Serum Lipid and Lipoprotein Concentrations in Obese Dogs

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ABSTRACT. Serum lipid and lipoprotein concentrations in 10 obese and 16 control dogs were examined. The serum triglyceride (TG) concentration in obese dogs was significantly higher than in control dogs. The serum concentrations of TG and phospholipid (PL) in beta lipoprotein and PL in pre-beta lipoprotein were significantly higher in obese dogs, while the serum PL concentration in alpha$_1$ lipoprotein was significantly lower in obese animals. In the serum total cholesterol concentration in obese dogs, a higher tendency for beta and pre-beta lipoproteins and lower tendency for alpha$_1$ lipoprotein were observed. These abnormal lipoprotein profiles were similar to those in diabetes mellitus in men and acute pancreatitis in dogs.—KEY WORDS: canine, hypertriglyceridemia, lipid, lipoprotein, obesity.

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Obesity is defined as the excessive fat deposition in adipose tissue. It occurs when caloric intake is in excess of energy consumption. Obesity is not only a nutritional disease but also the trigger of other diseases. Obesity is sometimes related to endocrine disorders, especially to diabetes mellitus [11]. Furthermore, dogs with pancreatitis are often obese [1]. A trend toward an increased concentration of serum triglyceride (TG) in obese subjects is reported [13].

Recently, there has been an increase in the population of hypernutritional dogs since reared as companion animals. Consequently, there may be a greater incidence of diseases related to obesity than previously. Obesity may lead to the development of hyperlipidemia in men [6]. It is reported that hyperlipidemia preceded and apparently caused pancreatitis [9]. Studies of lipid and lipoprotein metabolism in obese dogs are therefore necessary to clarify the etiology and pathology of diseases related to obesity, but serum lipid and lipoprotein profiles are still limited [2]. The purpose of this study is to define the characteristics of serum lipid and lipoprotein concentrations in obese dogs.

MATERIALS AND METHODS

Animals: Ten obviously obese dogs (4 males and 6 females) with no clinical abnormality were selected from dogs referred to the University of Osaka Prefecture Veterinary Teaching Hospital. They were over two years old. Obesity was assessed by the method of Edney and Smith [7]. Briefly, lean—little body fat evident, skeletal structure obvious; optimum—moderate amount of body fat, rib cage easily palpated but not showing too obviously; obese—rib cage not visible when dog moves, bones of chest barely palpable; gross—unable to feel ribs, large amounts of subcutaneous fat can be grasped by hand, obvious incapacity due to excess fat. Sixteen normal beagle dogs (3 males and 13 females; over two years old) were used as controls.

Samples: The animals were fasted for at least twelve hours before blood sampling. Blood samples were collected by jugular venepuncture into three tubes; a tube containing ethylenediaminetetraacetic acid disodium salt dehydrate (EDTA-2Na; final concentration 1 mg/m*l* of blood), a heparin tube and a serum tube. The white blood cell count (WBC), red blood cell count (RBC), platelet, hematocrit and hemoglobin were measured in a Particle Counter (Model PC-608, Erma Inc., Tokyo). Plasma biochemical examination, including the measurement of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin (T. Bil), blood urea nitrogen (BUN), glucose, albumin and amylase, was performed by COBAS READY® (Nippon Roche, Tokyo). For lipid analysis, the sera were stored at −70°C. For lipoprotein analysis, EDTA-2Na 0.37 mg and sucrose 18.5 mg were added to one m*l* of serum and frozen at −20°C until analyses.

Serum lipid analysis: TG and total cholesterol (TC) were determined by Cleantech TG-S kit (Iatron Co., Ltd., Tokyo) and Monotest Cholesterol kit (Boehringer Mannheim, Germany), respectively. Phospholipid (PL) was assayed with a Phospholipid-test kit (Wako Pure Chemical Industries, Ltd., Osaka) and non-esterified fatty acid (NEFA) was estimated with a Nescort NEFA Kit-U (Nippon Shoji Kaisha, Osaka). The concentration of total lipids (TL) was expressed as the sum of TG, TC and PL.

Serum lipoprotein analysis: Agarose film electrophoresis of serum samples was performed by the Ciba Corning Gel Electrophoresis System (Ciba Corning Diagnostics Corp., Palo Alto, CA) at 90 V with Universal PHAB Buffer (Ciba Corning Diagnostic Corp.; pH 8.6) for 45 min. The films were enzymatically [17] stained with Co-Trigly.A., Co-Cholest.A. and Co-PL.A. (Nippon Chemiphar Co., Ltd., Tokyo). TL staining was performed by the enzymatic formazan method, mixing three reagents [17]. The distribution patterns of lipoproteins were determined in a Densito-Pattern Analyzer (Model EPA-3000, Maruzen Petrochemical Co., Ltd., Tokyo) at a

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wavelength of 650 nm. Serum lipoproteins were separated by agarose film electrophoresis into origin lipoprotein, beta lipoprotein, pre-beta lipoprotein and alpha$_1$ lipoprotein. The lipid concentrations in each lipoprotein were calculated by multiplying the concentration of serum lipid (Table 4) by the fractional rate of each lipoprotein measured with a densitometer.

Statistical analysis: Differences between the two groups were analyzed by Student's *t*-test. When variances were unequal, Cochran-Cox's test was used.

RESULTS

Breed and body condition: The breed and body condition of the dogs are shown in Table 1. Obese dogs were classified as obese (n=5) and grossly obese (n=5). However, there was no significant ($p>0.05$) difference in hematological, biochemical, serum lipid and lipoprotein analyses between the obese and gross groups (data not shown).

Hematological examinations: In hematological values, there was no significant difference ($p>0.05$) between the control and obese dogs (Table 2).

Biochemical examinations: The AST, ALT, ALP, T.Bil, glucose, albumin and amylase values in obese dogs were not significantly ($p>0.05$) different from those in control dogs (Table 3). The BUN levels in the obese dogs were significantly ($p<0.05$) higher than those in the control dogs (Table 3).

Serum lipid concentrations: Serum lipid concentrations in the two groups are shown in Table 4. The serum TG levels in the obese dogs were significantly ($p<0.01$) higher than in the control dogs. In the other lipid levels, there were no significant differences ($p>0.05$) between the two groups.

Serum lipoprotein concentrations: The serum lipoprotein concentrations in the control and obese dogs are shown in Table 5. In the control dogs, beta lipoprotein contained the most serum TG. The major constituents of alpha$_1$ lipoprotein were TC and PL. No lipid was found in the origin fraction. In obese dogs, the serum TG concentration in beta lipoprotein was significantly ($p<0.01$) higher than in control dogs. The serum PL concentrations in beta and pre-beta lipoproteins were also significantly ($p<0.05$, $p<0.01$, respectively) higher than in control dogs. Conversely, the serum PL concentration in alpha$_1$ lipoprotein was significantly ($p<0.01$) lower than that in control dogs. In the serum TC concentration, there was a tendency to higher beta and pre-beta lipoproteins and lower alpha$_1$ lipoprotein in obese dogs, as compared with those in control dogs.

Table 1. Breed and body condition of dogs

Animals/Breed	Body condition			
	Lean	Optimum	Obese	Gross
Control dogs (n=16)				
Beagle	2	14	0	0
Obese dogs (n=10)				
Mongrel	0	0	2	2
Pug	0	0	0	2
Shiba Inu	0	0	1	0
Shetland Sheepdog	0	0	0	1
Shih Tzu	0	0	1	0
Yorkshire Terrier	0	0	1	0

Table 2. Hematological values in dogs

Hematological examination	Obese dogs (n=10)	Control dogs (n=16)
WBC ($\times 10^2$/mm^3)	134.1±54.5[a)]	100.3±37.3
RBC ($\times 10^4$/mm^3)	631.9±140.4	624.6±109.0
Platelet ($\times 10^4$/mm^3)	34.8±18.9	23.1±15.9
Hematocrit (%)	44.1±10.0	41.1±9.4
Hemoglobin (g/d*l*)	13.6±3.2	13.1±2.3

WBC: white blood cell count, RBC: red blood cell count.
a) Mean±Standard deviation (S.D.)

Table 3. Biochemical values in dogs

Biochemical examination	Obese dogs (n=10)	Control dogs (n=16)
AST (IU/*l*)	18.3±17.0[a)]	13.2±5.0
ALT (IU/*l*)	44.1±19.6	47.1±25.6
ALP (IU/*l*)	112.9±77.9	120.8±45.2
T. Bil (mg/d*l*)	0.29±0.09	0.39±0.18
BUN (mg/d*l*)	17.9±8.1*[b)]	10.2±2.6
Glucose (mg/d*l*)	108.7±11.9	110.9±12.9
Albumin (g/d*l*)	3.1±0.6	2.9±0.5
Amylase (IU/*l*)	606.7±203.7	453.9±180.8

AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, ALP: Alkaline phosphatase, T. Bil: Total bilirubin, BUN: Blood urea nitrogen.
a) Mean±S.D.
b) Significantly different from control dogs.
*: $p<0.05$.

Table 4. Serum lipid levels in dogs

Lipid	Obese dogs (n=10)	Control dogs (n=16)
TG (mg/d*l*)	100.2±44.3**[b)]	35.0±10.7[a)]
TC (mg/d*l*)	268.1±149.9	199.3±32.6
PL (mg/d*l*)	447.2±108.0	476.5±47.8
TL (mg/d*l*)	815.6±259.2	710.7±81.3
NEFA (μEq/*l*)	869.2±273.4	826.3±345.2

TG: Triglyceride, TC: Total cholesterol, PL: Phospholipid, TL: Total lipids, NEFA: Non-esterified fatty acid.
a) Mean±S.D.
b) Significantly different from control dogs.
**: $p<0.01$.

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Table 5. Total lipid content and each lipid component in serum lipoprotein fractions

Lipids/ Lipoprotein	Obese dogs (n=10)	Control dogs (n=16)
TG (mg/dl)		
origin	4.9±9.2[a]	n.d.[b]
	(4.9)	
pre-beta	4.4±4.1	3.1±2.1
	(5.3)	(9.3)
beta	88.0±45.4**[c]	28.8±10.6
	(85.9)	(81.7)
alpha$_1$	3.0±2.9	3.0±1.7
	(3.9)	(9.0)
TC (mg/dl)		
origin	n.d.	n.d.
pre-beta	94.8±149.5	15.4±20.5
	(26.3)	(7.1)
beta	35.0±33.7	12.3±7.7
	(13.7)	(6.4)
alpha$_1$	138.4±57.3	171.5±30.0
	(60.0)	(86.5)
PL (mg/dl)		
origin	n.d.	n.d.
pre-beta	77.2±62.4**	7.1±12.0
	(16.4)	(1.6)
beta	80.2±63.5*	32.1±20.5
	(18.4)	(7.0)
alpha$_1$	289.8±114.8**	437.3±63.6
	(65.1)	(91.5)
TL (mg/dl)		
origin	n.d.	n.d.
pre-beta	181.5±160.3	71.7±79.5
	(20.2)	(10.1)
beta	143.7±129.1	62.4±33.7
	(17.3)	(8.8)
alpha$_1$	490.4±227.1	576.6±102.5
	(62.5)	(81.2)

TG: Triglyceride. TC: Total cholesterol, PL: Phospholipid, TL: Total lipids.
The fractional rate for each lipoprotein is given in parentheses.
a) Mean±S.D.
b) n.d.: Not detected.
c) Significantly different from control dogs.
*: $p<0.05$, **: $p<0.01$.

DISCUSSION

The relationship of an abnormal lipoprotein profile to diseases has been reported in human clinical medicine [6, 9]; hyperlipemia is related to diabetes, ischemic heart disease, nephrotic syndrome, hepatic disease and endocrine disorders [8]. Recent improvement in serum lipoprotein electrophoretic analysis [17] has contributed to better understanding of abnormal lipid metabolism than usual staining. However, canine lipid and lipoprotein analysis has not often been carried out in veterinary clinical medicine.

Basic studies on canine lipoprotein are being done in foxhounds and mongrel dogs by Mahley and Weisgraber [10]. They fractionate canine plasma lipoprotein by ultracentrifugation combined with electrophoresis and show the difference between canine and human lipoproteins [10]. Canine serum lipoproteins in idiopathic hyperlipidemia [14, 19] and in diseases accompanied with hyperlipidemia [2, 15, 18] are studied. It has been reported that obesity predisposes to diabetes [11] and pancreatitis [1] in dogs; but studies on serum lipoprotein in obese dogs are limited [2].

It has been reported that the degree of obesity relates to the level of fasting plasma insulin and the response of insulin to glucose [12], both of which affect lipoprotein metabolism. In this study, obese dogs were classified further into two conditions: obese and grossly obese. However, there was no significant difference in hematological, biochemical, serum lipid and lipoprotein analysis between them (data not shown). The degree of obesity may therefore not affect the levels of serum lipid and lipoprotein.

In hematological and biochemical values, there was no significant difference between obese and control dogs except the BUN level. The BUN level increased in obese animals, but was not abnormal. It was considered, therefore, that obesity in the dogs in this study was not accompanied by diabetes or other diseases.

Serum lipid levels have been investigated in normal dogs [2, 3, 5, 10, 15, 16]. The serum lipoprotein concentration and class are affected by breed [5] and diet [20]. While these influences were not determined in this study, the TG level might not be affected by food intake because the blood sample was collected after fasting for more than twelve hours. The human TC concentration depends on age and gender. In a previous study, we found that the concentrations of TC, PL and TL in alpha$_1$ lipoprotein in seven month old beagles were significantly lower and that of NEFA was significantly higher than those in beagles over two years old (data not shown). These result suggested that lipid metabolism in young dogs was different from that in adults. To avoid this influence, the dogs used in this research were over two years old. It has been reported that the mean cholesterol in the alpha$_1$ lipoprotein (HDL) concentration in entire bitches is greater than that in intact males [2]. In the lipid and lipoprotein concentrations, however, there were no significant differences between males and females (data not shown). The concentrations of TG and TC observed in the present control dogs were similar to those in other reports [2, 3, 5, 10, 16]. The serum PL level in control dogs was higher than that in others [3, 10, 16], but the serum NEFA value in control dogs was lower than that in another study [3]. These differences may be attributable to the difference in breed, diet or methods used.

The higher TG level in beta lipoprotein than that in pre-beta lipoprotein observed in control dogs might indicate the swift metabolism of pre-beta lipoprotein into beta lipoprotein by lipoprotein lipase (LPL). The main constituents of alpha$_1$ lipoprotein was found to be serum TC and PL and this finding was similar to those in another

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report [10]. As for the distribution of TL, alpha$_1$ lipoprotein was indicated to be a major lipid carrier in dogs because the canine alpha$_1$ lipoprotein was shown to have a high lipid content.

The higher serum TG level in obese dogs than in control dogs was attributed to increased TG in beta lipoprotein. Moreover, an increase in PL and a tendency to an increase in TC in pre-beta lipoprotein were observed in obese animals. An increase in serum TG is reported to be attributable to excessive production of pre-beta lipoprotein due to high caloric intake in obese subjects [8]. These results may be associated with accelerated synthesis of pre-beta lipoprotein and depressed catabolism of beta lipoprotein. Furthermore, the decrease in PL and the tendency to a decrease in TC in alpha$_1$ lipoprotein observed in obese animals suggest depressed secretion of alpha$_1$ lipoprotein from liver and intestine and/or decreased catabolism of pre-beta lipoprotein to alpha$_1$ lipoprotein. Origin (chyromicron) and pre-beta lipoprotein (VLDL) are catabolized to beta (LDL) and alpha$_1$ (HDL) lipoprotein by LPL [4]. The reduction in LPL activity may contribute to the increase in pre-beta lipoprotein and the decrease in alpha$_1$ lipoprotein. These abnormal lipoprotein profiles in obese dogs may therefore be attributable to increased synthesis of pre-beta lipoprotein, a decrease in LPL activity and suppressed catabolism of beta lipoprotein. Further studies on detail of the mechanism of changes in serum lipid and lipoprotein in obese dogs are needed.

Changes in serum lipid and lipoprotein in obese dogs were similar to those in human diabetes [6] and those in canine acute pancreatitis [15, 18]. As LPL activity is regulated by insulin, severe insulin deficiency may contribute to hypertriglyceridemia [8]. Canine acute pancreatitis associated with primary hypertriglyceridemia is reported [18]. Obesity may lead to the development of hyperlipidemia in man [6], and pancreatitis is often seen in obese dogs [1]. These suggest that obesity may predispose to diabetes and acute pancreatitis in dogs.

In obese dogs, hypertriglyceridemia, increased beta and pre-beta lipoprotein and decreased alpha$_1$ lipoprotein were seen. These may be attributable to increased synthesis of pre-beta lipoprotein, decreased LPL activity and suppressed catabolism of beta lipoprotein.

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