Evaluation of intestinal permeability and gluten sensitivity in Soft-Coated Wheaten Terriers with familial protein-losing enteropathy, protein-losing nephropathy, or both

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Objective—To evaluate intestinal permeability and gluten sensitivity in a family of Soft-Coated Wheaten Terriers (SCWT) affected with protein-losing enteropathy (PLE), protein-losing nephropathy (PLN), or both.

Animals—6 affected adult dogs.

Procedure—Intestinal biopsy specimens, urine protein-to-creatinine ratio, serum concentrations of albumin and globulin, and concentration of α_1-protease inhibitor in feces were evaluated before, during, and 13 weeks after daily administration of 10 g of gluten for 7 weeks. Eosinophils and lymphocytes-plasmacytes were enumerated in intestinal biopsy specimens. Intestinal permeability was evaluated before and during the sixth week of gluten administration via cellobiose-mannitol and chromium-EDTA absorption tests.

Results—Serum globulin concentration decreased significantly after prolonged administration of gluten. Although not significant, there was an increase in lymphocytes-plasmacytes and a decrease in eosinophils in intestinal biopsy specimens. Furthermore, these counts were greater than those reported for clinically normal dogs. Gluten administration did not increase intestinal permeability.

Conclusions and Clinical Relevance—Daily administration of gluten was associated with a significant decrease in serum globulin concentration in SCWT affected with PLE or PLN, but other variables remained unchanged. Although enhanced wheat-gluten sensitivity may be one factor involved in the pathogenesis of PLE or PLN in SCWT, this syndrome does not appear to be the result of a specific sensitivity to gluten. (*Am J Vet Res* 2000;61:518–524)

A familial syndrome of **protein-losing enteropathy (PLE)**, **protein-losing nephropathy (PLN)**, or both, has been reported in **Soft-Coated Wheaten Terriers (SCWT)**.[1] In SCWT, PLE is associated with inflammatory bowel disease with varying morphologic characteristics, lymphangiectasia, or both, and PLN is the result of glomerulonephritis. In 1 study[1] of 222 affected dogs, 76 (34%) had PLE, 85 (38%) had PLN, and 61 (27%) had PLE and PLN. Prevalence of subclinical enteric or renal disease in dogs that had only clinical manifestations of PLN or PLE, respectively, was not determined. Clinicopathologic abnormalities associated with this syndrome vary, depending on whether the dog has PLE or PLN.[1,a] The pathogenetic mechanisms of PLE and PLN in SCWT are unknown. Preliminary findings for renal biopsy specimens obtained from SCWT with PLN are consistent with those of immune-complex disease of the glomerulus. Because of the association of PLN and PLE in SCWT, gastrointestinal tract disease could predispose dogs to immune-complex formation and PLN. Examples of disorders that could affect the intestines and cause immune-complex-mediated glomerulonephritis include systemic lupus erythematosus, inflammatory bowel disease with absorption of intestinal-derived antigens (eg, food antigens and bacterial antigens), and food hypersensitivities.[2-4]

Food hypersensitivity reactions are a documented cause of enteropathy in humans and dogs and can be occult or associated with enteritis of varying severity, including PLE.[3,5-7] In humans, food hypersensitivities result from type-I or combined type-III and -IV hypersensitivity reactions, although a particular patient can have all 3 types of hypersensitivity reactions.[8] Food hypersensitivities may develop because of failure to suppress the intestinal mucosal immune responses to food antigens or from increased intestinal permeability with absorption of intact or incompletely digested food allergens.[9] Enhanced intestinal permeability can be a primary event or can be secondary to disruption of intestinal mucosal integrity.[10-14] Glomerulonephritis has been reported in association with food hypersensitivity in humans.[4,5,15-19]

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We believe that PLE-PLN in SCWT is the result of a food hypersensitivity. We arrived at this belief because of several findings. First, 1 affected SCWT did not have clinical signs for a period of years while being fed a gluten-free diet,[b] but that dog developed mild proteinuria and lymphocytic enteritis within several days of adding gluten, a potential food allergen, to the diet. Second, SCWT have a high relative risk factor for food allergy dermatitis.[20] Third, many owners of affected SCWT report that these dogs have to be fed elimination diets to avoid or reduce clinical signs. Some diets are gluten-free; however, many owners have to feed diets that are free of other potential allergens before clinical improvement is detected. If affected SCWT are sensitive to multiple food antigens, as suggested by results of the owner-performed food trials, increased intestinal permeability to food antigens as a primary or secondary event is a possible cause for the allergic response. Thus, the PLE-PLN syndrome in SCWT may result from a defect in intestinal permeability causing increased absorption of allergens from the gastrointestinal tract. The resulting food hypersensitivity may lead to enteric disease, which progresses to PLE. Glomerulonephritis, which causes PLN, may result from glomerular deposition of circulating immune complexes, which form after intestinal absorption of antigens or in situ formation of immune complexes.

The purpose of the study reported here was to evaluate intestinal permeability and gluten sensitivity in a family of SCWT affected with PLE-PLN. We chose to use wheat gluten during this study because it is a potential food allergen that may increase intestinal permeability in gluten-sensitive dogs and because there is a general belief perpetuated among breeders of SCWT that this syndrome is caused by sensitivity to wheat gluten.

Materials and Methods

Dogs—All dogs were cared for in accordance with principles outlined in the NIH Guide for the Care and Use of Laboratory Animals. All studies involved the use of 6 SCWT (3 female, 3 male) that were part of a colony of SCWT maintained at the North Carolina State University College of Veterinary Medicine. The 6 dogs were members of 2 litters that resulted from matings involving 2 SCWT, both of which were clinically affected with PLE-PLN. At the time of this study, 5 dogs were 17 months old, whereas 1 male was 24 months old. Four random-source adult male dogs served as control dogs for the intestinal permeability study. Adequate health of control dogs was verified through physical examination and evaluation of results of a CBC, serum biochemical analyses, urinalysis, and parasitologic examination of feces for intestinal parasites. All dogs were fed a standard diet[c] free of wheat gluten for at least 6 weeks before initial evaluation as well as throughout the study.

Since birth, these dogs had undergone clinicopathologic evaluation every 3 months, consisting of a CBC, serum biochemical analyses, urinalysis, determination of urine protein-to-creatinine ratio, parasitologic examination of feces for intestinal parasites, and determination of concentration of fecal α_1-protease inhibitor.[d] Urine protein:creatinine was evaluated only when the urine specimen was free of evidence of hemorrhage or inflammation, as determined by examination of urine sediment. All assays were performed in accordance with standard laboratory methods, with reported reference ranges being those established for our laboratory. Values for fecal α_1-protease inhibitor represented the mean for 3 samples collected during 3 consecutive days; an established reference range was used.[21] In addition, duodenal and gastric biopsy specimens were obtained via gastroduodenoscopy. Renal ultrasonography was conducted every 6 months; renal biopsy specimens were obtained every 12 months, using an ultrasound-guidance technique.

From the time of birth until initiation of the study, all 6 SCWT had mild evidence of PLE as manifested by increased values for fecal α_1-protease inhibitor. These increases were sporadic in 5 SCWT and persistent in only 1 SCWT. When analyzed as the percentage of time an increased value for fecal α_1-protease inhibitor was detected for all samples collected from each dog, the median percentage was 50 (first quartile, 29%; third quartile, 67%). Values in all dogs ranged from 0.04 to 45.5 μg/g of feces (mean ± SEM, 7.88 ± 1.46; reference range, 0.23 to 5.67 μg/g). Hypoglobulinemia (≤ 2.5 g/dl) was detected in only 2 SCWT in 1 sample each since the dogs became 12 months old; none of the dogs had hypoalbuminemia (≤ 2.5 g/dl). Protein-losing nephropathy was detected in 1 SCWT on the basis of sporadic increases in urine protein:creatinine (> 1.0) and histologically diagnosed glomerulonephritis. Although a diagnosis of PLN was not made in the remaining 5 SCWT, 3 had questionable increases in urine protein:creatinine (1 sample each; ratios of 0.53, 0.53, and 0.55; reference range for questionable values = 0.5 to 1.0). Two of these 3 dogs had mild changes in the glomeruli that were evident histologically. One SCWT had renal dysplasia. Clinical signs in all 6 dogs were limited to rare or infrequent vomiting, diarrhea, or inappetence, or a combination of the 3 signs.

At the time of publication, 3 dogs were 62 months old, 1 dog was 67 months old, and 2 dog had died. As they became older, all 6 dogs developed more substantial evidence of PLE. Median percentage of time an increased value for fecal α_1-protease inhibitor was detected for all samples collected was 53.5 (first quartile, 42.5%; third quartile, 63.5%). Values for 96 samples from all dogs ranged from 0.04 to 50.9 μg/g of feces (mean ± SEM, 7.88 ± 1.46 μg/g of feces). Infrequent hypoglobulinemia was detected in all 6 dogs; infrequent hypoalbuminemia was detected in 4 dogs. Inflammatory bowel disease was documented in all 6 dogs, characterized by eosinophilic and lymphocytic-plasmacytic infiltrates. At the time of publication, a definitive diagnosis of PLN, as determined by the aforementioned definition, was established in 2 additional dogs (ie, 3 of the 6 SCWT had PLN); proteinuria was persistent in 2 of these 3 dogs. The dog with renal dysplasia was euthanatized because of progressive renal failure.

Unfortunately, clinically normal SCWT were not available to serve as control dogs for this study. Dogs affected with this familial syndrome have been identified when they were only 6 months old; however, some dogs have not been identified until they were 12 years old. There is not a specific genetic or mechanistic test currently available to allow for an early, accurate diagnosis of the disease. Until such a test is available, it would be nearly impossible to know for certain that a specific dog was not affected, and only dogs that are not affected would be suitable control dogs.

Study design and clinical evaluation—Beginning 6 weeks before initiation of the study, dogs were fed the standard diet. Dogs then were fed the standard diet plus 10 g of gluten/d for a 7-week period (weeks 0 to 7), and dogs subsequently were fed only the standard diet for an additional 13 weeks (ie, weeks 8 to 20). The following tests were performed in all SCWT 1 week before, at the end of 7 weeks of administration of gluten, and 13 weeks after (ie, week 20) gluten administration: CBC, serum biochemical analyses, urinalysis, determination of urine protein:creatinine, parasitologic examination that used a flotation technique to detect intestinal para-

sites in feces, and determination of concentration for fecal α_1-protease inhibitor.

Intestinal permeability studies—Intestinal permeability was assessed 3 weeks before and 6 weeks after initiation of gluten administration, using cellobiose-mannitol[13] and **chromium-EDTA (Cr-EDTA)**[22] absorption. The Cr-EDTA solution was formulated by using a modification of a technique reported elsewhere.[22] Chromium chloride • 6 H_20 (28.4 g dissolved in 400 ml of deionized water) and EDTA tetra sodium salt (40 g dissolved in 600 ml of deionized water) were combined and heated for 1 hour at 100 C. Excess EDTA was neutralized with 1*M* $CaCl_2$ (final pH 7.4). Once cooled, the solution was brought to a volume of 2 L with deionized water.

Food was withheld overnight. Dogs then were given an approximately isotonic solution containing 25 mg of Cr-EDTA, 5 g of D(+)cellobiose,[e] and 2 g of D-mannitol[f] via intragastric administration. Dogs were placed in a metabolism cage. Water was available ad libitum. After 6 hours and again at 24 hours, all urine was collected from the dogs by means of bladder catheters and from the floor of the cage. Food was provided after the 6-hour collection. Total volumes of urine produced at 6 and 24 hours were recorded. A urine preservative[g] (1 ml of a 100% solution; wt/vol) was added to collected urine. A 10-ml aliquot of urine from the 6-hour collection was removed and stored at –20 C until analyzed for cellobiose and mannitol. The remainder of the 6-hour collection was pooled with the 24-hour collection. A 10-ml aliquot from the pooled collection was removed and stored at –20 C until analyzed for chromium.

Urine mannitol concentrations were determined, using a spectrophotometric technique.[13] Mannitol was oxidized to formaldehyde by addition of periodic acid.[h] Formaldehyde was reacted with chromotropic acid[i] to form a purple complex, and absorbance was read at 570 nm.[j] Urine cellobiose concentrations were determined by converting cellobiose to D-glucose, using β-glucosidase,[k] and measuring the D-glucose concentration via a commercial kit[l] that uses the hexokinase method. Percentages for urinary recovery of the doses of cellobiose and mannitol administered were calculated, and the ratio of cellobiose recovery-to-mannitol recovery was determined.

Urine chromium concentrations were determined, using atomic absorption spectrophotometry. Samples were diluted 1:50 with water. The method of standard addition was used to document that there was not any detectable matrix interference. Concentrations of chromium were calculated from a linear standard curve. All samples were assayed within the range of linearity on the standard curve. Percentage of urinary recovery for each dose of Cr-EDTA administered was calculated.

Morphometric analysis of duodenal specimens—Duodenal biopsy specimens were obtained via duodenoscopy 1 week before, during (week 7), and 13 weeks after (ie, week 20) gluten administration. Biopsy specimens were fixed in formalin solution, and 5-µm-thick slices were stained with hematoxylin and eosin. Longitudinally oriented villi were selected at random for cell counts. Mononuclear cells in the lamina propria (lymphocytes, plasmacytes) were counted in a 3,600-μm^2 area defined by a calibrated ocular reticle grid, whereas eosinophil counts were determined in an area of 10,000 μm^2. For each dog at each sample collection period, cells in ≥ 3 villi were counted. Each villus represented a specific biopsy section, whenever possible, and 2 counts, representing the top half and bottom half of the villus, were conducted for each villus. Each biopsy specimen was scored without knowledge of whether the sample represented the period before or after gluten administration.

Gluten administration—The 6 SCWT were given 10 g of gluten,[m] PO, once daily for 7 weeks.[23-25] During the 7-week period of gluten administration, stool consistency, appetite, and episodes of vomiting were recorded daily.

Statistical analyses—Data were tested for normality of distribution, using the Shapiro-Wilk W test. On the basis of that analysis, nonparametric tests were selected for analysis of the data. The Wilcoxon signed-rank test for paired data was used to compare differences at weeks –1 (before gluten administration) and 7 (during gluten administration) as well as weeks 7 and 20 (13 weeks after cessation of gluten administration) for each of the following variables: serum concentrations of albumin and globulin, concentration of fecal α_1-protease inhibitor, urine protein:creatinine, and eosinophil and lymphocyte-plasmacyte counts in duodenal biopsy specimens. The Wilcoxon signed-rank test for paired data also was used to compare differences in cellobiose-mannitol and Cr-EDTA absorption from data obtained from SCWT before (week –3) and during (week 6) gluten administration. The Wilcoxon rank-sums test was used to compare differences in cellobiose-mannitol and Cr-EDTA absorption from data obtained from SCWT before (week –3) gluten administration and from control dogs. Significance was defined as $P < 0.05$.

Results

None of the 6 SCWT had more than an infrequent episode of vomiting, diarrhea, or inappetence during gluten administration. Subjectively, there was an increase in the frequency of these signs in 3 of 6 dogs.

Serum globulin concentrations were significantly ($P = 0.03$) decreased in SCWT after 7 weeks of gluten administration, compared with values obtained before gluten administration (**Table 1**). All 6 dogs had a decrease in serum globulin concentration after gluten administration (**Fig 1**), but in only 3 dogs did the concentrations decrease to less than the reference range. These same 3 dogs had serum albumin concentrations that decreased to less than the reference range during week 7. However, median serum albumin concentration during week 7 was not significantly ($P = 0.19$) different from that before gluten administration and

Table 1—Selected clinicopathologic findings* in 6 Soft-Coated Wheaten Terriers for samples obtained before (week –1), at the end of 7 weeks of administration of 10 g of gluten/d (week 7), and 13 weeks after cessation of gluten administration (week 20)

		Wk			*P* value	
Variable	**Reference range**	**–1**	**7**	**20**	**–1 vs 7**	**7 vs 20**
Fecal α_1-protease inhibitor (µg/g of feces)	0.23–5.67	4.3 (1.1, 16.7)	18.1 (3.2, 32.2)	4.8 (3.0, 17.6)	0.22	0.69
Urine protein:creatinine	< 0.5	0.12 (0.05, 0.24)	0.18 (0.11, 0.37)	0.18 (0.05, 0.58)	0.16	0.69
Serum albumin (g/dl)	2.5–4.0	3.05 (2.97, 3.33)	2.75 (2.47, 3.22)	3.10 (2.90, 3.40)	0.19	0.25
Serum globulin (g/dl)	2.5–4.5	2.95 (2.75, 3.45)	2.50 (2.22, 2.72)	2.55 (2.33, 2.78)	0.03	0.78

*Data expressed as median (first quartile, third quartile).

remained within the reference range. In 2 of the 3 dogs with hypoalbuminemia and hypoglobulinemia, serum globulin and albumin concentrations returned to within reference ranges after cessation of gluten administration. However, median serum albumin and globulin concentrations were not significantly (P = 0.25 and 0.78, respectively) different between weeks 7 and 20.

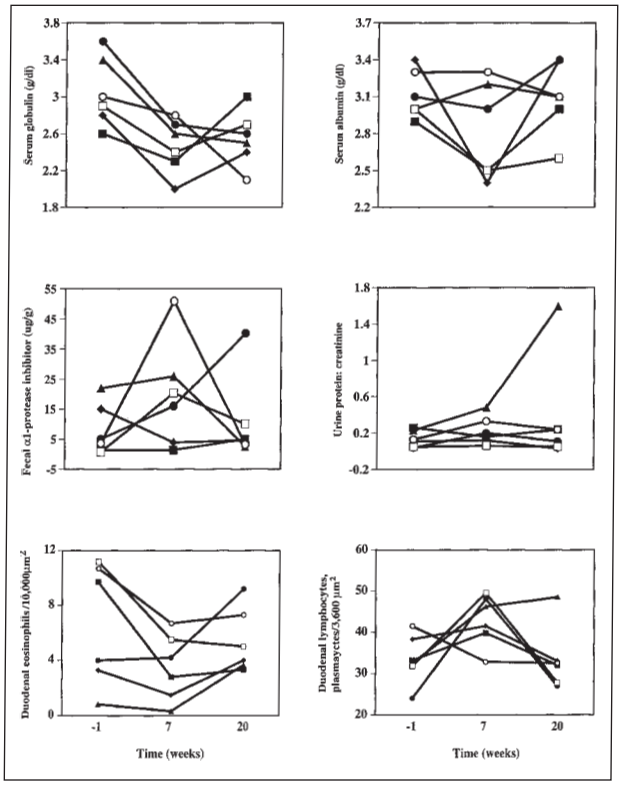

Figure 1—Concentrations of serum globulin, serum albumin, and fecal α_1-protease inhibitor; urine protein-to-creatinine ratio, and eosinophil and lymphocyte-plasmacyte counts in duodenal biopsy specimens for 6 Soft-Coated Wheaten Terriers before (week –1), at the end of 7 weeks of administration of 10 g of gluten/d (week 7), and 13 weeks after cessation of gluten administration (week 20). Each dog is represented by the same unique symbol in each graph.

Median concentration of fecal α_1-protease inhibitor did not differ significantly (P = 0.22) before and during gluten administration (weeks –1 and 7, respectively) or between the median concentrations obtained during and after gluten administration (weeks 7 and 20; P = 0.69). Immediately before gluten administration, only 2 dogs had abnormal values for fecal α_1-protease inhibitor; however, 4 dogs had increased values after gluten administration. Values for fecal α_1-protease inhibitor remained increased in 2 dogs after cessation of gluten administration, but these were not the same dogs that had abnormal values before administration. It is interesting that 2 of the 3 dogs that had decreased serum albumin and globulin concentrations had values for fecal α_1-protease inhibitor that were within the reference range during and 13 weeks after cessation of gluten administration.

Urine protein:creatinine did not differ significantly during the study. In fact, the dog with PLN was the only dog that had a value for urine protein:creatinine (1.6) outside the reference range; it was detected 13 weeks after cessation of gluten administration.

Intestinal permeability did not differ significantly between SCWT and control dogs or between SCWT before and during gluten administration, as determined on the basis of results of cellobiose-mannitol and Cr-EDTA absorption tests (**Table 2**). This suggests that 10 mg of gluten given daily for 6 weeks did not stimulate an increase in intestinal permeability or that the cellobiose-mannitol and Cr-EDTA absorption tests were not sufficiently sensitive to detect increases in permeability.

Although significant differences were not evident during evaluation of mucosal biopsy specimens, lymphocyte-plasmacyte counts increased in duodenal biopsy specimens obtained after 7 weeks of gluten administration, compared with data for specimens obtained before gluten administration. Subsequently, values decreased after cessation of gluten administra-

Table 2—Percentage urinary recovery of chromium for a 24-hour collection and cellobiose-to-mannitol ratio for a 6-hour collection in 4 control dogs and 6 Soft-Coated Wheaten Terriers (SCWT) 3 weeks before and 6 weeks after initiation of administration of 10 g of gluten/d

				P value	
Variable	**SCWT wk –3**	**SCWT wk 6**	**Control dogs**	**Wk –3 vs wk 6**	**Wk –3 vs control dogs**
Chromium recovery (% of dose)*	16.6 (14.4, 24.5)	11.4 (6.1, 18.8)	15.4 (7.5, 21.2)	0.44	0.39
Cellobiose:mannitol*	0.044 (0.037, 0.052)	0.051 (0.029, 0.080)	0.066 (0.043, 0.086)	0.69	0.2

*Data expressed as median (first quartile, third quartile)

Table 3—Eosinophil and lymphocyte-plasmacyte counts in intestinal biopsy specimens obtained from 6 Soft-Coated Wheaten Terriers before (week –1), at the end of 7 weeks of administration of 10 g of gluten/d (week 7), and 13 weeks after cessation of gluten administration (week 20)

		Wk		*P* value	
Variable	**–1**	**7**	**20**	**–1 vs 7**	**7 vs 20**
Eosinophils/10,000 µm²*	6.85 (2.68, 10.83)	3.5 (1.2, 5.8)	4.1 (3.18, 7.78)	0.06	0.09
Lymphocytes-plasmacytes/3,600 µm²*	32.9 (29.9, 39.1)	43.9 (38.1, 48.4)	33.1 (32.0, 42.7)	0.16	0.09

*Data expressed as median (first quartile, third quartile). Values (mean ± SD) reported[26] for clinically normal dogs were as follows: eosinophils, 0.3 ± 0.3 cells/5,000 µm²; lymphocytes, 27.4 ± 5.8 cells/5,000 µm²; and plasmacytes, 2.9 ± 3.7 cells/5,000 µm².

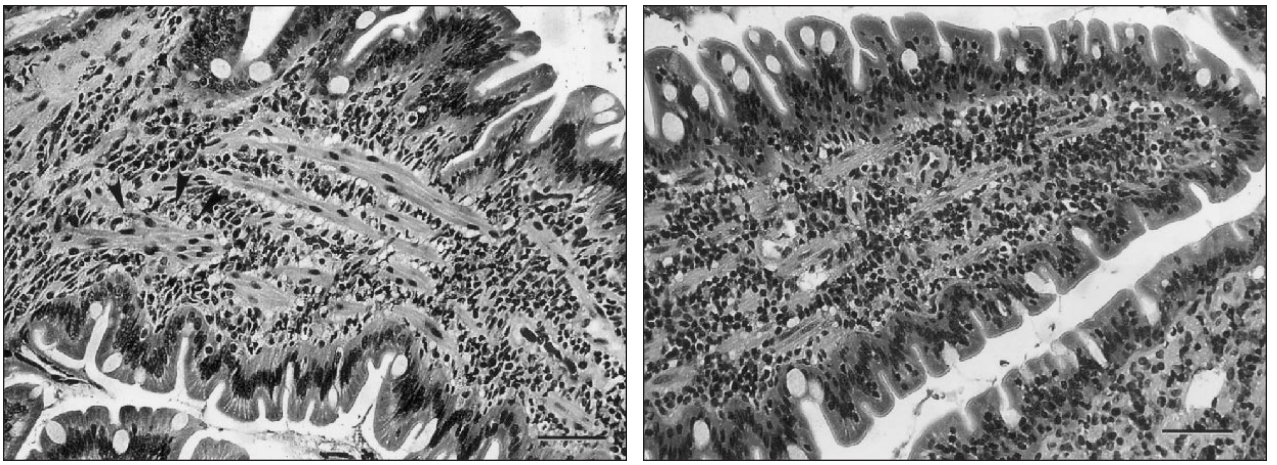

Figure 2—Photomicrographs of a duodenal villus in a specimen obtained from a male Soft-Coated Wheaten Terrier affected with protein-losing enteropathy and protein-losing nephropathy. Specimens were collected before gluten administration (left) and at the end of 7 weeks of administration of 10 g of gluten/d (right). Notice the relative increase in eosinophils before gluten administration (arrowheads), whereas eosinophils are less obvious in the specimen obtained after gluten administration. Also, notice the relative increase in lymphocytes-plasmacytes after gluten administration. H&E stain; bar = 50 µm.

tion and were similar to values for specimens obtained before gluten administration (**Table 3, Fig 2**). Lack of a control group of clinically normal age-matched dogs prevented more rigid assessment of the relative number of cells. However, the median number of lymphocytes-plasmacytes after 7 weeks of gluten administration was clearly greater than has been reported[26] for a group of clinically normal dogs and was similar to the number reported for dogs with lymphocytic-plasmacytic enteritis.[26] These observations indicated that although not a cause of a detectable increase in intestinal permeability, gluten administration tended to provoke an infiltration of mononuclear inflammatory cells, which may suggest an immunologic response to this dietary change. Median eosinophil counts in duodenal biopsy specimens were markedly higher than the reference range at all time points. Although eosinophil counts decreased slightly after gluten administration, the difference was not significant.

Discussion

The SCWT reported here had a significant decrease in serum globulin concentration associated with gluten administration. Although there was a slight decrease in median serum albumin concentration and 3 dogs developed hypoalbuminemia after gluten administration, the difference was not significant. The mechanism by which hypoglobulinemia developed after gluten administration is unknown. On the basis of the fact that the SCWT of this report were mildly affected with PLE-PLN, enteric loss of globulin is the most plausible mechanism. Enteric loss of protein can result from several mechanisms, including mucosal erosion or ulcers, lymphatic obstruction, and increased mucosal permeability to protein secondary to cell damage or cell loss. Mucosal damage, cellular damage, or increased mucosal permeability was not detected in dogs of this study. Although lymphangiectasia was not reported, lymphatic obstruction cannot be excluded. Leakage of specific serum proteins in patients with PLE appears to be independent of the molecular weight of the protein, and the reduction in serum concentration of various proteins may differ. Reduced serum concentration of a particular protein develops when the rate of enteric loss exceeds the body's synthetic capability. Enteric loss of protein has a greater effect on the serum concentrations of proteins that typically have a low catabolic rate, such as IgG, IgM, and IgA.[2] It is possible that hepatic synthesis of albumin allowed for maintenance of the serum albumin concentration, but globulin synthesis, which has a slower rate, was unable to maintain the serum globulin concentration. It is also possible that hypoglobulinemia developed during the study as a result of disease progression that was unrelated to gluten administration.

Intestinal permeability, as measured by cellobiose-mannitol and Cr-EDTA absorption studies, was not increased in 6 SCWT affected with PLE-PLN, compared with control dogs. The SCWT were evaluated during the early stages of the disease and had only minimal clinical manifestations of disease. Because increased intestinal permeability was not detected early in the course of the disease, it is unlikely that abnormal intestinal permeability is involved in the early pathogenic events that initiate this syndrome. It is possible that changes in intestinal permeability may have been detected in the SCWT of this report if we had used more sensitive tests of intestinal permeability.[27] We selected the tests used in this study because they were easy to conduct, and results of these tests were abnormal in humans with celiac disease[28] and Irish Setters with gluten-sensitive enteropathy.[11,13] The Cr-EDTA absorption test used in our study is similar to that used in other studies, except we measured chromium via atomic absorption instead of radioactive counting. The cellobiose-mannitol absorption test is more sensitive than the Cr-EDTA absorption test; however, if there is defective permeability in the distal portion of the intestinal tract, bacterial degradation of the sugars may prevent absorption of the sugars and lead to a falsely low ratio.[28] The Cr-EDTA absorption test is not affected by bacterial activity.

We are not aware of any study in which investiga-

tors evaluated potential interference of absorption between Cr-EDTA and mannitol-cellobiose absorption when administered concurrently, as was done in this study. Values for the cellobiose-mannitol and Cr-EDTA absorption tests obtained for control dogs used in the study reported here are similar to those reported for clinically normal dogs.[10,13] Therefore, it is unlikely that any of the compounds used in our study interfered with absorption of the other compounds.

Irish Setters with gluten-sensitive enteropathy have jejunal damage and an increase in cellobiose-mannitol excretion after 6 weeks of gluten administration at the rate of 1 g of gluten/d.[13] It is unlikely that gluten was administered to the dogs in the study reported here at a dose that was too low or for a duration that was too short for an effect to be detected. It is possible that increases in intestinal permeability secondary to inflammatory bowel disease are late in the course of the disease, but this question was not addressed during the current study.

In this study, we elected to administer wheat gluten to SCWT affected with PLE-PLN, because it is a potential food allergen and some SCWT owners and breeders observed that their dogs were intolerant of wheat-containing products. Subsequent to this study, we have documented that the 6 SCWT reported here have food hypersensitivities to multiple food allergens, as determined by use of gastroscopic food sensitivity testing and oral provocation.[29] Therefore, PLE-PLN of SCWT is not a disease with selective sensitivity to gluten, which is the case in gluten-sensitive enteropathy of Irish Setters or celiac disease in humans. However, it is interesting that serum globulin concentration was significantly decreased and duodenal lymphocyte-plasmacyte counts increased slightly after gluten administration, suggesting sensitivity to gluten in the SCWT of this report. The return of intestinal lymphocyte-plasmacyte counts after cessation of gluten administration to values that were similar to those obtained before gluten administration provides evidence that the increase detected after gluten administration was caused by a mucosal reaction to gluten. It is possible that the patterns detected in this study would have achieved significance if a larger population of affected SCWT had been available for the study. The number of intestinal intraepithelial lymphocytes increases within hours of an oral gluten challenge in humans with celiac disease but not in healthy control subjects.[30] Similarly, clinically normal dogs do not have changes in height of the jejunal villi or alkaline phosphatase and aminopeptidase N activities of the jejunum after prolonged administration of gluten.[31]

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[e]Cellobiose, Sigma Chemical Co, St Louis, Mo.
[f]D-mannitol, Sigma Chemical Co, St Louis, Mo.
[g]Mucolex, X, VWR Scientific Products, Pittsburgh, Pa.
[h]Periodic acid, Sigma Chemical Co, St Louis, Mo.
[i]Chromotropic acid, Sigma Chemical Co, St Louis, Mo.
[j]UV-160 spectrophotometer, Shimadzu, Kyoto, Japan.
[k]β-glucosidase, Sigma Chemical Co, St Louis, Mo.
[l]IL glucose kit, Instrumentation Laboratory Monarch, Lexington, Mass.
[m]Gluten derived from wheat, Sigma Chemical Co, St Louis, Mo.

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