NOTE

Effects of proportions of carbohydrates and fats in diets on mucin concentration and bile composition in gallbladder of dogs

Riho SHIKANO1), Koichi OHNO1), Takuro NAGAHARA1), Itsuma NAGAO1), Hiroto TOYODA1), Taisuke NAKAGAWA2), Yuko GOTO-KOSHINO1), James K CHAMBERS3), Hirotaka TOMIYASU1)*, Hajime TSUJIMOTO1)

1)Department of Veterinary Internal Medicine, Graduate School of Agricultural and Sciences, The University of Tokyo, Tokyo, Japan
2)Veterinary Medical Center, Graduate School of Agricultural and Sciences, The University of Tokyo, Tokyo, Japan
3)Department of Veterinary Pathology, Graduate School of Agricultural and Sciences, The University of Tokyo, Tokyo, Japan

ABSTRACT. The associations of diet compositions with mucin secretion in gallbladder have not been investigated in dogs. This study aimed to examine the effects of a low-carbohydrate diet (LC) and a low-fat diet (LF) on bile mucin concentration and composition of gallbladder bile in six clinically healthy beagle dogs. After feeding of both diets, the bile mucin concentration was significantly decreased. In addition, there were significant decreases in the concentrations of taurochenodeoxycholic acid in bile, which is considered to promote mucin secretion, after feeding of both diets. The present study suggested that the proportions of carbohydrate and fat in diet affect the composition of gallbladder bile in dogs.

KEYWORDS: bile acid, canine, low-carbohydrate diet, low-fat diet, mucin

Gallbladder diseases in dogs include gallbladder mucocele, bile sludge, and cholelithiasis. Gallbladder mucocele is a disease of excessive accumulation of viscous mucus in the gallbladder, and bile sludge is an accumulation of echogenic material without acoustic shadowing in the gallbladder. Cholelithiasis is a disease with stones in the gallbladder due to crystallization or precipitation of substances in the bile. In humans, cholesterol gallstones are common, but gallstones observed in dogs are primarily composed of mucin, bilirubin and calcium [13]. Because the main component of these mucus and substances has been found to be mucin in dogs, the excessive mucin secretion has been thought to be associated with the occurrences of gallbladder disease [2, 3, 17].

Mucin is secreted by the mucosal epithelium of the gallbladder and is essential for protection of the mucosal epithelium [5, 17, 18]. Mucin secretion is affected by inflammation of the gallbladder and changes in the composition of bile acids in humans [23]. The major bile acids in dogs include taurocholic acid (TCA), taurodeoxycholic acid (TDCA), and taurochenodeoxycholic acid (TCDCA), and it has been reported that TDCA and TCDCA promote mucin secretion from the gallbladder epithelium [14, 24]. Therefore, it is reasonable to hypothesize that increased TDCA and TCDCA concentrations in the gallbladder of dogs would be associated with the occurrence of canine gallbladder diseases.

A previous study in dogs showed that feeding a high-fat and high-cholesterol diet increased TCDCA concentrations in bile, although feeding a low-fat diet decreased TDCA concentrations and increased tauroursodeoxycholic acid (TUDCA) concentrations [11]. Actually, previous studies also showed the associations between diet and formation of gallstones, and feeding a high-carbohydrate diet was shown to be associated with formation of pigment gallstone in hamsters [16] and in humans [19]. Also in prairie dogs, feeding a high-carbohydrate diet induced increased TCDCA concentrations and formation of bile sludge and gallstone [1]. In dogs, low-protein, high-cholesterol, high-carbohydrate, and methionine-deficient diet induced bile sludge and pigment gallstones [8]. However, there has been no study that investigated the effects of the proportion of carbohydrates and fats in diet on composition of gallbladder bile in dogs.

Therefore, the purpose of this study was set to examine the changes in the concentration of mucin and the composition of bile acids in gallbladder bile after feeding of two diets with different proportions of carbohydrates and fats.

Six beagle dogs kept in our laboratory were used in this study. Four dogs were castrated male and two dogs were spayed female,
the median age was 7 (1–7) years old, and the median body weight was 10.0 (8.2–12.0) kg. The signalment of each dog is shown in Supplementary Table 1. Before this study, they were fed a commonly available total nutritional diet twice a day for 1 month. They were kept in cages and the temperature of the room was kept at 25°C. All dogs had no clinical sign and underwent physical examination, complete blood counts, plasma biochemical analysis, thoracic X-ray examinations and abdominal ultrasonographic examination. Since bile sludge is observed in the gallbladders of clinically healthy dogs [4], dogs with bile sludge were not excluded in the present study. Five dogs were considered healthy based on the results of these examinations, and one dog continued to show elevated alkaline phosphatase (ALP). The dog with elevated ALP was not excluded from the present study, because the histopathological examination of the liver tissue of the dog did not show any abnormalities. All experimental and animal care procedures complied with the policies outlined in the Guide to Animal Use and Care of the University of Tokyo (Approval No. P20-069).

This study was designed as an open, randomized, 2 × 2 crossover trial. Six dogs were arbitrarily allocated into two groups of three dogs each and fed a low-carbohydrate diet (LC) or low-fat diet (LF) for 4 weeks, and they were fed the other diet for 4 weeks after a 4-week washout period according to the previous studies [11, 16]. The LC and LF used in this study were commercially available therapeutic diets (Canine glycobalance canned dog food, Gastrointestinal low fat wet; Royal Canin Japon, Tokyo, Japan). The diet that was used during washout period was also a commercially available therapeutic diet that was not low-carbohydrate or low-fat one (Select Skin Care; Royal Canin Japon) (Supplementary Table 2). Caloric intake during the feeding period was constant, and the animals were fed twice a day.

Before and after each 4-week feeding periods, we measured body weight of dogs, collected blood and gallbladder bile samples, and measured the volume of bile sludge. Five mL of blood samples were collected and plasma was immediately separated to measure ALP, alanine aminotransferase (ALT), γ-glutamyltranspeptidase (GGT), aspartate aminotransferase (AST), calcium (Ca), glucose, blood urea nitrogen (BUN), creatinine, lipase, albumin, total bilirubin, total cholesterol (T.Cho) and triglyceride using Fuji Dri-Chem 5000V (Fujifilm Corp., Tokyo, Japan).

During the collection of gallbladder bile, dogs were sedated with medetomidine (0.01 mg/kg, i.v.) and midazolam (0.1 mg/kg, i.v.), and gallbladder bile was obtained via ultrasound-guided cholecystocentesis [10]. After 12–18 hr of fasting, all bile in the gallbladder was collected. The concentration of mucin in bile was measured using Fecal Mucin Assay Kit (Cosmo Bio Ltd., Tokyo, Japan) [6]. The concentrations of each bile acid, total bile acids, bilirubin, phospholipid, cholesterol, and calcium in the gallbladder bile were measured at a commercial testing laboratory (SRL, Tokyo, Japan). Bile acids and bilirubin, phospholipids and cholesterol, and calcium were measured by high-performance liquid chromatography, spectrophotometry, and atomic absorption spectrophotometry, respectively.

For the measurements of the volume of bile sludge, long axis image of the gallbladder was obtained by ultrasonography (HI VISION Ascendus, HITACHI, Tokyo, Japan) under sedation after 12–18 hr of fasting, and the proportions of bile sludge area in the gallbladder area were calculated using software (Image J version 1.53, https://imagej.nih.gov/ij/). Then, the volumes of bile sludge were approximated by multiplying the calculated proportions of bile sludge by the volumes of the gallbladder that were calculated according to the elliptic method; $V = 0.53 \times a \times b \times c$, $V$: volume of the gallbladder, $a$: maximum length of the gallbladder in the long axis, $b$: maximum width of the gallbladder in the long axis, $c$: maximum depth of the gallbladder in the short axis [7].

The results of blood examinations, bile mucin concentration, proportion of each bile acid, and volume of bile sludge were compared between before and after feeding periods of each food by Wilcoxon signed rank test using statistical software (JMP, SAS Institute, Cary, NC, USA). Values of $P<0.05$ were considered significant.

No significant change in body weight was observed after 4-week feeding period of LC or LF, although two dogs (Dog 3 and Dog 6) had mild decrease of appetite and diarrhea for several days during the feeding period of LC.

Results of blood examinations showed that ALP ($P=0.03$), GGT ($P=0.03$), and BUN ($P=0.03$) were significantly decreased after feeding of LC, and T.Cho was significantly decreased after feeding of LF ($P=0.03$) (Supplementary Table 3).

The median bile mucin concentration was significantly decreased from 37.5 (21.0–52.6) mg/dL to 12.0 (4.9–19.1) mg/dL after feeding of LC ($P=0.03$, Fig. 1). Also after feeding of LF, the median bile mucin concentration was significantly decreased from 21.7 (15.9–37.7) mg/dL to 17.2 (11.8–31.1) mg/dL ($P=0.03$, Fig. 1). When examined the changes in the concentrations of bile acids, there was no significant change in the concentration of TCA after feeding of LC or LF (Fig. 2A). However, the concentrations of TCDCA ($P=0.03$, Fig. 2B), which promotes mucin secretion, and total bile acid ($P=0.03$, Supplementary Table 4) were significantly decreased after feeding of LC. There were also significant decreases in the concentrations of TCDCA ($P=0.03$), TDCA ($P=0.03$), which also promotes mucin secretion, TUDCA ($P=0.03$), tauroliothocholic acid (TLCA) ($P=0.03$), and total bile acids ($P=0.03$) in bile after feeding of LF (Fig. 2B and 2C, and Supplementary Table 4), and the concentration of glycochenodeoxycholic acid (GCDC) were significantly increased ($P=0.03$) after feeding of LF (Supplementary Table 4). These results indicated the possibility that the decrease of TCDCA and TDCA in bile resulted in the decrease of mucin secretions into gallbladder bile. Previous studies showed that
CYP7a1 and CYP8b1 were associated with bile acid synthesis [9, 22], and CYP7a1 mRNA expression was significantly increased by feeding of high-fat and high-cholesterol diet [11]. Considering these findings, it was suggested that the changes in the proportions of carbohydrates and fats in the diets might induce the changes in the expression of molecules related to the bile acid synthesis pathway, such as CYP7a1 and CYP8b1. Additionally, the expression of MUC5ac, one of the major canine mucin subtypes, was increased in bile of dogs with gallbladder mucocele compared to healthy dogs [12]. Since we did not examine the concentrations of each mucin subtype in bile in this study, it is needed to examine the associations of changes in diet compositions with the expressions of each mucin subtype. The decrease of total bile acid concentration was thought to be derived from the decreases in the concentrations of TCA or TCDA after feeding of the diets, because these bile acids account for 31.5–35.5% of total bile acids in dogs [24].

In this study, glycine was contained in LF but not in LC and maintenance diet, and the difference in the intake amounts of glycine might result in the difference of changes in the concentrations of GCDA, which is glycine-conjugated bile acids. In the present study, the concentrations of TUDCA and TLCA were significantly decreased after feeding of LF. On the other hand, a previous study showed that the concentrations of TUDCA decreased and those of TLCA increased after the feeding of high-fat and high-cholesterol diet [11]. Although the causes of the differences in the results were unclear, the composition of fat in the diet may affect the concentrations of TUDCA and TLCA in gallbladder bile.

There was a tendency to decrease in the bile calcium concentration (P=0.06) (Supplementary Table 4) after feeding of LC, and there were significant decreases in the concentrations of bilirubin (P=0.03), and calcium (P=0.03) in bile after feeding of LF (Supplementary Table 4). There was no significant change in blood bilirubin concentration after feeding LF (Supplementary Table 3). In humans, it has been reported that ionized calcium concentration in bile is positively correlated to calcium concentration in bile [20] and the concentrations of ionized calcium in bile increase following to the increase of bile mucin concentration [20]. Therefore, it was possible that the decrease of bile mucin concentration induced the decrease of bile calcium concentration also in dogs after feeding of LC and LF. Bilirubin is present in the blood as unconjugated forms and in the gallbladder as conjugated forms after it undergoes glucuronide conjugation in the liver [1]. Therefore, the results in this study suggested that the glucuronic acid conjugation capacity in the liver may have decreased after feeding of LF due to uncovered mechanisms. However, other mechanisms should be also investigated because blood bilirubin concentration did not significantly change after feeding of LF.

We examined the changes in the proportions of each bile acid in gallbladder bile, and it was shown that the proportions of TCA (P=0.03) and glycchoolic acid (GCA) (P=0.03) in bile were significantly increased and that of TCDCA was significantly decreased (P=0.03) after feeding of LC (Supplementary Fig. 1A and 1B, and Supplementary Table 5). After feeding of LF, the proportions of TCA (P=0.03) and GCDA (P=0.03) were significantly increased, and those of TCDCA (P=0.03), TUDCA (P=0.03), and TLCA (P=0.03) were significantly decreased (Supplementary Fig. 1A and 1B, and Supplementary Table 5). However, there was no significant change in the proportion of TCA after feeding of LC or LF (Supplementary Fig. 1C). The increase of the proportion of TCA after feeding of LC and LF and that of GCA after feeding of LF were thought to be derived from the decreased concentrations of total bile acids without significant changes of TCA and GCA concentrations.

Change in the bile sludge volumes were various among dogs, and when statistically analyzed, there was no significant change in the volume of bile sludge after feeding of LC or LF (Supplementary Fig. 2). In humans, it has been suggested that various factors other than mucin secretions such as Ca²⁺ and Na⁺ may be involved in the formation of bile sludge [1, 15, 20, 21], and further studies are needed to investigate the detailed mechanisms that induce the formations of bile sludge in dogs.

There are several limitations in this study. The proportions of both carbohydrates and fats were different between LC and LF, and it was unclear which of the two components had greater effects on the results obtained in this study. It should be also noted that the proportion of protein was relatively high in both LC and LF. Therefore, further studies using diets where the proportions of either carbohydrate or fat are changed are needed to clarify the effects of carbohydrates or fats in diets on the gallbladder of dogs. It is also a limitation in this study that floating mobile bile sludge in the gallbladder may not be accurately captured as bile sludge by the software. In addition, the proportions of bile sludge in the gallbladder were estimated based on the long axis image alone. Therefore,
further studies are needed to examine more accurate changes in the bile sludge volumes after feeding of LC and LF.

In conclusion, this study showed that both LC and LF decreased the concentration of mucin in gallbladder bile, accompanied by a decrease in the concentration of the bile acids that promote mucin secretion in gallbladder. In the future, it is needed to conduct long-term feeding trials in dogs with bile sludge or gallbladder diseases to examine the preventive or therapeutic benefits of LC or LF in canine gallbladder disease.

CONFLICT OF INTEREST. The authors have nothing to disclose.

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