Serum cholecystokinin concentrations in dogs with naturally acquired pituitary-dependent hyperadrenocorticism

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OBJECTIVE
To determine serum cholecystokinin (CCK) concentrations in dogs with pituitary-dependent hyperadrenocorticism (PDH) and to evaluate associations among CCK concentration, PDH, and gallbladder mucocele (GBM).

ANIMALS
14 client-owned dogs with PDH and 14 healthy dogs.

PROCEDURES
Dogs were separated into 4 groups: healthy dogs without gallbladder sludge (group A; n = 7), healthy dogs with gallbladder sludge (group B; 7), dogs with PDH and gallbladder sludge (group C; 8), and dogs with PDH and GBM (group D; 6). Serum CCK concentrations were then measured before and 1, 2, and 4 hours after consumption of a high-fat meal. Concentrations in dogs with PDH were also measured before and after trilostane treatment. Results were compared among groups and assessment points.

RESULTS
Preprandial serum CCK concentrations in group C were significantly lower than those in groups A, B, and D, but no significant differences in postprandial CCK concentrations were identified among the groups 1, 2, or 4 hours after the meal. With respect to trilostane treatment of dogs with PDH, no significant differences were identified between pre- and post-trilostane serum CCK concentrations in group C or D. Median CCK concentration after trilostane treatment was higher in group D than in group C, but this difference was not significant.

CONCLUSIONS AND CLINICAL RELEVANCE
The outcomes in this study did not support the hypothesis that a low circulating CCK concentration affects the development of GBM in dogs with PDH. (*Am J Vet Res* 2016;77:1101–1107)

Gallbladder mucocele refers to the abnormal accumulation of inspissated bile and mucus in the gallbladder lumen, with organ overdistention and a distinct ultrasonographic appearance.[1] This disorder is common to humans and dogs alike, but little is known about its pathogenesis or potential causative factors in dogs. Gallbladder mucocele is commonly accompanied in dogs by gallbladder sludge, which is often considered an incidental finding identified during abdominal ultrasonography.[2] Although its clinical importance in dogs remains controversial,[2] gallbladder sludge in humans is widely regarded as the starting point for GBM and various other gallbladder disorders.[3]

ABBREVIATIONS

ALP	Alkaline phosphatase
AUC	Area under the curve
CCK	Cholecystokinin
GBM	Gallbladder mucocele
PDH	Pituitary-dependent hyperadrenocorticism

Gallbladder sludge may similarly contribute to the pathogenesis or progression of GBM in dogs,[4] and gallbladder dysmotility may be involved in the initial formation of gallbladder sludge in both dogs and humans. Gallbladder dysmotility is, in turn, influenced by changes in gallbladder structure or contents, autonomic nerve function, or circulating concentrations of endogenous hormones.[3,4] For example, CCK is an endogenous peptide hormone that controls gallbladder motility.[5] The CCK stimulates gallbladder contraction and relaxation of the sphincter of Oddi, promoting bile flow via the common bile duct into the duodenum.[6] A lack of CCK induces gallbladder hypomotility and prolongs the residence time of excess cholesterol in the organ, leading to rapid crystallization and precipitation of solid cholesterol crystals in CCK-deficient mice.[7] Both contraction and relaxation of the gallbladder are strongly correlated with plasma CCK concentration in humans,[8] and the diminished release of CCK contributes to gallstone formation in humans.[9]

Hyperadrenocorticism is a common endocrinopathy that affects middle-aged to older dogs.[10,11] The most common clinical signs of chronic hyperadrenocorticism are polyuria, polydipsia, polyphagia, dermatologic abnormalities, abdominal distention, and signs of lethargy.[11,12] A striking association between hyperadrenocorticism and GBM in dogs was identified in a study,[13] in which dogs with hyperadrenocorticism were 29 times as likely to have GBM as were dogs without hyperadrenocorticism. Several other studies[14–16] have been conducted to examine the relationship between GBM and hypercortisolemia in Beagles with iatrogenic hyperadrenocorticism. Microbiologic evaluation of gallbladder bile in dogs with iatrogenic hyperadrenocorticism revealed no correlation between the presence of GBM and bactibilia,[14] and iatrogenic hyperadrenocorticism did not trigger gallbladder sludge formation.[15] However, healthy Beagles that received exogenous hydrocortisone in another study[16] had a significant increase in unconjugated bile acids concentration and decrease in total taurine-conjugated bile acids concentration within the gallbladder. Therefore, a shift to a more caustic bile acid profile may irritate the gallbladder epithelium,[17] with subsequent mucinous hyperplasia and eventual mucocele formation. Nonetheless, whether hypercortisolemia is the primary cause of canine GBM, as opposed to the secondary cause of an underlying gallbladder dysfunction or gallbladder dysmotility, remains unclear.

The purpose of the study reported here was to assess whether any differences in circulating CCK concentrations existed between dogs with naturally acquired PDH and healthy dogs. We also evaluated whether low circulating CCK concentration was associated with GBM in dogs with PDH.

Materials and Methods

Animals

Client-owned dogs brought to the veterinary teaching hospital of Chungbuk National University between September 2011 and March 2013 were eligible for inclusion in this case-control study. To be included as cases, dogs were required to have a new diagnosis of untreated PDH and ultrasonographic evidence of gallbladder sludge or GBM. Dogs with evidence of concurrent disease other than GBM were excluded from the study. Sexually intact bitches were also excluded to avoid possible confounding effects of sex hormones on serum CCK concentration. Cases were further classified as having PDH and gallbladder sludge or having PDH and GBM.

To be included as controls, dogs were required to be healthy, as determined via physical examination, indirect measurement of systolic blood pressure, examination of fecal specimens for parasites (flotation technique), heartworm antigen testing, CBC and serum biochemical analyses (including amylase, lipase, and pancreas-specific lipase activity), urinalysis, ACTH response testing, thyroid function testing (plasma total thyroxine, free thyroxine, and thyroid-stimulating hormone concentrations), and diagnostic imaging (thoracic and abdominal radiography and abdominal ultrasonography). Controls were also required to have the same body condition score as the cases. They were included regardless of whether they had gallbladder sludge, but those with GBM were excluded. Controls were further classified as having or not having gallbladder sludge.

The study was approved by the University Ethics Committee of Chungbuk National University. Informed consent was obtained from all dogs' owners prior to inclusion of their dogs in the study.

Diagnosis of PDH

The diagnosis of PDH was made on the basis of established criteria.[11,18–20] First, a tentative diagnosis of hyperadrenocorticism was made with consideration of each dog's medical history, physical examination findings, and hematologic, serum biochemical, and urinalysis results. All dogs with suspected hyperadrenocorticism had polyuria (urine output > 50 mL/kg/d) and polydipsia (water intake > 100 mL/kg/d). All dogs fulfilled at least 4 of the following clinicopathologic criteria: high serum ALP activity, high serum alanine aminotransferase activity, hypercholesterolemia, hypertriglyceridemia, hyperglycemia, and urine specific gravity < 1.020.

All dogs with suspected hyperadrenocorticism received ACTH-stimulation and low-dose dexamethasone suppression testing. Blood samples were collected for ACTH-stimulation testing before and 1 hour after IV administration of synthetic ACTH[a] (0.25 mg/dog). Blood samples were collected for the suppression test before (baseline) and 4 and 8 hours after IV administration of dexamethasone[b] (0.01 mg/kg). A diagnosis of hyperadrenocorticism was made on the basis of a high serum cortisol concentration 8 hours after dexamethasone administration. A diagnosis of PDH specifically was made when the serum cortisol concentration was < 1.5 µg/dL at 4 hours after dexamethasone administration or was < 50% of the baseline concentration at 4 or 8 hours after dexamethasone administration.[19,20]

Serum was harvested from blood samples. Serum cortisol concentrations were measured by use of a chemiluminescent immunoassay-based autoanalyzer,[c] as described elsewhere.[21] All dogs with PDH were also examined by abdominal ultrasonography.[d] Pituitary-dependent hyperadrenocorticism was differentiated from functional adrenal tumor on the basis of the results of the low-dose dexamethasone suppression test and abdominal ultrasonographic evaluations of the adrenal glands, as described elsewhere.[11,18]

Serum insulin concentration was also measured with a commercial kit[e] in accordance with the manufacturer's instructions (intra-assay variation, 1%; inter-assay variation, 7%; lower detection limit, 1.266 µU/mL).[19] Assay results were quantified by use of an automated microplate reader.[f]

Ultrasonographic evaluation

Ultrasonographic evaluation of all dogs was performed after food had been withheld for 12 hours. Ultrasonographic images were obtained with dogs positioned in dorsal recumbency by use of a ventral subcostal and right-sided intercostal approach. Longitudinal and transverse images of the gallbladder were obtained, permitting evaluation for the presence of gallbladder sludge and GBM. Gallbladder sludge was defined as mobile echogenic material within the gallbladder lumen without acoustic shadowing,[2,3] and GBM was defined as immobile material within the gallbladder lumen with a finely striated or stellate pattern.[1,4] The examination was performed for each dog by 2 investigators (DC and JC) independently, and final ultrasonographic appearance was achieved through consensus of their findings. For dogs with PDH, follow-up ultrasonographic examination was repeated in the same manner without knowledge of treatment received.

Measurement of serum CCK concentration

All dogs were hospitalized for at least 48 hours and food was withheld for at least 12 hours before blood samples were collected for measurement of serum CCK concentration. Great care was taken to avoid exposing the dogs to any sight or smell of food. After collection of a preprandial blood sample (0 hours), each dog was immediately fed one-fourth of a can of high-fat canned dog food.[g] Postprandial blood samples were then collected 1, 2, and 4 hours after feeding, with measurement points selected on the basis of a previous report.[22] Serum was separated from clotted whole blood samples by centrifugation at 1,200 × *g* for 10 minutes within 1 hour after sample collection and stored at –80°C until analyzed.

Serum CCK concentration was measured with an ELISA kit[h] in accordance with the manufacturer's instructions (intra-assay variation, < 5%; interassay variation, < 3%; and lower detection limit, 0.35 pmol of CCK/L of serum). All samples and standards were assayed in duplicate with an automated microplate reader.[i]

Evaluation of serum CCK concentration before and after trilostane treatment

All dogs with PDH in the study received treatment with trilostane[j] to inhibit cortisol production by the adrenal glands.[19,20] The initial trilostane dose was 0.5 to 1.0 mg/kg, administered PO every 12 hours. Subsequently, the dose was adjusted on the basis of the ACTH-stimulated serum cortisol concentration and whether clinical signs improved or resolved. All treated dogs were reassessed after an additional 4 weeks of treatment to determine any effects on serum CCK concentration.

Statistical analysis

All statistical analyses were performed with statistical software.[k] The Kolmogrov-Smirnov test was performed for data with a normal distribution. Results were reported as median (interquartile range) for nonnormally distributed data. A value of $P < 0.05$ was considered significant for all analyses, unless otherwise indicated.

The Kruskal-Wallis test was used to compare preprandial serum CCK concentrations among 4 groups of dogs: healthy dogs with gallbladder sludge, healthy dogs without gallbladder sludge, dogs with PDH and gallbladder sludge, and dogs with PDH and GBM. The Mann-Whitney *U* test with Bonferroni adjustment for multiple comparisons was then performed, for which a value of $P < 0.008$ was considered significant.

To compare changes in serum CCK concentrations from before to after the meal among the 4 groups, the Friedman test with Dunn post hoc test was used. Two-way ANOVA and the post hoc Tukey honest significant difference test were performed to evaluate the influences of assessment point and dog group on serum CCK concentration. The integrated AUC was calculated for each time-versus-CCK concentration curve. The Wilcoxon signed rank test for matched pairs was used to compare changes in all analytes between before and after trilostane treatment within the 2 groups of dogs with PDH. The Mann-Whitney *U* test was used to compare all variables between 2 groups, with values of $P < 0.05$ considered significant.

Results

Fifty-three client-owned dogs with PDH were initially considered as cases for the study. Four dogs were excluded because follow-up evaluation was refused by owners. Forty-nine dogs with gallbladder sludge (n = 40) or GBM (9) were initially selected on the basis of results of ultrasonographic evaluation. Because all 9 dogs with PDH and GBM were overweight (body condition score, 7/9) and to control for this variable, 28 overweight dogs with gallbladder sludge or GBM were subsequently selected for the study. Eleven of the 28 dogs were excluded because of evidence of concurrent disease other than GBM (ie, chronic kidney disease plus pancreatitis [n = 3], cranial cruciate ligament rupture and patellar luxation [2], myxomatous mitral valve disease [2], diabetes mellitus [2], pancreatitis alone [1], or immobile material in the gallbladder other than GBM [1]). Three sexually intact bitches were also excluded. Consequently, 14 dogs with PDH were included in the study. The group with gallbladder sludge included 8 dogs, and the group with GBM included 6 dogs **(Table 1)**.

Fourteen healthy controls with the same body condition score as the cases (7/9) were included in the study. The group with gallbladder sludge included 7 dogs, and the group without gallbladder sludge included 7 dogs (Table 1).

Comparisons among all 4 groups

Significant differences were identified in preprandial serum CCK concentrations for all pairwise

Table 1—Characteristics of healthy client-owned dogs without (n = 7) and with (7) ultrasonographic evidence of gallbladder sludge and of dogs with PDH and gallbladder sludge (8) or GBM (6).

Characteristic	Healthy without gallbladder sludge	Healthy with gallbladder sludge	With PDH and gallbladder sludge	With PDH and GBM
Reproductive status				
Sexually intact male	2	2	2	2
Neutered male	2	2	3	2
Sexually intact female	0	0	0	0
Spayed female	3	3	3	2
Age (y)	12.0 (10.0–13.0)	12.0 (10.0–13.0)	12.0 (10.0–13.0)	12.0 (10.0–13.0)
Body weight (kg)	9.20 (4.56–11.75)	8.94 (4.08–11.40)	7.75 (3.56–14.00)	9.85 (5.20–14.20)
Breed				
Miniature Schnauzer	2	2	2	2
Shih Tzu	2	2	2	2
Pomeranian	1	1	1	1
Mixed	1	1	1	1
Other	1	1	2	0

Values represent median (range) for age and body weight and counts for all other variables.

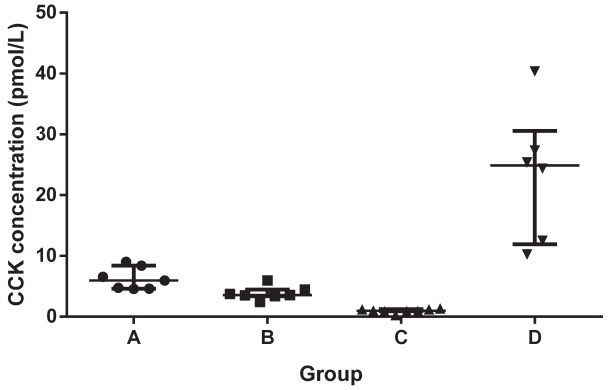

Figure 1—Dot plots of preprandial serum CCK concentrations in individual client-owned dogs that were healthy and lacked (group A; n = 7) or had (group B; 7) gallbladder sludge identified via ultrasonography or had PDH and gallbladder sludge (group C; 8) or GBM (group D; 6). Long horizontal bars indicate the median, and shorter horizontal bars represent the interquartile range. Significant ($P < 0.008$) differences were identified for all pairwise comparisons (Mann-Whitney *U* test with Bonferroni adjustment).

comparisons among the 4 groups of dogs. The median CCK value for dogs with PDH and gallbladder sludge was significantly ($P < 0.001$) lower than that in healthy dogs with or without gallbladder sludge or dogs with PDH and GBM, and the median value for dogs with GBM was the highest of all 4 groups **(Figure 1).**

Serum CCK concentrations in the 4 groups were reevaluated 1, 2, and 4 hours after the dogs consumed a fatty meal, revealing significant ($P < 0.001$) differences between pre- and postprandial values in all groups except dogs with PDH and GBM **(Figure 2).** Of note, although preprandial serum CCK concentrations differed significantly ($P < 0.001$) among the 4 groups, no significant intergroup differences were identified for postprandial concentrations 1, 2, or 4 hours after the meal. The integrated AUC for dogs with PDH and GBM was significantly ($P = 0.003$) greater than that of dogs with PDH and gallbladder sludge; no other significant differences were identified with respect to this variable.

Effect of trilostane treatment

All 14 dogs with PDH received trilostane treatment. Regarding those with gallbladder sludge, ACTH-stimulated serum cortisol concentrations were within the target range (2 to 5.5 µg/dL) for 5 dogs after 16 weeks and for 3 dogs after 20 weeks of treatment. Regarding the dogs with GBM, target values were achieved for 2 dogs after 16 weeks and for 4 dogs after 20 weeks of treatment. By 16 weeks after treatment initiation, polyuria, polydipsia, and polyphagia had resolved in all dogs and alopecia and abdominal distention had improved. However, serial ultrasonographic evaluation revealed no evidence of improvement in gallbladder sludge or GBM in affected dogs.

Comparisons of serum CCK concentrations in dogs with PDH before and after trilostane treatment revealed no significant differences for those with gallbladder sludge ($P = 0.25$) or those with GBM ($P = 0.09$; **Table 2**). The median CCK concentration after trilostane treatment was still higher for dogs with GBM, although there was no significant difference between pre- and posttreatment values.

Prior to trilostane treatment, serum glucose ($P = 0.009$) and insulin ($P = 0.01$) concentrations and serum ALP activity ($P = 0.008$) were significantly higher in dogs with GBM than in dogs with gallbladder sludge. After treatment, there were no significant differences between the 2 groups in any variable (Table 2).

After trilostane treatment, all dogs had significant decreases from pretreatment values in serum activities of ALP, alanine transferase, and γ-glutamyltransferase and serum concentrations of total cholesterol, glucose, and insulin. However, serum triglycerides concentration was unaffected by this treatment (Table 2).

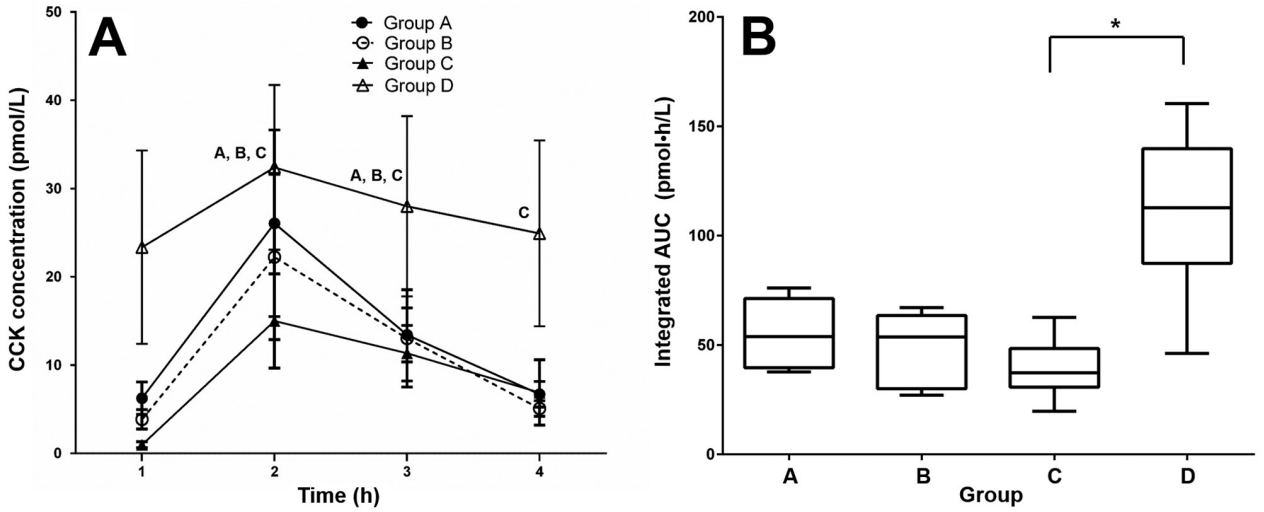

Figure 2—Serum CCK concentrations at various points before (0 hours) and after consumption of a high-fat meal (A) and box plots of integrated AUCs of serum CCK values measured during the first 4 hours after that meal for individual client-owned dogs that were healthy and without (group A; n = 7) or with (group B; 7) gallbladder sludge identified via ultrasonography or had PDH and gallbladder sludge (group C; 8) or GBM (group D; 6; B). *Median values for the indicated groups differed significantly (P = 0.008; Mann-Whitney U test with Bonferroni adjustment). $^{A–C}$Letters represent the group for which the corresponding median value differed significantly (P < 0.001) from that at 0 hours.

Table 2—Median (interquartile range) values of various analytes measured in serum samples from dogs with PDH and gallbladder sludge (n = 8) or GBM (6) before and after trilostane treatment.

	With gallbladder sludge			With GBM		
Analyte	**Before**	**After**	***P* value**	**Before**	**After**	***P* value**
CCK (pmol/L)	0.96 (0.89–1.23)	1.88 (0.37–2.69)	0.25	25.84* (11.92–33.17)	21.64 (6.098–32.24)	0.09
Cortisol (μg/dL)	10.85 (9.12–12.03)	2.90 (1.99–3.84)	0.008	11.52 (6.70–16.25)	3.53 (3.02–4.16)	0.03
Insulin (μU/mL)	8.85 (6.10–13.43)	3.27 (1.87–4.74)	0.01	18.40* (13.17–20.65)	5.94 (2.79–8.90)	< 0.001
ALP (U/L)	658.0 (284.8–2,000)	347.0 (128.8–1,048.0)	0.008	3,287* (753.0–7,385.0)	952.5 (134.0–1,923.0)	0.03
Alanine aminotransferase (U/L)	158.5 (88.2–202.0)	38.5 (18.2–139.5)	0.008	241.0 (142.3–381.0)	95.0 (41.5–107.5)	0.03
γ-Glutamyltransferase (U/L)	7.0 (3.0–11.0)	3.0 (2.0–6.0)	0.03	11.5 (5.8–34.5)	7.5 (4.2–10.0)	0.04
Total cholesterol (mg/dL)	258.0 (213.5–319.5)	205.0 (144.8–268.5)	0.02	312.5 (277.3–348.5)	244.0 (180.3–287.0)	< 0.001
Glucose (mg/dL)	123.0 (98.0–126.0)	89.0 (83.0–98.0)	< 0.001	158.0* (124.9–223.0)	98.50 (89.6–109.5)	< 0.001
Triglycerides (mg/dL)	133.0 (56.5–215.5)	127.0 (54.8–249.3)	0.73	233.0 (105.5–395.8)	186.0 (86.8–297.0)	0.09

*Value differs significantly (P < 0.05) from the respective value for dogs with PDH and gallbladder sludge.
Values of P < 0.05 were considered significant for all comparisons.

Discussion

Mice with deficiencies in CCK and CCK-1(A) receptors have gallbladder hypomotility and sludge formation.[7,23] In humans, CCK administration can prevent gallbladder sludge buildup during parenteral feeding,[24] and serum CCK concentration is positively correlated with gallbladder sludge formation and gallbladder stones.[9,25] Therefore, many investigators have proposed that a decrease in gallbladder motility mediated by a low circulating CCK concentration is a predisposing factor for accumulation of biliary sludge[3,26] and development of GBM.[27] Consequently, we hypothesized that dogs with PDH and GBM might also have a low circulating CCK concentration.

However, whereas results of the present study indicated that preprandial serum CCK concentrations in dogs with PDH and gallbladder sludge were decreased relative to those of healthy dogs, no differences were identified in postprandial CCK concentrations. Dogs with PDH also had differences in serum

CCK concentrations measured before and after trilostane treatment. These findings suggested that hypercortisolemia is unlikely to cause a decrease in serum CCK concentration in dogs. Moreover, CCK concentrations in dogs with PDH and GBM were unexpectedly high, contradicting the supposition that low amounts of hormones in systemic circulation might contribute to GBM development.

Given the link between serum CCK concentration and gallbladder motility, we could not conclusively explain why serum CCK concentrations were higher in dogs with PDH and GBM than in dogs with PDH but without GBM. We can speculate that the observed increase in serum CCK concentration might have resulted from a feedback mechanism related to the decrease or loss of gallbladder contractility following persistent GBM. Humans with gallbladder stones can be classified into 2 subgroups with regard to gallbladder emptying: contractors and noncontractors.[9,28] Contractors have a low plasma CCK concentration relative to that of noncontractors or healthy individuals as well as a high degree of CCK receptor expression.[9,28] In contrast, noncontractors have high plasma CCK concentrations and a low degree of CCK receptor expression relative to that of contractors or healthy individuals.[9,28] Therefore, high production of CCK in dogs with PDH and GBM might be attributable to a noncontractile GBM, although the pathogenesis of gallbladder stones differs from that of GBM. Additional research into whether serum CCK concentration increases secondary to GBM will be necessary to improve our understanding of the pathogenesis of GBM in dogs.

The present study revealed that serum glucose and insulin concentrations were higher in dogs with PDH and GBM than in those with gallbladder sludge, whereas previous investigations revealed that hyperinsulinemia and hyperglycemia were risk factors for gallbladder disorders in humans and mice.[29,30] Moreover, gallbladder dysmotility is purportedly caused by a diminished sensitivity of the gallbladder to circulating CCK in humans with diabetes mellitus.[29,30] Therefore, an increase in serum insulin and glucose concentrations resulting from chronic hypercortisolemia in dogs with hyperadrenocorticism might increase the risk of GBM. However, no improvement in gallbladder sludge and GBM in dogs with PDH treated with trilostane was identified through serial ultrasonographic evaluation, even though serum glucose and insulin concentrations decreased after treatment. The study follow-up period may simply have been too brief to detect changes in gallbladder properties via ultrasonography, and therefore, any potential trilostane-mediated alterations must be reevaluated through longer-term studies. However, to the best of our knowledge, no reports exist of improvement or resolution of GBM in dogs with hyperadrenocorticism after pharmacological intervention.

A high serum triglycerides concentration in humans is believed to promote a decrease in sensitivity of the gallbladder smooth muscle to CCK, which, as previously mentioned, is a risk factor for gallbladder dysmotility and gallbladder disorders, particularly gallbladder stones.[31] A particularly high serum triglycerides concentration can similarly accompany GBM in dogs,[4,32] and a retrospective case-control study[33] revealed an association between hypertriglyceridemia or hypercholesterolemia and GBM in that species. In contrast, we detected similar serum total cholesterol and triglyceride concentrations in dogs with PDH and GBM, compared with dogs with PDH with a relatively innocuous GBM, although our failure to detect significant differences between these groups might have reflected a type II error or the relatively small number of dogs used. In addition, this discrepancy between study results might have been attributable to qualitative differences between study populations.

The lack of other differences in the study reported here must be interpreted with caution in light of the small sample size. A major concern is that the serial evaluation of postprandial serum CCK concentrations after a high-fat meal revealed no significant difference among the study groups at any assessment point. A power calculation to determine an appropriate sample size for the study was not initially performed because no published data existed regarding serum CCK concentrations in dogs with hyperadrenocorticism when the study was conceived. Although post hoc analysis of the data revealed a statistical power of 99% for detecting differences in serum CCK concentrations between 2 groups containing 14 dogs with PDH, analyses with a larger number of subjects are still required to confirm the findings.

The present study was additionally limited by the fact that measurement of the amount of CCK in circulation in conscious dogs is problematic because dietary and neuromodulatory factors can affect CCK production. For example, even the sight or smell of food could influence CCK concentration in the bloodstream,[34,35] even though a carefully controlled environment was used to minimize this possibility. Also, CCK in plasma from peripherally obtained blood samples from dogs[36,37] and humans[38] appears to be molecularly heterogenous, which could potentially hamper measurements of circulating CCK concentration via antibody-dependent assays, including the ELISA used in the present study.

We found no evidence for the contribution of CCK to the pathogenesis of GBM in dogs with PDH in the study reported here. Additional investigation with a larger number of subjects is needed to clarify the association between hypercortisolemia, serum CCK concentration, and development of GBM in dogs.

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Drs. Noh and Kim designed the study, performed the research, analyzed the data, and wrote the manuscript. Dr. J. Chang performed the research and analyzed the data. Dr. Kang designed the study, performed the research, analyzed the data, was involved

in drafting the manuscript, and approved the revisions. Drs. D. Chang and Yang performed the research and analyzed the data.

The authors declare that there were no conflicts of interest.

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Footnotes

a. Synacthen, Novartis, Basel, Switzerland.
b. Dexamethasone, Je Il Pharm Co, Daegu, Korea.
c. Immulite 1000, Siemens, Los Angeles, Calif.
d. ProSound Alpha 5, Aloka Co, Tokyo, Japan.
e. Milliplex MAP canine kit, Millipore Co, Billerica, Mass.
f. Luminex200, Luminex Co, Billerica, Mass.
g. Hill's a/d, Hill's Pet Nutrition Inc, Topeka, Kan.
h. Uscn Life Science Co Ltd, Whuan, China.
i. ELx 808, BioTek Instruments Inc, Winooski, Vt.
j. Vetoryl, Arnolds Veterinary Products, Shrewsbury, England.
k. Prism 6 for Windows, version 6.05, Graph-Pad Software, La Jolla, Calif.

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