

Relationship of hyperlipidemia and gallbladder sludge scores in dogs with hyperadrenocorticism

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Abstract

Hyperadrenocorticism (HAC) and hyperlipidemia have been identified as the causes of cholestatic disease in dogs. However, the possibility of direct association of HAC with a cholestatic disease or secondary hyperlipidemia-induced cholestatic disease in dogs with HAC has not been elucidated. This study aimed to evaluate the differences in serum triglyceride (TG), total cholesterol (TC) and basal cortisol (BC) concentrations based on the gallbladder (GB) sludge score in dogs with HAC. This study was a retrospective cohort study. In total, 36 dogs with pituitary-dependent hyperadrenocorticism (PDH) were divided into six groups (GB scores 0–5) based on the ultrasonographic evaluation of the GB at the time of diagnosing HAC. Serum concentrations of TG, TC and BC were measured and compared among the six groups. Additionally, correlation analysis was conducted to determine the relationships between the variables. The median serum TG concentration was significantly higher ($P = 0.003$) in the cholestatic disease group (GB score 1–5) (median, 102.0 [range, 36.0–1121] mg/dL) than that in the normal group (GB score 0) (median, 50.0 [range, 50.0–56.0] mg/dL). There was a positive correlation between serum TG concentrations and the GB sludge score ($P = 0.035$, $r_s = 0.353$). Furthermore, there was no significant difference in serum TC and BC concentrations among the groups and no correlation between serum TC or BC concentration and the GB sludge score was observed. Our results suggest that an increased serum TG concentration could be associated with the severity of cholestatic disease in dogs with HAC.

Keywords: canine, cholestatic disease, gallbladder dysmotility, hypertriglyceridemia

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Introduction

Hyperadrenocorticism (HAC) is a relatively common endocrine disease in elderly dogs, with a prevalence of more than 1 in 100 dogs diagnosed with HAC (Willeberg and Priester, 1982; Bellumori *et al.*, 2013). In cases of HAC among canines, pituitary-dependent hyperadrenocorticism (PDH) accounts for 80-85% and the remaining proportion is attributed to adrenocortical tumors (AT) and iatrogenic HAC (Owens and Drucker, 1977; Ling *et al.*, 1979; Behrend *et al.*, 2013). HAC is known to increase the risk of forming gallbladder (GB) sludge and GB mucoceles in dogs (Pike *et al.*, 2004; Mesich *et al.*, 2009). A GB mucocele is an abnormal intraluminal accumulation of concentrated bile or mucus in a distended GB (Besso *et al.*, 2000; Mehler and Bennett, 2006). The predisposing factors for GB sludge and mucocele formation include hypothyroidism, gallbladder dysmotility, dyslipidemias and exogenous steroid administration (Aguirre *et al.*, 2007). Among these predisposing factors, hyperlipidemia has been reported to cause cholestatic disease in dogs (Kutsunai *et al.*, 2014).

However, the direct association between HAC and cholestatic disease or secondary hyperlipidemia-induced cholestatic disease in dogs with HAC has not been elucidated. Therefore, this study examined whether hyperlipidemia affects cholestatic disease in dogs with HAC. This study aimed to evaluate differences in serum triglyceride (TG) and total cholesterol (TC) levels in dogs with HAC, as hyperlipidemia is defined as increased TG, TC or both levels in blood (Xenoulis and Steiner, 2010). Furthermore, we evaluated basal cortisol (BC) concentrations according to the GB sludge score to identify the association between hypercortisolemia and cholestatic disease in dogs with HAC.

Materials and Methods

Case Selection: In total, 62 dogs with naturally occurring HAC who presented to the Veterinary Teaching Hospital of Chungbuk National University between October 2014 and June 2022 were examined. This study was approved by the University Ethics Committee (CBNUA-2001-22-02). Of the 62 dogs with HAC, 7 were diagnosed with AT using endocrine function tests and ultrasonographic evaluations were excluded (Behrend *et al.*, 2013). In addition, 19 dogs diagnosed with PDH were excluded for one or more of the following reasons: (i) the presence of other concurrent endocrine diseases such as hypothyroidism and diabetes mellitus that could cause either cholestatic disease or hyperlipidemia; (ii) not being in a fasting state for at least 12 h; (iii) administration of drugs that could cause hyperlipidemia, such as glucocorticoids and phenobarbital; (iv) administration of ursodiol which could improve cholestatic disease; or missing medical records.

Overall, the HAC group consisted of 36 dogs with PDH. They were divided into six groups according to the ultrasonographic evaluation of the GB at the time of HAC diagnosis: GB sludge score 0 (n = 3), GB sludge score 1 (n = 11), GB sludge score 2 (n = 4), GB sludge score 3 (n = 7), GB sludge score 4 (n = 2), and GB sludge

score 5 (n = 9). The ultrasonographic evaluation of the GB was based on a semi-objective scoring system, which estimates the percentage of the total GB volume filled with biliary sludge (BS): score 0 for no BS, score 1 for BS filling <25% of GB volume, score 2 for BS filling 26% to 50% of GB volume, score 3 for BS filling 51% to 75% of GB volume, score 4 for BS filling >75% of GB volume and score 5 for GB mucocele (Butler *et al.*, 2022). After ultrasonographic evaluation of the GB, the 36 dogs with PDH were also sub-classified into either three or two groups. The three groups were composed of the normal (GB sludge score 0) group, cholestasis (GB sludge score 1-4) group, and GB mucocele (GB sludge score 5) group. The two groups were the normal (GB sludge score 0) group and the cholestatic disease (GB sludge score 1-5) group.

Diagnosis: HAC was diagnosed as described previously (Resuch and Feldman, 1991; Feldman *et al.*, 1996; Behrend *et al.*, 2013). The provisional diagnosis of HAC was based on the patient's history, physical examination, urinalysis and blood analysis at admission. The main clinical signs in dogs diagnosed with HAC were polyuria and polydipsia. Each dog diagnosed with HAC had at least four abnormal findings: abdominal distension, symmetrical alopecia, urine specific gravity <1.020, increased activity of serum alkaline phosphatase (ALP), increased activity of serum alanine aminotransferase (ALT), hypertriglyceridemia (HTG) and hypercholesterolemia.

A final diagnosis of HAC was confirmed using an adrenocorticotrophic hormone (ACTH) stimulation test, low-dose dexamethasone suppression test (LDDST) or both. For the ACTH stimulation test, blood samples were collected before and 1 h after IV administration of 250 µg of synthetic tetracosactrin (Synacthen; Dalim Pham Co., Wonju, Republic of Korea). HAC was diagnosed if the serum cortisol concentration was >22 µg/dL 1 h after synthetic tetracosactrin administration (Rodriguez *et al.*, 2011). For the LDDST, blood samples were collected before and 4 and 8 h after IV administration of 0.01 mg/kg dexamethasone (Dexamethasone®; Je Il Pham Co., Daegu, Republic of Korea). HAC was diagnosed if the serum cortisol concentration was >1.5 µg/dL at 8 h after dexamethasone administration (Behrend *et al.*, 2013; Oh *et al.*, 2022).

PDH was diagnosed if the serum cortisol concentration was <50% of the pre-value concentration 4 h after dexamethasone administration. All dogs were evaluated using abdominal ultrasonography. Ultrasonographic findings suggestive of PDH are bilaterally enlarged adrenal glands (Bennaim *et al.*, 2018). Enlargement of the right and left adrenal gland was confirmed by ultrasonographic evaluation of adrenal gland width (>0.60 and >0.62 cm for dogs that weighed ≤12 kg; >0.72 and >0.70 cm for dogs that weighed >12 kg, respectively) (Bento *et al.*, 2016). Ultrasonographic findings that suggested AT were of moderate asymmetry in the adrenal glands and contralateral adrenal atrophy with a smaller adrenal gland width of <5 mm. Consequently, PDH was differentiated from AT based on the results of

endocrine tests and ultrasound images of the adrenal glands as previously described (Resuch and Feldman, 1991; Feldman *et al.*, 1996; Behrend *et al.*, 2013).

HTG was defined as a serum TG concentration above the reference interval (21-116 mg/dL), and hypercholesterolemia was defined as serum TC concentration above the reference interval (135-270 mg/dL) (Kim *et al.*, 2019). Blood samples for HTG and hypercholesterolemia testing were obtained after a minimum of 12 h of fasting.

Statistical Analysis: Data was analyzed using GraphPad Prism version 9.4.1 (GraphPad Software Inc., San Diego, CA, USA). A normality test was conducted using the Shapiro-Wilk test to determine whether data was normally distributed. However, following statistical analyses were performed using non-parametric tests because many groups did not follow a normal distribution. Continuous variables are expressed as medians with ranges. Categorical variables (sex and breed) were described as frequencies. *P* values were calculated using two-tailed tests and 95% confidence intervals (CI) for differences between medians were evaluated. The Kruskal-Wallis test or Mann-Whitney U test was performed to compare continuous variables (age, body condition score on a 9-point scale, activity of serum ALT, and activity of serum ALP) among groups divided according to the GB sludge scoring system.

The serum concentrations of TG, TC and BC were compared between groups using the Kruskal-Wallis test with Dunn's multiple comparisons test or Mann-Whitney U test. Correlations of the GB sludge score with TG, TC and BC were evaluated using Spearman's correlation tests. Fisher's exact test was conducted to examine the differences in the proportions of cholestatic disease (GB sludge score 1-5) between the HTG and normal TG groups and between the hypercholesterolemia and normal TC groups. *P* < 0.05 was considered statistically significant.

Results

The basic characteristics of the 36 dogs with PDH are shown in Table 1. There were no statistically significant differences in age, body condition score, serum ALT and ALP levels and fasting blood glucose concentration among the six groups. Of the 36 dogs, 19 were female (10 intact and 9 spayed) and 17 were male (2 intact and 15 spayed). The breeds included Shih Tzu (*n* = 12), Maltese (*n* = 7), Yorkshire Terrier (*n* = 4), two Miniature Pinschers, two mixed-breed dogs, two Pomeranians, two Miniature Poodle, one Beagle, one Boston Terrier, one Dachshund, one Pekingese, and one Spitz.

When comparing the six groups (Table 2), there were no significant differences in the median serum concentrations of TG, TC and BC between the groups.

When comparing the three groups (GB score 0, GB score 1-4, and GB score 5), there were no statistically significant differences in age, body condition score, serum ALT level, serum ALP level and fasting blood glucose concentration among the three groups (Table 3). However, there was a significant difference in the median serum TG concentration among the three groups. After conducting Dunn's multiple comparisons test, the median serum TG concentration was found to be significantly higher in the cholestasis group (GB score 1-4) (median, 107.0 [range, 36.0-436.0] mg/dL) than that in the normal group (GB score 0) (median, 50.0 [range, 50.0-56.0] mg/dL). The median serum TC concentration and median serum BC concentration did not differ between the groups.

In comparing the two groups (GB score 0 and GB score 1-5), there were no statistically significant differences in age, body condition score, serum ALT level, serum ALP level and fasting blood glucose concentration between the two groups. The median serum TG concentration was significantly higher in the cholestatic disease group (GB score 1-5) (median, 102.0 [range, 36.0-1121] mg/dL) than that in the normal group (GB score 0) (median, 50.0 [range, 50.0-56.0] mg/dL). The median serum TC and the median serum BC concentrations did not differ between the groups (Table 4).

The correlation analysis showed a positive correlation between the serum TG concentration and GB sludge score (*P* = 0.035, *r_s* = 0.353) (Table 5). However, there was no correlation between serum TC concentration and the GB sludge score (*P* = 0.050, *r_s* = 0.329). Similarly, there was no correlation between serum BC concentration and the GB sludge score (*P* = 0.577, *r_s* = -0.096).

In comparing the proportions of cholestatic disease (GB score 1-5) (Table 6), the proportions of cholestatic disease did not differ between the HTG and normal TG groups. The proportions of cholestatic disease did not differ between the hypercholesterolemia and normal TC groups.

In comparing the GB score 0-2 and GB score 3-5 groups, there were no statistically significant differences in the age, body condition score, serum ALT level, serum ALP level and fasting blood glucose concentration between the groups. The median serum TG concentration was significantly higher in the 3-5 group (median, 127 mg/dL [range, 58-1121] mg/dL) than that in the GB score 0-2 group (median, 88 mg/dL [range, 36-200] mg/dL). The median serum TC concentration was significantly higher in the GB score 3-5 group (median, 338.5 [range, 203-1479] mg/dL) than that in the GB score 0-2 group (median, 231 [range, 127-495] mg/dL). The median serum BC concentration did not differ significantly between the groups.

Table 1 Basic characteristics of 36 dogs with pituitary-dependent hyperadrenocorticism (PDH)

Variables	GB score 0 (n = 3)	GB score 1 (n = 11)	GB score 2 (n = 4)	GB score 3 (n = 7)	GB score 4 (n = 2)	GB score 5 (n = 9)	P value
Median age (range), years	14 (9-14)	11 (4-15)	12.5 (10-13)	12 (9-16)	13.5 (12-15)	12 (8-16)	0.750
Median BCS (range)	5 (5-8)	5 (4-8)	5 (4-6)	6 (2-9)	6.5 (6-7)	6 (2-8)	0.826
Median serum ALT (range), IU/L	71 (71-93)	64 (34-1121)	105.5 (74-142)	73 (40-658)	215.5 (52-379)	102 (16-968)	0.873
Median serum ALP (range), IU/L	1229 (1229-1809)	882 (77-3270)	1111 (692-1396)	1264 (328-24030)	2438 (1295-3580)	1258 (77-3131)	0.679
Median serum glucose (range), mg/dl	108 (104-121)	103 (66-118)	109.5 (96-124)	104 (82-129)	113.5 (105-122)	112 (101-119)	0.454
Sex, n (%)							
F	2 (66.7)	4 (36.4)	0	2 (28.6)	1 (50)	1 (11.1)	
SF	0	2 (18.2)	2 (50)	0	0	5 (55.6)	
M	0	1 (9.1)	0	1 (14.3)	0	0	
CM	1 (33.3)	4 (36.4)	2 (50)	4 (57.1)	1 (50)	3 (33.3)	
Breed, n (%)							
Maltese	0	2 (18.2)	1 (25)	2 (28.6)	0	2 (22.2)	
Miniature Pinscher	2 (66.7)	0	0	0	0	0	
Mixed breed	0	0	0	2 (28.6)	0	0	
Pomeranian	0	0	1 (25)	0	0	1 (11.1)	
Miniature Poodle	0	0	0	1 (14.3)	0	1 (11.1)	
Shih Tzu	0	5 (45.5)	2 (50)	1 (14.3)	1 (50)	3 (33.3)	
Yorkshire Terrier	0	1 (9.1)	0	1 (14.3)	1 (50)	1 (11.1)	
Other purebreeds	1 (33.3)	3 (27.3)	0	0	0	1 (11.1)	

ALP, alkaline phosphatase; ALT, alanine aminotransferase; BCS, body condition score; CM, castrated male; F, female; GB, gallbladder; M, male; SF, spayed female.

Table 2 Comparison of variables to GB scores

Variables	GB Score 0	GB Score 1	GB Score 2	GB Score 3	GB Score 4	GB Score 5	P value
Median TG (range), mg/dL	50.0 (50.0-56.0)	90.0 (36.0-200.0)	109.5 (51.0-133.0)	145.0 (87.0-436.0)	149.5 (102.0-197.0)	97.0 (58.0-1121)	0.058
Median TC (range), mg/dL	228.0 (214.0-228.0)	235.0 (191.0-439.0)	244.5 (127.0-495.0)	359.0 (274.0-1264)	388.0 (348.0-428.0)	306.0 (203.0-1479)	0.107
Median BC (range), µg/dL	6.95 (6.60-6.95)	7.20 (2.50-13.20)	8.00 (2.70-11.30)	6.93 (2.29-23.80)	5.75 (4.60-6.90)	4.82 (1.50-9.80)	0.890

BC, basal cortisol; GB, gallbladder; TC, total cholesterol; TG, triglyceride.

Table 3 Comparison of variables in cholestasis (GB score 1-4) and GB mucocoele (GB score 5)

Variables	Normal (GB score 0)	Cholestasis (GB score 1-4)	GBM (GB score 5)	P value
Median age (range), yr	14 (9-14)	12 (4-16)	12 (8-16)	0.823
Median BCS (range)	5 (5-8)	5.5 (2-9)	6 (2-8)	0.922
Median serum ALT (range), IU/L	71 (71-93)	73.5 (34-1121)	102 (16-968)	0.967
Median serum ALP (range), IU/L	1229 (1229-1809)	1229 (77-24030)	1258 (77-3131)	0.909
Median serum glucose (range), mg/dl	108 (104-121)	104.5 (66-129)	112 (101-119)	0.369
Median TG (range), mg/dL	50.0 (50.0-56.0)	107.0 ^a (36.0-436.0)	97.0 (58.0-1121)	0.033*
Median TC (range), mg/dL	228.0 (214.0-228.0)	296.5 (127.0-1264)	306.0 (203.0-1479)	0.374
Median BC (range), µg/dL	6.95 (6.60-6.95)	6.915 (2.29-23.80)	4.820 (1.50-9.80)	0.546

*P < 0.05, considered statistically significant.

^aP = 0.028, compared with the normal (GB score 0) group.

ALP, alkaline phosphatase; ALT, alanine aminotransferase; BC, basal cortisol; BCS, body condition score; GB, gallbladder; GBM, GB mucocoele; TC, total cholesterol; TG, triglyceride.

Table 4 Comparison of variables in cholestatic disease (GB score 1–5)

Variables	Normal (GB score 0)	Cholestatic disease (GB score 1–5)	P value
Median age (range), yr	14 (9-14)	12 (4-16)	0.648
Median BCS (range)	5 (5-8)	6 (2-9)	0.712
Median serum ALT (range), IU/L	71 (71-93)	74 (16-1121)	0.946
Median serum ALP (range), IU/L	1229 (1229-1809)	1240 (77-24030)	0.738
Median serum glucose (range), mg/dl	108 (104-121)	108 (66-129)	0.615
Median TG (range), mg/dL	50.0 (50.0-56.0)	102.0 (36.0-1121)	0.003*
Median TC (range), mg/dL	228.0 (214.0-228.0)	300.0 (127.0-1479)	0.176
Median BC (range), µg/dL	6.95 (6.60-6.95)	6.6 (1.5-23.8)	0.797

* $P < 0.05$, considered statistically significant.

ALP, alkaline phosphatase; ALT, alanine aminotransferase; BC, basal cortisol; BCS, body condition score; GB, gallbladder; TC, total cholesterol; TG, triglyceride.

Table 5 Respective correlations between serum triglyceride concentration, serum total cholesterol concentration, or serum basal cortisol concentration and GB sludge score

Variables	Spearman r_s	P value
Between TG and GB sludge score	0.353	0.035*
Between TC and GB sludge score	0.329	0.050
Between BC and GB sludge score	-0.096	0.577

* $P < 0.05$, considered statistically significant.

BC, basal cortisol; GB, gallbladder; TC, total cholesterol; TG, triglyceride.

Table 6 Comparisons of the proportions of cholestatic disease (GB score 1–5) in hypertriglyceridemia and hypercholesterolemia

Variables	HTG (n = 14)	Normal TG (n = 22)	Hypercholesterolemia (n = 21)	Normal TC (n = 15)
Cholestatic disease (GB score 1-5), n (%)	14 (100)	19 (86.4)	21 (100)	12 (80)
P value	0.267		0.064	

GB, gallbladder; HTG, hypertriglyceridemia; TC, total cholesterol; TG, triglyceride.

Table 7 Comparison of variables in the GB score 0–2 and GB score 3–5 groups

Variables	GB score 0–2	GB score 3–5	P value
Median age (range), yr	11.5 (4-15)	12 (8-16)	0.294
Median BCS (range)	5 (4-8)	6 (2-9)	0.470
Median serum ALT (range), IU/L	72.5 (34-1121)	87.5 (16-968)	0.557
Median serum ALP (range), IU/L	1195 (77-3270)	1280 (77-24030)	0.343
Median serum glucose (range), mg/dl	105 (66-124)	111 (82-129)	0.261
Median TG (range), mg/dL	88 (36-200)	127 (58-1121)	0.025*
Median TC (range), mg/dL	231 (127-495)	338.5 (203-1479)	0.011*
Median BC (range), µg/dL	6.95 (2.5-13.2)	5.74 (1.5-23.8)	0.476

* $P < 0.05$, considered statistically significant.

ALP, alkaline phosphatase; ALT, alanine aminotransferase; BC, basal cortisol; BCS, body condition score; GB, gallbladder; TC, total cholesterol; TG, triglyceride.

Discussion

Our study showed that the severity of cholestatic disease correlated with serum TG concentration in dogs with HAC. In humans, patients with HTG had impaired GB motility compared to healthy controls (Jonkers *et al.*, 2003). Additionally, impaired GB

motility was improved by appropriate TG-lowering treatment with fish oil and bezafibrate (Jonkers *et al.*, 2003). In humans, it has been established that HTG induces insulin resistance (Li *et al.*, 2014) and insulin resistance leads to GB dysmotility (Boland *et al.*, 2002; Nakeeb *et al.*, 2006). Similarly, HTG has been reported

to induce insulin resistance in dogs (Xenoulis *et al.*, 2011). Insulin controls the Na⁺-K⁺ pump, which affects osmotic and ionic homeostasis and causes dysfunction of GB myocytes (Sweeney and Klip, 2001). These studies suggest that increased serum TG concentration might induce GB dysmotility and thereby affect the development of cholestatic disease. However, the present study did not evaluate insulin resistance, although fasting blood glucose concentration was not different among different GB sludge scores and no diabetic dogs with HAC were noted in the present study. Further studies are needed to investigate the association between serum TG concentration, insulin resistance and cholestatic disease in dogs with HAC considering GB motility. Although there were no significant differences in the proportion of cholestatic disease, all dogs with HTG had cholestatic disease (GB sludge score 1-5) in this study. This result also supports the idea that increased serum TG concentration could be associated with the severity of cholestatic disease in dogs with HAC.

Our findings showed no correlation between serum TC concentration and the severity of cholestatic disease in dogs with HAC. It has been previously reported that the oversaturation of bile with cholesterol induces cholestatic disease in dogs (Behrend, 2015). However, serum TC concentrations did not differ between dogs with cholestatic disease and controls (Kutsunai *et al.*, 2014). Similarly, serum TC concentrations did not differ between dogs with cholestatic disease and control dogs after administering hydrocortisone (Kook *et al.*, 2012). Despite HAC being known to cause hypercholesterolemia in dogs, our study suggests that increased serum TC concentration might not be associated with the severity of cholestatic disease in dogs with HAC, as supported by previous studies (Kook *et al.*, 2012; Kutsunai *et al.*, 2014). In comparing the proportions of cholestatic disease, all dogs with hypercholesterolemia had cholestatic disease (GB sludge score 1-5). This result may be due to the small sample size in the normal (GB sludge score 0) group. If the sample size in the normal (GB sludge score 0) group were larger, some dogs with hypercholesterolemia might have had GB sludge scores of 0. The median serum TC concentration was significantly higher in the GB sludge score 3-5 group than that in the GB sludge score 0-2 group. Nonetheless, this does not necessarily imply that serum TC concentration was associated with cholestatic disease severity in dogs with HAC, as the median serum TC concentration did not differ among the six groups, and there was no correlation between serum TC concentration and GB sludge score. Therefore, the present study suggests that increased serum TC concentration may not be associated with the severity of cholestatic disease in dogs with HAC and previous reports (Kook *et al.*, 2012; Kutsunai *et al.*, 2014) support this finding.

Our results showed that serum BC concentration was not correlated with the severity of cholestatic disease in dogs with HAC. In dogs, the inhibition of glucocorticoids induced a significant decrease in GB motility and normal motility was recovered with the administration of hydrocortisone (Potapov *et al.*, 1976). Cortisol has been suggested to improve GB dysmotility

which induces cholestatic disease. In another study, hydrocortisone was administered to investigate the effects of iatrogenic HAC on the development of cholestatic disease in dogs (Kook *et al.*, 2012). There were no differences in the ratio of GB sludge formation between the administered and control groups in that study (Kook *et al.*, 2012), indicating that cortisol does not affect the formation of GB sludge. Furthermore, there were no differences in serum BC concentrations among normal, cholestasis and GB mucocele dogs (Kim *et al.*, 2017), indicating that HAC is not directly associated with cholestatic disease.

To the best of our knowledge, no study has clarified the correlation between HTG and cholestatic disease in dogs without HAC. However, some studies have shown that serum TG and TC levels were not different between dogs with and without GB sludge (Secchi *et al.*, 2012; Tsukagoshi *et al.*, 2012), indicating that GB sludge may not cause HTG. Therefore, HTG might be a cause of GB sludge, not a consequence. In human patients with HTG, bile appears to be supersaturated with cholesterol compared with controls (Ahlberg *et al.*, 1980). HAC is a well-known cause of HTG in dogs (Xenoulis and Steiner, 2010). Furthermore, iatrogenic HAC induced by hydrocortisone could cause shifts toward higher concentrations of the more hydrophobic unconjugated bile acids (chenodeoxycholic acid and deoxycholic acid) and lower concentrations of the amphipathic taurine-conjugated bile acids in GB bile (Kook *et al.*, 2011), although it is not known whether the changed bile composition is caused by induced hypercortisolemia or secondary HTG. Therefore, a higher prevalence of HTG in dogs with HAC than in dogs without HAC could be a cause of GB sludge. However, basal cortisol was not correlated with GB sludge score in our study, although HAC is reported as a risk factor for GB sludge (Cook *et al.*, 2016). Above all, the formation of GB sludge might be caused by the change in bile composition associated with secondary HTG in dogs with HAC. However, further studies are required for comparing the bile composition between normotriglyceridemic and hypertriglyceridemic dogs without HAC, to elucidate the effect of HTG on the formation of GB sludge in dogs with HAC. In other words, studies should determine whether the cause of GB sludge is a direct effect of hypercortisolemia or secondary HTG in dogs with HAC. Furthermore, HAC progress slowly and are often present for several months before diagnosis.

Our study has some limitations. First, there is a possibility that cholestatic disease induced an increase in serum TG concentration. Generally, impairment of GB emptying and consequent cholestatic disease increases serum TG and TC concentrations (Tsukagoshi *et al.*, 2012; Kutsunai *et al.*, 2014). However, only serum TG concentrations correlated with the severity of cholestatic disease and serum TC concentrations were not observed in the present study. Further studies are needed to elucidate whether an increase in serum TG concentration is the cause or consequence of cholestatic disease in dogs with HAC. Secondly, the sample size was small for some groups. Therefore, we also subdivided the 36 dogs with PDH into three groups. Thirdly, dogs with AT, which causes HAC, were excluded from this study. Dogs with AT

secrete various adrenal sex hormones that induce clinical signs similar to those observed in dogs with PDH (Frank *et al.*, 2015) and differences in sex hormone profiles may cause inter-dog variability (Hill *et al.*, 2005). On this basis, dogs with PDH could be more uniform than dogs with AT that secrete various adrenocortical hormones. Fourthly, the serum BC concentration changes with time and may vary depending on the measurement time (Nakeeb *et al.*, 2006). Therefore, our results regarding serum BC concentrations might not be accurate.

In conclusion, our results indicate that the GB sludge score correlates with serum TG concentration in dogs with PDH. Furthermore, we suggest that an increased serum TG concentration may be associated with the severity of cholestatic disease in dogs with HAC. However, further large cohort studies are needed to clarify the mediating effect of TG on the development of cholestatic disease in dogs with HAC.

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