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NOTE

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Clinofibrate improved canine lipid metabolism in some but not all breeds

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ABSTRACT. The objectives of this study were to assess if Clinofibrate (CF) treatment improved lipid metabolism in dogs, and to clarify whether its efficacy is influenced by canine characteristics. We collected medical records of 306 dogs and performed epidemiological analyses. Lipid values of all lipoproteins were significantly decreased by CF medication, especially VLDL triglyceride (TG) concentration (mean reduction rate=54.82%). However, 17.65% of dogs showed drug refractoriness in relation to TG level, and Toy Poodles had a lower CF response than other breeds (OR=5.36, 95% CI=2.07–13.90). Therefore, our study suggests that genetic factors may have an effect on CF response, so genetic studies on lipid metabolism-related genes might be conducted to identify variations in CF efficacy.

KEY WORDS: clinofibrate, descriptive epidemiology, drug response, dyslipidemia, Toy Poodle

High serum cholesterol (Cho) and triglyceride (TG) concentrations in dogs are caused by various factors such as lack of exercise, high fat diets, obesity, neutralization, age, diseases and breed [6, 21, 24]. Miniature Schnauzer and Shetland Sheepdog are breeds that are well known to have a high risk of contracting hypertriglyceridemia and hypercholesterolemia [13, 25]. These days, total Cho and TG concentrations are measured in animal clinics as part of health management for dogs, because they are correlated with some diseases such as diabetes mellitus, hypothyroidism, protein-losing nephropathy and liver disease [1, 7].

Canine serum lipid is transported in four groups of lipoproteins: chylomicrons (CM), very low density lipoproteins (VLDL), low density lipoproteins (LDL), and high density lipoproteins (HDL) [7, 24]. In Japan, a lipoprotein analysis service is used for the measurement of canine lipid levels. When dogs require treatment, they are prescribed some lipid metabolism medicines that were developed for humans, such as Clinofibrate (CF) which is well known for treatment of high VLDL-TG concentration.

Fibrates are very effective for treating high VLDL-TG concentration. Their TG-lowering effect is caused by activation of the peroxisome proliferator activated receptor α (PPARα), the nuclear hormone receptor superfamily that regulates lipid and lipoprotein metabolism [16]. There have been various studies about the effects on dogs of fibrates such as Fenofibrate and Bezafibrate [3, 17], but there have been few epidemiological studies with CF.

In this study we have two objectives: (1) to assess how CF treatment improves lipid metabolism in dogs, and (2) to clarify whether the CF response is influenced by canine characteristics such as breed, age, sex, neuter status, or by the Body Condition Score (BCS) which is used to measure canine obesity.

Three hundred six canine medical records were obtained from Spectrum Lab Japan (Tokyo, Japan) which provides examination services for animals. We created a database of individual dog's information (age, sex, neuter status, BCS, breed, lipid value of each lipoprotein (CM, VLDL, LDL, HDL) and fasting time) that was recorded when they received the lipoprotein analysis service (LipoTEST) from 2008 to 2016. The evaluation of BCS was carried out by each dog's veterinarian using a 5-point scale (1: thin, 2: underweight, 3: ideal, 4: overweight, 5: obese). LipoTEST was performed by Skylight Biotech Inc. (Akita, Japan), and Cho and TG concentrations were measured using gel filtering high performance liquid chromatography [12, 20].

To ensure that the results were not affected by data from dogs that had eaten recently, we excluded samples if the fasting time was less than eight hours. Recent research indicated that CM-TG concentrations were higher when fasting times were less than eight hours, due to exogenous lipids [21]. To evaluate CF efficacy, we collected records of each lipoprotein's lipid concentrations before and after the first medication, ensuring that the two measurements were performed within one year. In addition, all dogs were prescribed CF at 10 mg/kg/BID by each veterinarian. The database was divided into four subsets based on breed. There were three main breed subsets for Miniature Schnauzers, Toy Poodles and Shih Tzus, and a fourth subset containing 26 additional breeds

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Table 1. Details of dog samples

Records of 306 dogs		N	%
Breed	Miniature Schnauzer (MSC)	62	20.26
	Toy Poodle (TPO)	47	15.36
	Shih Tzu (STZ)	25	8.17
	Other (OTR)	172	56.21
Sex	Male (M)	128	41.83
	Female (F)	176	57.52
	Unknown	2	0.65
Neuter status	Intact	55	17.97
	Neutered	249	81.37
	Unknown	2	0.65
BCS	2: underweight	6	1.96
	3: ideal	93	30.39
	4: overweight	124	40.52
	5: obese	51	16.67
	Unknown	32	10.46

BCS, Body Condition Score with 5-point scale (1–5).

Table 2. Canine characteristics and lipid concentrations of each breed

		MSC (62 dogs)	TPO (47 dogs)	STZ (25 dogs)	OTR (172 dogs)	Total (306 dogs)
Age		9.75[a)] (2.00–12.75)	6.17[b)] (1.58–14.08)	11.25 (2.00–14.58)	9.00 (1.50–16.92)	9.08 (1.50–16.92)
Sex		10 MI, 16 MN	5 MI, 21 MN	3 MI, 8 MN	11 MI, 54 MN	29 MI, 99 MN
		6 FI, 30 FN	2 FI, 18 FN, 1 UR	5 FI, 8 FN, 1 UR	13 FI, 94 FN	26 FI, 150 FN, 2 UR
BCS	2	2	1	0	3	6
	3	26	17	3	47	93
	4	21	18	15	70	124
	5	6	3	4	38	51
	Unknown	7	8	3	14	32
Total cholesterol		294.19 (163.45–800.74)	292.19 (139.47–1,076.07)	288.69 (152.16–896.25)	269.48 (132.86–2,287.30)	276.47 (132.86–2,287.30)
Total triglyceride		633.90[c)] (108.95–6,011.71)	374.61 (95.76–2,507.12)	405.77 (139.40–1,697.16)	277.34 (79.69–5,095.56)	355.20 (79.69–6,011.71)

MSC, Miniature Schnauzer; TPO, Toy Poodle; STZ, Shih Tzu; OTR, Other Breed; MI, Male intact; MN, Male neutered; FI, Female intact; FN, Female neutered; UR, unreported sex status; BCS, Body Condition Score with 5-point scale (1−5). All lipid values show pre-dosage concentrations. Values are median (range) for age (years), total cholesterol concentration (mg/d*l*), and each triglyceride concentration (mg/d*l*). a) Significantly different from STZ ($P<0.05$). b) Significantly different from the other three breed groups ($P<0.001$). c) Significantly different from OTR ($P<0.00001$).

and mixed breeds.

All statistical analyses were performed using EZR version 1.35 (Saitama Medical Center, Jichi Medical University, Saitama, Japan) statistics software which has expanded R and R commander functions [8]. $P<0.05$ was considered to be significant. To evaluate the reduction in lipid level from using CF, we applied the Wilcoxon signed-rank test because all differences between lipid concentrations before and after medication showed non-normal distribution. Total TG reduction rate ((before dosage−after dosage)/before dosage) also showed non-normal distribution, so we performed a multivariate logistic regression analysis in relation to drug response, instead of a multiple regression analysis in relation to total TG reduction rate. Then we divided samples into two groups according to the presence or absence of drug efficacy using the total TG reduction rate. For the grouping, we created a model of whether the total TG reduction rate was positive or not. Multivariate logistic regression analysis was used to calculate odds ratios (ORs) and 95% confidence intervals (95% CIs) after controlling simultaneously for potential confounders. Variables considered in the models were a dog's characteristics (age, breed, BCS, sex, and neuter status) and pre-dose total TG concentration. We excluded 34 records with incomplete information about dog characteristics from the Multivariate logistic regression analysis. For age, we divided samples into two groups; up to seven years old and more than seven years old. Samples with total TG concentration of more than 500 mg/d*l* were defined as severe dyslipidemia.

Three hundred six dogs met the criteria for the data set, comprising 62 Miniature Schnauzers, 47 Toy Poodles, 25 Shih Tzus and 172 others. The dogs' characteristics and lipid data are summarized in Tables 1 and 2. Miniature Schnauzers, which have a hereditary tendency to suffer from hypertriglyceridemia [13, 24, 25], had higher total TG levels than any other breed group ($P<0.00001$). Toy Poodles were younger than the other breed groups ($P<0.001$), and Shih Tzus were older than Miniature Schnauzers ($P<0.05$) (Table 2).

There was little knowledge about the efficacy of CF on each lipoprotein, so we evaluated the effects using the Wilcoxon signed-

Table 3. Cholesterol concentrations before and after Clinofibrate medication

		Pre-dosage lipid value (mg/d*l*)	Post-dosage lipid value (mg/d*l*)	Difference (mg/d*l*) (Wilcoxon signed-rank test)
Cholesterol	Total	Median 276.47	Median 222.32	Median 46.60
		Mean 318.91	Mean 253.41	V=39,300, $P=1.81 \times 10^{-24}$
		Range 132.86–2,287.30	Range 66.62–1,695.04	
	CM	Median 2.68	Median 0.84	Median 1.22
		Mean 6.61	Mean 3.22	V=38,162, $P=2.67 \times 10^{-21}$
		Range 0.15–141.74	Range 0.04–68.54	
	VLDL	Median 27.07	Median 13.55	Median 8.95
		Mean 52.48	Mean 27.33	V=40,308, $P=1.80 \times 10^{-27}$
		Range 6.39–1,775.01	Range 1.33–905.41	
	LDL	Median 44.77	Median 25.00	Median 10.79
		Mean 69.61	Mean 50.52	V=36,165, $P=2.72 \times 10^{-16}$
		Range 4.57–513.16	Range 2.04–535.46	
	HDL	Median 190.45	Median 169.27	Median 16.48
		Mean 190.05	Mean 172.34	V=36,286, $P=1.41 \times 10^{-16}$
		Range 49.03–314.91	Range 60.82–314.69	

CM, Chylomicrons triglyceride concentration; VLDL, Very low density lipoprotein triglyceride concentration; LDL, Low density lipoprotein triglyceride concentration; HDL, High density triglyceride lipoprotein triglyceride concentration.

Table 4. Triglyceride concentrations before and after Clinofibrate medication

		Pre-dosage lipid value (mg/d*l*)	Post-dosage lipid value (mg/d*l*)	Difference (mg/d*l*) (Wilcoxon signed-rank test)
Triglyceride	Total	Median 355.20	Median 161.85	Median 125.15
		Mean 554.05	Mean 308.44	V=40,703, $P=1.06 \times 10^{-28}$
		Range 79.89–6,011.71	Range 22.53–4,356.71	
	CM	Median 36.61	Median 8.82	Median 18.25
		Mean 94.21	Mean 45.53	V=38,077, $P=4.53 \times 10^{-21}$
		Range 0.61–2,327.52	Range 0.19–1,137.74	
	VLDL	Median 266.04	Median 124.87	Median 102.39
		Mean 412.41	Mean 230.68	V=40,897, $P=2.59 \times 10^{-29}$
		Range 67.55–4,457.40	Range 4.84–3,539.90	
	LDL	Median 20.36	Median 17.61	Median 2.91
		Mean 32.59	Mean 23.33	V=31,208, $P=6.17 \times 10^{-7}$
		Range 4.07–333.07	Range 3.13–237.98	
	HDL	Median 10.63	Median 5.47	Median 3.49
		Mean 15.56	Mean 8.86	V=39,715, $P=1.10 \times 10^{-25}$
		Range 2.30–178.34	Range 0.98–92.06	

CM, Chylomicrons triglyceride concentration; VLDL, Very low density lipoprotein triglyceride concentration; LDL, Low density lipoprotein triglyceride concentration; HDL, High density triglyceride lipoprotein triglyceride concentration.

rank test. All statistical analyses showed significant reductions in lipoprotein concentrations after CF treatment (Tables 3 and 4). These results clarified that CF can improve dogs' lipid metabolism in each lipoprotein, and particularly decrease VLDL-TG concentration (Median difference=102.39, $P=2.59 \times 10^{-29}$; Table 4). CF improved VLDL-TG concentrations in 256 of the 306 dogs, with a mean reduction rate of 54.82% (median reduction rate=57.43) (data not shown). Cho and TG concentrations were reduced in 239 and 252 dogs, respectively, with mean reduction rates of 27.00 and 53.70% (median reduction rate=24.15 and 54.71, respectively) (data not shown). Fibrates have a similar molecular structure to human PPARα agonist, which regulates some lipid metabolism related genes such as *lipoprotein lipase* (*LPL*), *apolipoprotein C3* (*APOC3*), and *apolipoprotein A5* (*APOA5*) [16, 23]. These genes regulate the quantity of VLDL, consist abundantly with TGs, so fibrates are particularly able to decrease VLDL-TG levels. The dog PPARα is likely to be similar to human PPARα. There is a 97% homology between dog PPARα and human PPARα amino acid sequences [14], so the CF treatment in the dogs may have acted as a dog PPARα agonist causing homeostasis of lipid metabolism, similar to the effect of human CF treatment.

However, 17.65% of all dogs (n=54) showed CF refractoriness in total TG level, so we performed multivariate logistic regression analyses using dog characteristics and pre-dosage total TG values. The model comprised 272 dogs, of which 48 showed drug refractoriness. Sample information in the model is summarized in Table 5. Multivariate logistic regression analysis indicated that breed and pre-dosage total TG values were independently related to CF response, but other dog characteristics were not associated with CF efficacy (Table 6). Toy Poodles had a higher risk of showing CF refractoriness compared with the other

Table 5. Comparisons between canine characteristics of dogs with and without drug response

Canine characteristics		Drug response	
		Yes (224 dogs)	No (48 dogs)
Breed	MSC	44	11
	TPO	25	13
	STZ	19	2
	OTR	136	22
Age		9.08 (1.50–16.92)	9.34 (3.00–15.08)
	<7	60	10
	≥7	164	38
Sex		23 MI, 70 MN 21 FI, 110 FN	3 MI, 19 MN 3 FI, 23 FN
BCS	2	5	1
	3	71	22
	4	108	14
	5	40	11
T-Cho		276.47 (132.86–2,287.30)	295.66 (139.47–1,076.07)
T-TG		369.45 (83.84–6,011.71)	281.55 (79.69–2,895.11)
VLDL-TG		294.94 (70.30–4,457.40)	220.37 (67.55–1,852.84)

MSC, Miniature Schnauzer; TPO, Toy Poodle; STZ, Shih Tzu; OTR, Other Breed; MI, Male intact; MN, Mail neutered; FI, Female intact; FN, Female neutered; BCS, Body Condition Score with 5-point scale (1−5); T-Cho, Total cholesterol concentration; T-TG, Total triglyceride concentration; VLDL-TG, Very low density lipoprotein triglyceride concentration. T-Cho, T-TG, and VLDL-TG showed pre-dosage concentrations. Values are median (range) for age (years), total cholesterol concentration (mg/d*l*), and each triglyceride concentration (mg/d*l*).

Table 6. Risk factors for Clinofibrate refractoriness

Risk factors		Odds ratio (95% CI)	*P*-value
(Intercept)		0.08 (0.02–0.33)	<0.001
Age	<7	Reference	
	≥7	2.44 (0.98–6.08)	<0.1
BCS	All		0.19
	2	0.46 (0.04–5.16)	
	3	Reference	
	4	0.48 (0.22–1.04)	
	5	1.12 (0.46–2.74)	
Breed	All		<0.005
	OTR	Reference	
	MSC	1.91 (0.79–4.60)	0.15
	STZ	0.71 (0.15–3.43)	0.72
	TPO	5.36 (2.07–13.90)	<0.001
Before Total-TG	<500	Reference	
	≥500	0.37 (0.17–0.82)	<0.05
Neuter status	Intact	Reference	
	Neutered	1.56 (0.59–4.15)	0.37
Sex	Female	Reference	
	Male	1.09 (0.55–2.16)	0.80

BC3S, Body Condition Score with 5-point scale (1–5); OTR, Other Breed; MSC, Miniature Schnauzer; STZ, Shih Tzu; TPO, Toy Poodle. Before Total-TG shows pre-dosage concentration (mg/d*l*).

breed groups, with an OR of 5.36 (95% CI=2.07–13.90). Toy Poodles are well known to be susceptible to diabetes mellitus [22], a disease related to dyslipidemia [1, 24]. Furthermore, there was a greater CF response with the severe dyslipidemia group than with the dogs without severe dyslipidemia (OR=0.37, 95% CI=0.17–0.82). This result is in accordance with a previous study on Bezafibrate response for canine dyslipidemia [3].

The reason why Toy Poodles had lower CF response than the other breeds may have been due to anamnesis, such as diabetes mellitus. The present study did not take anamnesis into account, but other studies have shown that insulin-resistant dogs have lower *LPL* mRNA expression levels than insulin-sensitive dogs [4, 18]. So diabetes mellitus might have affected CF efficacy in the current study. Furthermore, there have been various studies about the relationship between canine diabetes mellitus and some gene variations such as *cytotoxic T lymphocyte-associated antigen 4* and *dog leukocyte antigen* [2, 9, 19]. So genetic variants may possibly be related to CF response. Further analysis is needed with such disease information included in the dataset.

Although the current study considered fasting time in order to exclude effect of exogenous lipids, it was not possible to eliminate environment factors such as exercise. However, despite this the results seem to indicate that genetics can affect drug efficacy, because breed was most strongly correlated with CF resistance.

In recent years pharmacogenetic studies have often been conducted on humans, and the relevance of drug response and gene polymorphisms in fibrates has also been clarified. For example, human *APOA5* mutations have been shown to be correlated with the fibrates efficacy [5, 11]. *APOA5* is a target gene for PPARα, and human *APOA5* has a PPAR response element (PPRE) in the promoter region [23]. Furthermore, some studies have indicated that human *APOA5* has a big influence on lipid and lipoprotein metabolism [10, 15]. Therefore, it is possible that a similar response occurs in dogs.

In conclusion, this study suggests that genetic factors may affect CF response. Therefore, genetic studies might be performed on some genes which related to lipid metabolism and diabetes mellitus to identify variations in CF efficacy in dogs. Such studies, together with further epidemiological studies are needed to make better treatment plans for lipid metabolism disorders in dogs.

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