

## Lipoprotein cholesterol and triglyceride concentrations associated with dog body condition score; effect of recommended fasting duration on sample concentrations in Japanese private clinics

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**ABSTRACT.** The objectives of this study were to survey clinics' guidance about recommended fasting duration (FD) prior to lipoprotein analysis, and to characterize lipoprotein cholesterol and triglyceride concentrations in obese and overweight dogs categorized on the basis of the 5-point body condition score (BCS) scale. A dataset was created from lipoprotein analysis medical records of 1,538 dogs from 75 breeds in 354 clinics from 2012 to 2013. A phone survey was conducted to obtain the clinics' FD. Two-level linear mixed-effects models were applied to the data. Over 50% of the clinics said they recommended fasting for 12 hr or more. Dogs in clinics with FD 12 hr or more had lower chylomicron triglyceride concentrations than those in clinics with FD less than 8 hr ( $P=0.05$ ). Mean ( $\pm$  SEM) BCS at sampling was  $3.7 \pm 0.02$ . Obese and overweight dogs had higher very low density lipoprotein (VLDL) and high density lipoprotein (HDL) cholesterol and triglyceride concentrations than ideal dogs ( $P<0.05$ ), but no such difference was found for low density lipoprotein cholesterol and triglyceride concentrations ( $P\geq 0.07$ ). Across all BCS, as dog age rose from 0 to 8 years old, HDL cholesterol concentrations decreased by 13.5 mg/dl, whereas VLDL triglyceride concentrations increased by 81.7 mg/dl ( $P<0.05$ ). In conclusion, FD of 8 hr or less may affect lipoprotein lipid concentrations. Obese and overweight dogs were characterized as having high VLDL and HDL cholesterol and triglyceride concentrations.

**KEY WORDS:** body condition score, canine, epidemiology, lipids, lipoprotein

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High cholesterol and triglyceride concentrations in dogs have been associated with health disorders, such as obesity and endocrine diseases, and are transported by lipoproteins [1, 9]. Canine lipoproteins can be categorized into four classes based on their hydrate density [13, 21]: chylomicrons (CM), very low density lipoproteins (VLDL), low density lipoproteins (LDL) and high density lipoproteins (HDL). A lipoprotein analysis service has been provided across Japan since 2005, and it has been suggested that the lipoprotein analysis could be an important tool for identifying appropriate therapies to improve the quality of life of dogs with dyslipidemia [14]. However, recommendations of fasting duration (FD) prior to lipoprotein analysis vary considerably between private clinics, because each clinic has its own policy. This is a serious complicating factor, because increased cholesterol and triglyceride concentrations observed in dogs after a meal are physiological and transient, and these higher concentrations typically fall again within 7–12 hr [3, 10].

In order to identify a patient dog's obesity, a body condition score (BCS) system has been widely used by veterinarians in Japan. Also, an assessment of associations between lipoprotein lipids and other physiologic variables, such as

age, sex and breeds, can be useful to help the diagnosis and therapy of dyslipidemia. Dogs could also be categorized into different size groups based on their breed, such as large, medium and small size; this would take account of some breed effects [16, 19]. However, in Japan, no studies have used the same mixed-effects model to characterize lipoprotein cholesterol and triglyceride concentrations in obese and overweight dogs, and examine other variables for lipoprotein lipids by using medical records. Furthermore, no studies have used BCS to determine the correlations between obese/overweight dogs and ideal dogs for the concentrations of the 4 classes of lipoprotein cholesterol and triglycerides. Additionally, the clinic information would include the dog's location, dog owner's social status and veterinary health guidance; this dog-clinic relationship is a multi-level structure, because a health related treatment on an individual dog is performed within a clinic. Therefore, the objectives of the present study were 1) to survey FD variations in private clinics, 2) to characterize lipoprotein cholesterol and triglyceride concentrations in obese and overweight dogs by using a mixed-effects model, 3) to examine other factors associated with lipoprotein lipid concentrations and 4) to determine the correlations between concentrations of the four classes of lipoprotein cholesterol or triglycerides and dog BCS.

### MATERIALS AND METHODS

*Dog database, a phone survey for FD and lipoprotein analysis:* A dog database has been created at Meiji University (Kawasaki, Japan) by cooperating with a veterinary service

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(Spectrum Lab Japan, Tokyo, Japan). The database contains information on the veterinary service recorded about individual dog's characteristics (BCS, age, sex, neuter status and breed) when they received serum samples for lipoprotein analysis in 2012 and 2013 from veterinarians in private clinics throughout Japan. The database does not include dogs' rearing environments and food information. The BCS for each dog was evaluated by the dog's veterinarian using a 5-point scale (1: thin, 2: underweight, 3: ideal, 4: overweight and 5: obese). Website information and brochures about the BCS scale system are widely available to veterinary clinics across Japan, provided by PFI (Washington, D.C., U.S.A.) and a nutrition company (Hill's-Colgate JAPAN Ltd., Tokyo, Japan).

A phone survey was conducted from October to December 2013 of the 491 clinics which submitted the serum samples for lipoprotein analysis between January 2012 and December 2013, in order to obtain information about the clinics' FD. Information was obtained from 377 (76.8%) of the 491 clinics.

Lipoprotein analysis was performed by a commercial laboratory (Skylight Biotech Corporation, Akita, Japan) using a high performance liquid chromatography system with gel permeation columns. The analysis included dogs' cholesterol and triglyceride concentrations in each lipoprotein class [14, 20].

**Data and exclusion criteria:** The initial dataset included data of 2,658 dogs comprising 81 breeds collected from the 377 veterinary clinics which responded to the phone survey. The following data were not included in the final dataset: records of second visits or more (762 records) and records of dogs having a health problem, i.e. diabetes mellitus, hypothyroidism and hyperadrenocorticism (228 records). Also, the records of thin dogs (3 records) and underweight dogs (47 records) were excluded, because there were few of these dogs, and they were suspected of having a health problem. Records were also excluded when total cholesterol (673.8 mg/dl or higher) or triglyceride concentrations (1,104.7 mg/dl or higher) were greater than the respective means + 3 × SD (80 records) [4] to eliminate extremely high value samples having hypercholesterolemia or hypertriglyceridemia [21]. Thus, the final dataset included the records of 1,538 dogs in 75 breeds from 354 clinics. When BCS was used for the comparison of lipoprotein lipid concentrations, further records of dogs with no BCS information were excluded (130 records).

**Categories:** Veterinarians were asked to choose one of the following FD time periods that most closely matched their clinic's recommendation; less than 4 hr, 4–5.9 hr, 6–7.9 hr, 8–9.9 hr, 10–11.9 hr and 12 hr or more (Table 1). For statistical analysis, the FD data in the phone survey were categorized into 3 groups; less than 8 hr, 8 to 11.9 hr and 12 hr or more, because there were relatively few samples in the 4–5.9 and 6–7.9 hr FD groups. Obese, overweight and ideal dogs were defined as dogs having BCS 5, BCS 4 and BCS 3, respectively. Dogs were classified into 2 sex groups (male dogs or female dogs) and were also categorized into 2 neuter status groups (intact dogs or neutered dogs). The dogs

Table 1. Relative frequency of fasting duration of 1,580 dogs in 354 clinics prior to lipoprotein analysis

Fasting duration	Proportion of clinics, %	Proportion of dogs, %
Less than 4 hr	11.6	8.5
4–5.9 hr	5.1	10.8
6–7.9 hr	2.0	2.3
8–9.9 hr	9.6	8.5
10–11.9 hr	20.9	19.5
12 hr or more	50.8	50.4

were initially categorized into 6 body size groups (breed body size) based on the 75 breed names [16]: giant, large, medium, small, toy and unknown. However, in the present study, giant sized dogs (9 records) were included in the large sized dog group, because there were few samples. Finally, there was an unknown group that consisted of mixed breed dogs. Additionally, the dogs were categorized into 4 breed groups, namely Miniature Schnauzers, Shetland Sheepdogs, Mixed breeds and Other breeds. The 2 named breeds were selected, because they are known to be at high risk of having hyperlipidemia [21], and there were more than 90 dogs in each breed group. All other pure breeds were categorized into the Other breeds category group.

**Statistical analysis:** All statistical analyses were performed using SAS software (SAS Int. Inc., Cary, NC, U.S.A.). Two-level analysis was applied, using a clinic at level 2 and an individual dog at level 1, to take account of the hierarchical structure of the individual dogs within a clinic. There were 2 Models: one compared lipoprotein cholesterol and triglyceride concentrations between the FD groups (Model 1); the other model examined BCS and other risk factors for lipoprotein cholesterol and triglyceride concentrations (Model 2). A 2-level linear mixed-effects model using the MIXED procedure with least square means test was performed in the 2 Models. Additionally, a natural log-transformation was used on lipoprotein lipid concentrations, except for HDL cholesterol concentrations, to obtain a normal distribution for analyses, and then, the data were back-transformed in order to present the results.

Model 1 contained the FD groups as an explanatory variable, whereas in Model 2, the dog BCS groups were included as an explanatory variable, with a block of FD groups. In the preliminary analysis using Model 2, there were no differences between FD groups for cholesterol and triglyceride concentrations in any lipoprotein classes ( $P \geq 0.06$ ). Additionally, dog characteristics, such as age, sex, neuter status and breed body size, were included in both Models. In order to compare lipoprotein lipid concentrations between specific breeds, the breed group replaced the body size group in Model 2. Also, dog age was centered at its grand mean value. Quadratic expressions of age and 2-way interactions between BCS groups and dog characteristics were also examined in Model 2, but were then removed from the Model if they were not significant ( $P \geq 0.05$ ). Additionally, both Models included the clinic as a random intercept. To assess the variations in lipoprotein cholesterol and triglyceride

Table 2. Comparisons of lipoprotein cholesterol and triglyceride concentrations (mg/dl) between fasting duration (FD) groups

Measurements	FD<8 hr	8–11.9 hr	FD≥12 hr	P-value
	Mean ± SE*	Mean ± SE	Mean ± SE	
Number of clinics	66	108	180	
Number of dogs	331	431	776	
Cholesterol, mg/dl				
Chylomicron	0.9 ± 1.13	0.9 ± 1.11	0.8 ± 1.09	0.29
Very low density lipoprotein	11.0 ± 1.08	11.7 ± 1.07	11.4 ± 1.06	0.78
Low density lipoprotein	54.0 ± 1.08	51.6 ± 1.07	58.2 ± 1.06	0.24
High density lipoprotein	199.3 ± 3.97	198.9 ± 3.39	202.7 ± 2.83	0.51
Total concentration	279.1 ± 1.03	276.2 ± 1.03	289.9 ± 1.02	0.19
Triglyceride, mg/dl				
Chylomicron	9.8 ± 1.16 <sup>a)</sup>	9.2 ± 1.13 <sup>a,b)</sup>	7.2 ± 1.11 <sup>b)</sup>	0.05
Very low density lipoprotein	99.0 ± 1.10	101.1 ± 1.08	96.0 ± 1.06	0.82
Low density lipoprotein	15.5 ± 1.05	16.4 ± 1.04	15.7 ± 1.04	0.56
High density lipoprotein	5.8 ± 1.07	5.7 ± 1.06	5.2 ± 1.05	0.21
Total concentration	143.7 ± 1.08	148.8 ± 1.07	139.6 ± 1.06	0.69

\*Mean and SE were estimated by mixed-effects multivariable models. a, b) Means within a row with different letters differ ( $P \leq 0.05$ ).

concentrations that could be explained by the clinic, intra-class correlation coefficients (ICC) were calculated by the equation of Dohoo *et al.* [7]. Normality of the residuals in the final Models was evaluated by using normal probability plots [11]. Also, partial correlation analysis was performed for lipid concentrations in the 4 lipoprotein classes, using age as a controlled variable. Finally, a statistical power calculation was performed for a comparison of lipoprotein cholesterol and triglyceride concentrations between the FD groups by using the POWER procedure.

## RESULTS

**FD:** Of the 354 clinics in the final dataset, 50% recommended their dog owners to ensure FD of 12 hr or more before coming to the clinic for lipoprotein analysis. Table 2 shows comparisons of lipoprotein cholesterol and triglyceride concentrations between the 3 FD groups. Dogs in clinics with FD 12 hr or more had lower CM triglyceride concentrations than those in clinics with FD less than 8 hr ( $P=0.05$ ;  $0.32 \leq \text{Power} \leq 0.65$ ). However, there were no differences between any of the FD groups for VLDL, LDL and HDL cholesterol and triglyceride concentrations ( $P \geq 0.21$ ;  $0.07 \leq \text{Power} \leq 0.99$ ).

**BCS and other factors for lipoprotein lipid concentrations:** Mean ( $\pm$  SEM) BCS and age at sampling for 1,408 dogs in 347 clinics were  $3.7 \pm 0.02$  and  $8.4 \pm 0.10$  years old, respectively; the age range was between 0 and 17 years old. Obese and overweight dogs had higher VLDL and CM cholesterol concentrations than ideal dogs ( $P < 0.05$ ; Tables 3–5), and obese dogs also had higher HDL cholesterol concentrations than ideal dogs ( $P < 0.05$ ). Additionally, obese and overweight dogs had higher VLDL and HDL triglyceride concentrations than ideal dogs ( $P < 0.05$ ). However, no differences were found between dog BCS groups for LDL cholesterol and triglyceride concentrations ( $P \geq 0.07$ ).

Cholesterol and triglyceride concentrations in the four lipoprotein classes were associated with increased age ( $P < 0.05$ ; Tables 3 and 4). The HDL and LDL cholesterol concentrations decreased respectively from 203.9 mg/dl to 190.4 mg/dl and from 60.1 mg/dl to 40.0 mg/dl as the dog age rose from 0 to 8 and 7 years old, and then, both concentrations rose again as age increased further ( $P < 0.05$ ; Fig. 1A). In contrast, there were relatively small changes in VLDL and CM cholesterol concentrations with age. Additionally, there was a large rise of 81.7 mg/dl in VLDL triglyceride concentrations as dog age rose from 0 to 8 years old ( $P < 0.05$ ; Fig. 1B), but there were relatively small changes in HDL, LDL and CM triglyceride concentrations with age.

In addition to BCS and age, breed body size, sex and neuter status were also associated with lipoprotein cholesterol and triglyceride concentrations ( $P < 0.05$ ; Tables 3 and 4). Small sized dogs had the highest CM and VLDL cholesterol and triglyceride concentrations, and the highest HDL triglyceride concentrations ( $P < 0.05$ ). However, small and toy sized dogs had lower LDL and HDL cholesterol concentrations than large sized dogs ( $P < 0.05$ ). Additionally, female dogs had lower LDL cholesterol concentrations and higher LDL triglyceride concentrations than male dogs ( $P < 0.05$ ). Also, neutered dogs had higher VLDL cholesterol, lower LDL cholesterol, higher VLDL and HDL triglyceride concentrations than intact dogs ( $P < 0.05$ ). ICC analysis showed that the clinic effect explained 6.1–17.0% of the total variation in the lipoprotein cholesterol and triglyceride concentrations.

**Correlations between lipoprotein lipid concentrations in 4 classes:** Table 6 shows the correlation coefficients between cholesterol and triglyceride concentrations in the four lipoprotein classes for obese and overweight dogs, and for ideal dogs. For obese and overweight dogs no correlation was found between VLDL and HDL cholesterol concentrations ( $P=0.15$ ), but in ideal dog, higher HDL cholesterol concentrations were correlated with lower VLDL cholesterol

Table 3. Estimates of fixed effects, random effect variance and intraclass correlation coefficient (ICC) included in each final model for 4 classes of lipoprotein cholesterol concentrations

Fixed effects, variance and ICC	Cholesterol concentrations, mg/dl							
	Chylomicron		Very low density lipoprotein		Low density lipoprotein		High density lipoprotein	
	Estimate ( $\pm$ SE)	P-value	Estimate ( $\pm$ SE)	P-value	Estimate ( $\pm$ SE)	P-value	Estimate ( $\pm$ SE)	P-value
Intercept	-0.174 (0.124)	0.14	2.435 (0.082)	<0.01	3.864 (0.089)	<0.01	195.71 (4.40)	<0.01
Overweight dogs	0.393 (0.081)	<0.01	0.318 (0.054)	<0.01	0.031 (0.081)	0.59	3.52 (2.96)	0.23
Obese dogs	0.225 (0.113)	0.04	0.272 (0.076)	<0.01	0.070 (0.113)	0.39	9.74 (4.10)	0.01
Age, years old*	0.082 (0.015)	<0.01	0.044 (0.007)	<0.01	0.027 (0.008)	<0.01	0.30 (0.40)	0.48
Age $\times$ Age	-0.006 (0.003)	0.02	-0.006 (0.002)	<0.01	0.009 (0.002)	<0.01	0.28 (0.09)	<0.01
Female dogs	-0.040 (0.074)	0.58	-0.032 (0.050)	0.52	-0.117 (0.055)	0.03	-3.96 (2.70)	0.14
Neutered dogs	0.108 (0.085)	0.20	0.118 (0.057)	0.03	-0.274 (0.062)	<0.01	-4.08 (3.08)	0.18
Large and giant	-0.829 (0.197)	<0.01	-0.494 (0.132)	<0.01	0.805 (0.145)	<0.01	29.33 (7.18)	<0.01
Medium	-0.466 (0.147)	<0.01	-0.279 (0.099)	<0.01	0.216 (0.108)	0.04	15.67 (5.37)	<0.01
Small	0.061 (0.088)	0.48	0.229 (0.059)	<0.01	0.310 (0.065)	<0.01	1.41 (3.20)	0.65
Unknown†	-0.034 (0.149)	0.82	-0.083 (0.100)	0.40	0.163 (0.110)	0.13	-1.01 (5.45)	0.85
Clinic variance	0.22 (0.06)		0.08 (0.02)		0.08 (0.03)		0.22 (0.06)	
Dog variance	1.50 (0.07)		0.68 (0.03)		0.84 (0.04)		1.50 (0.07)	
ICC,%	12.8		11.0		8.6		7.7	

\*Age was centered at its grand mean value. †Unknown group consisted of mixed breed dogs.

Table 4. Estimates of fixed effects, random effect variance and intraclass correlation coefficient (ICC) included in each final model for 4 classes of lipoprotein triglyceride concentrations

Fixed effects, variance and ICC	Triglyceride concentrations, mg/dl							
	Chylomicron		Very low density lipoprotein		Low density lipoprotein		High density lipoprotein	
	Estimate ( $\pm$ SE)	P-value	Estimate ( $\pm$ SE)	P-value	Estimate ( $\pm$ SE)	P-value	Estimate ( $\pm$ SE)	P-value
Intercept	2.076 (0.150)	<0.01	4.529 (0.091)	<0.01	2.697 (0.056)	<0.01	1.609 (0.067)	<0.01
Overweight dogs	0.471 (0.099)	<0.01	0.369 (0.060)	<0.01	-0.037 (0.038)	0.33	0.167 (0.043)	<0.01
Obese dogs	0.251 (0.138)	0.06	0.351 (0.083)	<0.01	-0.120 (0.053)	0.02	0.148 (0.060)	0.01
Age, years old*	0.112 (0.018)	<0.01	0.053 (0.008)	<0.01	0.012 (0.005)	0.02	0.009 (0.006)	0.09
Age $\times$ Age	-0.008 (0.003)	0.01	-0.008 (0.002)	<0.01	0.003 (0.001)	0.02	-0.004 (0.001)	<0.01
Female dogs	-0.101 (0.090)	0.26	-0.025 (0.054)	0.64	0.093 (0.035)	<0.01	0.044 (0.039)	0.26
Neutered dogs	0.162 (0.103)	0.11	0.214 (0.062)	<0.01	-0.017 (0.040)	0.67	0.139 (0.045)	<0.01
Large and giant	-0.874 (0.239)	<0.01	-0.583 (0.145)	<0.01	-0.058 (0.092)	0.52	-0.335 (0.104)	<0.01
Medium	-0.472 (0.178)	<0.01	-0.294 (0.108)	<0.01	0.020 (0.069)	0.76	-0.151 (0.077)	0.05
Small	0.026 (0.107)	0.80	0.177 (0.065)	<0.01	0.060 (0.041)	0.14	0.032 (0.046)	0.48
Unknown†	0.018 (0.181)	0.91	-0.070 (0.110)	0.52	-0.019 (0.070)	0.78	-0.054 (0.079)	0.49
Clinic variance	0.32 (0.09)		0.13 (0.03)		0.02 (0.01)		0.08 (0.02)	
Dog variance	2.22 (0.10)		0.81 (0.04)		0.34 (0.02)		0.41 (0.02)	
ICC,%	12.7		13.8		6.1		17.0	

\*Age was centered at its grand mean value. †Unknown group consisted of mixed breed dogs.

concentrations ( $r=-0.09$ ;  $P<0.05$ ). In obese and overweight dogs, higher LDL cholesterol concentrations were correlated with higher VLDL and HDL triglyceride concentrations ( $0.07\leq r\leq 0.11$ ;  $P<0.05$ ), whereas in ideal dogs, there were no such correlations ( $P\geq 0.14$ ). In both obese/overweight dogs and ideal dogs, there were positive correlations between lipoprotein triglyceride concentrations in the four classes ( $0.22\leq r\leq 0.82$ ;  $P<0.05$ ). Additionally, comparisons of lipoprotein cholesterol and triglyceride concentrations between the 4 breed groups are shown in Supplementary Table 1.

## DISCUSSION

Approximately 51% of the clinics recommended their dog owners to ensure FD of 12 hr or more before taking a serum sample for lipoprotein analysis. These dogs in the clinics with FD 12 hr or more tended to have lower CM triglyceride concentrations than dogs in clinics with shorter recommended FD. The CM class of canine lipoproteins mainly contains triglycerides and is the lipoprotein class mainly responsible for transfer of dietary lipids [21]. Therefore, our study indicates that the 51% of clinics with FD of 12 hr or more could avoid the effect of transient hyperlipidemia after a meal on the lipoprotein analysis for CM triglyceride concentrations.

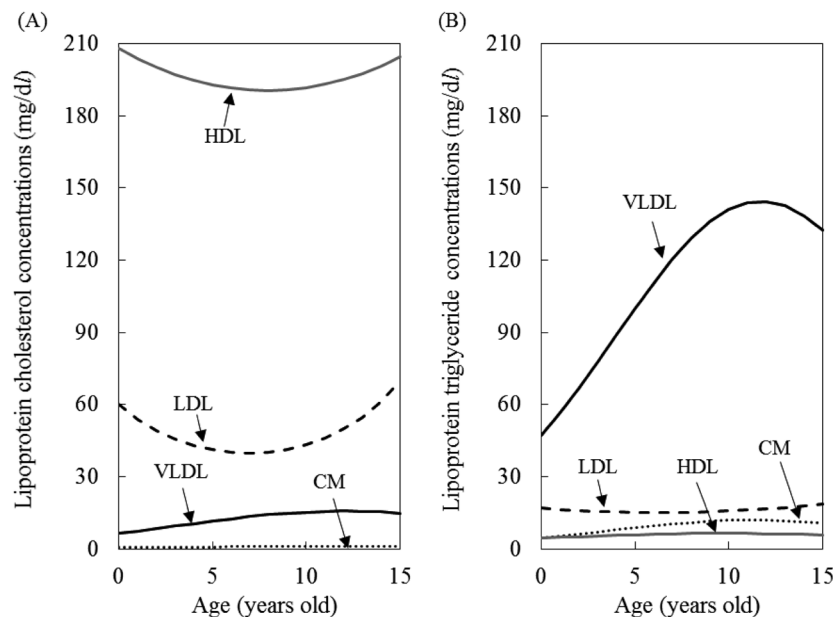


Fig. 1. Estimated cholesterol (A) and triglyceride (B) concentrations (mg/dl) in 4 lipoprotein classes\* of dogs at different ages. \*The 4 lipoprotein classes were chylomicrons (CM), very low density lipoproteins (VLDL), low density lipoproteins (LDL) and high density lipoproteins (HDL).

Table 5. Comparisons of lipoprotein cholesterol and triglyceride concentrations (mg/dl) in different dog body condition score groups

Measurements	Obese	Overweight	Ideal
	Mean $\pm$ SE*	Mean $\pm$ SE	Mean $\pm$ SE
Number of dogs	211	559	638
Cholesterol, mg/dl			
Chylomicron	0.9 $\pm$ 1.12 <sup>a</sup>	1.0 $\pm$ 1.09 <sup>a</sup>	0.7 $\pm$ 1.08 <sup>b</sup>
Very low density lipoprotein	12.7 $\pm$ 1.08 <sup>a</sup>	13.3 $\pm$ 1.06 <sup>a</sup>	9.7 $\pm$ 1.05 <sup>b</sup>
Low density lipoprotein	58.4 $\pm$ 1.09	56.2 $\pm$ 1.06	54.4 $\pm$ 1.06
High density lipoprotein	207.8 $\pm$ 4.19 <sup>a</sup>	201.6 $\pm$ 3.02 <sup>a,b</sup>	198.0 $\pm$ 2.84 <sup>b</sup>
Total concentration	296.5 $\pm$ 1.03 <sup>a</sup>	288.5 $\pm$ 1.02 <sup>a</sup>	275.9 $\pm$ 1.02 <sup>b</sup>
Triglyceride, mg/dl			
Chylomicron	8.8 $\pm$ 1.15 <sup>a,b</sup>	10.9 $\pm$ 1.11 <sup>a</sup>	6.8 $\pm$ 1.10 <sup>b</sup>
Very low density lipoprotein	115.1 $\pm$ 1.09 <sup>a</sup>	117.1 $\pm$ 1.07 <sup>a</sup>	81.0 $\pm$ 1.06 <sup>b</sup>
Low density lipoprotein	14.5 $\pm$ 1.05	15.7 $\pm$ 1.04	16.3 $\pm$ 1.04
High density lipoprotein	5.9 $\pm$ 1.06 <sup>a</sup>	6.0 $\pm$ 1.05 <sup>a</sup>	5.1 $\pm$ 1.04 <sup>b</sup>
Total concentration	157.6 $\pm$ 1.08 <sup>a</sup>	164.4 $\pm$ 1.06 <sup>a</sup>	124.6 $\pm$ 1.05 <sup>b</sup>

\*Mean and SE were estimated by mixed-effects multivariable models. a, b) Means within a row with different letters differ ( $P < 0.05$ ).

However, our study found 21.6% dogs in clinics with recommended FD of less than 8 hr, although food removal 8 to 12 hr prior to collection of samples is sufficient for general screening purposes [3, 10]. Therefore, it appears to be necessary for veterinarians to take more careful account of FD when they interpret lipoprotein concentrations in their patient dogs.

The present study showed that obese or overweight dogs had higher cholesterol and triglyceride concentrations in CM, VLDL and HDL than ideal dogs. Daily feeding of a

high-fat diet increases the production of CM by intestinal epithelial cells, and causes dog obesity [10] and abnormal insulin resistance [17]. For obese dogs with insulin resistance, an increased flux of non-esterified fatty acids from the adipose tissue results in enhanced hepatic triglyceride synthesis and reduced hepatic lipase and lipoprotein lipase activity, which are major enzymes involved in triglyceride clearance [2]. As a result, VLDL and HDL triglycerides are increased [1]. The increase of VLDL triglycerides raises VLDL cholesterol concentrations in circulating blood [2]. Additionally,



Table 6. Correlations between different cholesterol and triglyceride lipoprotein classes† concentrations of 1,353 dogs‡ categorized into either obese/overweight or ideal dog body condition classes

	Cholesterol concentrations				Triglyceride concentrations		
	CM	VLDL	LDL	HDL	CM	VLDL	LDL
Obese and overweight dogs (N=738)							
Cholesterol concentrations							
VLDL	0.81*	1.00	-	-	-	-	-
LDL	0.08	0.21*	1.00	-	-	-	-
HDL	-0.11*	-0.05	0.67*	1.00	-	-	-
Triglyceride concentrations							
CM	0.95*	0.74*	0.03	-0.08*	1.00	-	-
VLDL	0.81*	0.94*	0.11*	-0.03	0.82*	1.00	-
LDL	0.61*	0.58*	0.33*	-0.02	0.53*	0.50*	1.00
HDL	0.82*	0.71*	0.07*	-0.04	0.80*	0.73*	0.62*
Ideal dogs (N=615)							
Cholesterol concentrations							
VLDL	0.78*	1.00	-	-	-	-	-
LDL	0.01	0.12*	1.00	-	-	-	-
HDL	-0.11*	-0.09*	0.69*	1.00	-	-	-
Triglyceride concentrations							
CM	0.95*	0.75*	-0.02	-0.08*	1.00	-	-
VLDL	0.78*	0.96*	0.03	-0.06	0.80*	1.00	-
LDL	0.39*	0.28*	0.24*	-0.03	0.33*	0.22*	1.00
HDL	0.79*	0.74*	0.06	-0.02	0.76*	0.74*	0.45*

\* indicates  $P < 0.05$ . Partial correlation analysis was performed for the four lipoprotein classes, using age as a controlled variable.

†Lipoproteins were classified into four classes: chylomicrons (CM), very low density lipoproteins (VLDL), low density lipoproteins (LDL) and high density lipoproteins (HDL). ‡55 dogs did not have age information.

the reduction of hepatic lipase activity can contribute to high HDL cholesterol concentrations [2]. Therefore, in order to prevent lipoprotein lipid abnormalities, dog owners are recommended to provide a low-fat diet and appropriate exercise. In addition, veterinarians are recommended to prescribe medicines, such as fibrates, for obese dogs [18] with high VLDL and HDL cholesterol and triglyceride concentrations.

Our study showed that dog age affects VLDL triglyceride concentrations, and this indicates that triglyceride clearance in dogs of all BCS is suppressed as they get older. A feline study also reported that activities of lipoprotein lipase and hepatic lipase could be reduced more in senior cats than in younger ones [6]. Also, in the present study, there was an association between increased age and decreased HDL cholesterol concentrations. This association suggests that there is less uptake of cholesterol from body tissue in middle-aged and older dogs, because HDL transports cholesterol from body tissue to the liver [10]. Additionally, the relationship between increased age and decreased LDL cholesterol concentrations can be explained by a previous study showing greater hepatic LDL receptor activity in young dogs than in senior dogs [12]. The study showed that puppies contain 2 distinct lipoprotein receptors in their liver, an apoprotein B-E receptor which binds to both LDL and HDL cholesterol and an apoprotein E receptor which binds to only HDL cholesterol, whereas adult dogs had only the apoprotein E receptor [12]. Therefore, it is recommended that veterinarians take dog age into account when they interpret lipoprotein lipid concentrations.

Our study showed that small sized dogs had high CM, VLDL and HDL triglyceride concentrations, whereas large sized dogs had high LDL and HDL cholesterol concentrations. These results indicate that the small and large sized groups in our study contained some breeds which develop abnormal accumulations of triglycerides or cholesterol, e.g. Miniature Schnauzers (small size) and Golden Retrievers (large size) [21]. Additionally, our study showed low LDL cholesterol concentrations in female dogs, which is consistent with a previous study [15]. Also, the present study showed high VLDL and HDL triglyceride concentrations in neutered dogs, suggesting that a reduced metabolic rate due to neutering [8] impairs the clearance of triglycerides. Therefore, overall, it appears that the lipoprotein cholesterol and triglyceride concentrations are highly associated with physiologic factors, such as age, breed body size, sex and neuter status.

The negative correlations between HDL and VLDL cholesterol concentrations in the ideal dogs in our study suggest that increasing HDL cholesterol, which is carried from body tissue to the liver [10], is associated with a reduction in VLDL cholesterol synthesis in the liver [5]. In contrast, the lack of such negative correlations in obese and overweight dogs indicates that the relationships between HDL and VLDL cholesterol synthesis in these dogs are weaker than those in ideal dogs. Furthermore, the positive correlations between the triglyceride concentrations in the 4 lipoprotein classes for obese/overweight and ideal BCS groups show that the relationships for lipoprotein triglyceride synthesis did not

differ between obese/overweight and ideal dogs. In addition, the positive correlations between higher LDL cholesterol concentrations and both higher VLDL and HDL triglyceride concentrations in obese and overweight dogs suggest that enhanced hepatic triglyceride synthesis contributes to an increase in LDL synthesis. Additionally, our study showed a relatively high ICC of 6.1–17.0% for clinic variance in the lipoprotein lipid concentrations. This suggests that explained effects of the clinic, such as the dog's location, dog owner's social status or veterinary health guidance, can affect lipoprotein concentrations.

In conclusion, there appear to be some post-prandial effects of FD 8 hr or less on lipoprotein lipid concentrations. Also, the lipoprotein lipid concentrations differed depending on BCS, age, body size, sex and neuter status. Therefore, it is necessary for veterinarians to take account of these factors when they interpret lipoprotein lipid concentrations in their patient dogs.

Finally, it should be noted that there are some limitations in this present study because it is a cross-sectional study using veterinarian-submitted samples. Consequently, the dogs' rearing environments, food and any other nutrition were not taken into account in the analyses. Also, the level of agreement between the BCS evaluations conducted by the participating veterinarians was not assessed. However, even with such limitations, this research provides valuable information for veterinarians about the risk factors related to high lipoprotein cholesterol and triglyceride concentrations in dogs.

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