed, kennel environment, receiving a standard canine chow, rather than the home environ-

ment of most pet dogs in which various diets may be offered. Furthermore, fewer Beagles than cases were available and they were genetically more homogeneous, compromising the generality of the comparison. In addition, the biopsies were collected *postmortem*, which could have influenced their size or quality, thus introducing an additional confounding variable. However, every effort was made to recapitulate the *antemortem* biopsy technique and the biopsies from Beagles appeared not to be larger in size or better quality compared with those from clinical cases. The problem is further compounded by the various methods used by different authors to assess morphometric variables, making comparisons among studies challenging. For example, whereas the lamina propria density of mononuclear cells in our control group was similar to results derived from various breeds other than Beagles in studies adopting the same measurement protocol as ours,^{21,46,47} it differed from results cited in alternative studies.^{19,48} Similarly, the lamina propria density of eosinophils in our control group was concordant with values cited in 1 study,¹⁹ but was lower than values cited in 2 other studies, even though they adopted the same measurement protocol as ours.^{21,47} Nevertheless, the eosinophil density in our cases was also lower than values generally cited in the literature,^{21,49} suggesting that the difference in eosinophil counts between the groups was not attributable simply to an anomaly of the control dogs in this study. The issue of a suboptimal control group was circumvented in the examination of responses to treatment, because every case acted as its own control in this part of the study.

Provision of a hydrolyzed protein diet (a so-called "hypoallergenic" diet) is a recognized treatment strategy for canine CE.⁵⁰ Hydrolysis of the protein during the manufacturing process is thought to generate polypeptides of a size that are unlikely to stimulate the immune system. A proportion of dogs with CE will show full clinical remission in response to dietary treatment alone.^{51,52} All of the dogs in this study were rapidly diet-responsive, as demonstrated by a striking decrease in mean CCECAI from weeks 0 to 2, with a decrease in individual values in all of the dogs by the end of the study. Allenspach et al also showed resolution of clinical signs within 2 weeks of dietary treatment in dogs with diet-responsive CE,¹⁴ suggesting that dietary trials need not be continued for longer than 2 weeks before alternative diets or additional treatments are considered. In addition, there was an increase in mean body weight from weeks 0 to 6, with an increase in individual results in 12 of the 20 dogs, and a decrease in mean serum CRP concentration from weeks 0 to 2, with a decrease in individual results in 12 of 14 dogs in which CRP was measured. However, only 1 of 55 serum CRP assays yielded a concentration above the upper reference limit. This observation was at odds with data from 3 studies of IBD, which all suggested that increased serum CRP concentrations were common in dogs with CE.^{53–55} We surmise that the differ-

ence between these studies and ours was the more inflammatory nature of the disease in the former, in which all dogs had failed dietary and antibiotic treatment and required immunosuppressive treatment for clinical remission. A more recent study of both diet-responsive and steroid-responsive CE yielded data more concordant with ours, only 7 of 33 dogs having serum CRP concentrations above the upper limit of the reference interval.¹⁴ Similarly, many human patients with IBD have normal serum CRP concentrations.^{56,57} Despite these differences between studies, serum concentrations of CRP significantly decreased within the reference interval with treatment in all of them. Moreover, a recent examination of the biological variability in CRP in apparently healthy dogs suggested that sequential results from a given animal to determine trends may be more informative than absolute concentrations.¹⁶

Measurements of intestinal permeability and absorptive capacity proved to be unhelpful in the assessment of the cases in this study, showing no significant changes from weeks 0 to 6. This observation accords with the findings of Allenspach et al, who were unable to demonstrate differences in urinary L/R, X/G, or sucrose to methylglucose ratios before and after the treatment in dogs with both diet-responsive and steroid-responsive CE,⁵⁸ but contrasts with the findings of several other studies demonstrating higher permeability in dogs with CE of various etiologies.^{59–62} Several potential explanations can be offered to account for this disparity, including differences in measurement protocol. For example, voided urine samples were collected using a bowl rather than a metabolism cage in our study and that of Allenspach et al to ensure that the test was clinically practical, introducing inherent inaccuracies if urination was incomplete or part of the sample was lost during the process of collection. A blood test for intestinal permeability and function has been developed in the dog to facilitate sample collection,⁶³ but was unavailable to us in this study. Another difference among the various studies lies in the severity of the disease in the dogs recruited. Permeability changes may be more likely in dogs requiring immunosuppressive treatment, or in those with protein-losing enteropathy.^{61,62}

A number of histopathological observations were made after the initiation of dietary treatment in this study. There was a significant decrease in mean lamina propria mononuclear cell score. Quantitative variables also showed a difference, with a significant decrease in mean lamina propria eosinophil and mononuclear cell densities, the latter supporting the change in the corresponding qualitative score. Although the clinical relevance of relatively small changes in lamina propria cell densities remains unclear, eosinophils, lymphocytes, and plasma cells all are potential mediators of intestinal injury.⁶⁴ The decrease in numbers of eosinophils reconciled with the higher density of these cells at presentation and was compatible with amelioration of a hypersensitivity response to food antigens, whereas the decrease in mononuclear cells was compatible with gen-

eral amelioration of mucosal inflammation. Interestingly, these observations were at odds with recent studies that showed no significant changes in total lymphocyte counts and histopathological scores of duodenal biopsies from dogs with CE (including both diet-responsive and steroid-responsive disease) after periods of treatment ranging from 4 to 15 weeks.^{13,15,65} However, this study was likely to be more powerful than the previous study of diet-responsive enteropathy, examining 20 rather than 10 cases,¹⁵ and it applied a comprehensive qualitative and quantitative approach to the analysis of biopsies, being the first to apply the WSA-VA scoring system to a serial examination of intestinal biopsies from dogs with CE. Nevertheless, not all dogs showed changes in these variables that were concordant with those of the prevailing mean changes, and several of the examined variables showed no overall difference. Examples of the latter were lamina propria densities of CD3⁺ and IgA⁺ cells, which remained unchanged despite the decrease in numbers of mononuclear cells. We therefore surmised that neither T cells nor IgA-producing B cells and plasma cells were among the mononuclear cells showing a response to treatment. A previous study of duodenal lamina propria CD3⁺ T cells in dogs with both diet-responsive and steroid-responsive disease similarly showed no change with the inception of treatment.¹⁵ Additional studies would be required to distinguish the responsive populations of cells within the mononuclear fraction, which could include macrophages or B cells and plasma cells producing IgM, IgG, or IgE.

Of particular note in this study was the striking change in certain ultrastructural variables after the initiation of dietary treatment, including a decrease in the mean mitochondrial lesion score and intermicrovillar space, and an increase in the mean microvillous height. The mitochondrion is a sensitive sentinel of enterocyte injury, suggesting that the diet had a positive impact on epithelial cell health.³⁸ These observations also suggested that there was resolution of damage to the brush border, which serves a number of important digestive, absorptive, and antimicrobial functions.^{66,67} Ultrastructural lesions of the enterocyte have been documented in a variety of inflammatory enteropathies in humans, including celiac disease and IBD.^{41,68–70} This is the first time that such lesions have been assessed in diet-responsive CE of dogs before and after dietary treatment, although parallels can be drawn to similar lesions in gluten-sensitive enteropathy in Irish Setters,⁷¹ suggesting that they represent a common pathological end-point of intestinal inflammation attributable to a variety of different etiologies. Early work assessing the intestinal mucosa of children with celiac disease indicated that striking recovery of ultrastructural lesions may occur with dietary treatment, despite the persistence of lesions at the light microscopic level.⁶⁸ This observation correlated with clinical remission and underlines the important contribution of the brush border to global intestinal health, offering a potential explanation of the findings of previous studies of CE in the dog in which clinical remission occurred despite persistence of duode-

nal lesions at the light microscopic level. Ultrastructural lesions also appear to offer a sensitive window on impending disease, occurring before the observation of histological lesions in celiac disease.⁷² Taken together, the ultrastructural findings reported here are consistent with findings in the literature on human inflammatory enteropathies and emphasize some important limitations of light microscopy as a tool for the examination of the diseased intestine in the dog.

In conclusion, we documented a number of histological and ultrastructural lesions in diet-responsive CE in the dog and identified a significant, albeit subtle, decrease in lamina propria densities of eosinophils and mononuclear cells with dietary treatment. Furthermore, dietary treatment was associated with recovery of enterocyte mitochondrial and brush border health, documenting for the first time the resolution of key ultrastructural lesions with clinical remission of disease. As such, these data support the increasingly held view that architectural changes are at least as important as conventional measures of inflammation in the pathological assessment of CE and add credence to the notion that the principal benefit of intestinal biopsies in dogs with chronic gastrointestinal signs is to rule out infiltrative, ulcerative, and fungal disease rather than to catalog the type and number of lamina propria leukocytes.

Footnotes

- ^a Purina Veterinary Diets® HA Hypoallergenic® Canine Formula
^b Canine C-Reactive Protein ELISA Kit, BD BiosCences, San Jose, CA
^c Leica DFC300FX camera attached to Leica DM4000 microscope, Leica Microsystems, Milton Keynes, UK
^d Leica QWin® V3.4.0 image analysis software, Leica Microsystems, Milton Keynes, UK
^e Jeol Jem 100 Electron Microscope, Jeol, Welwyn Garden City, UK with an ES500W Erlangshen Model 782 camera system, Gatan, Abingdon, UK
^f Data were analyzed by the “stats”, “lme4”, and “multcomp” packages in R 2.11.0 (Vienna, Austria) except for those for the qualitative variables “villous stunting”, “mucosal fibrosis”, and “lamina propria mononuclear cells”, which were analyzed by the GLIMMIX procedure in SAS version 9.1 for PCs (SAS Institute Inc, Cary, NC).
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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Materials and methods.

Figure S1. Histopathological observations in dogs with chronic enteropathy before and after 6 weeks of a dietary trial.