

Correlation between plasma leptin concentration and body fat content in dogs

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Objective—To evaluate the relationship between plasma leptin concentration and body fat content in dogs.

Animals—20 spayed female Beagles that were 10 months old at the start of the experiment.

Procedure—Dogs were kept under regulated feeding and exercise conditions for 21 weeks, resulting in a wide range of body weights, body condition scores (BCS), and subcutaneous thicknesses. Plasma leptin concentration was measured by use of a canine leptin-specific ELISA test to evaluate its correlation to body fat content estimated by the deuterium oxide dilution method. Plasma concentrations of glucose, cholesterol, triglycerides (TG), and nonesterified fatty acids (NEFA) were also measured.

Results—Body fat content (9 to 60% of body weight) was positively and closely correlated ($r = 0.920$; $n = 20$; $P < 0.001$) to plasma leptin concentration (0.67 to 8.06 ng/ml), compared with other variables (ie, glucose, cholesterol, TG, and NEFA; $r = 0.142, 0.412, 0.074, \text{ and } 0.182$, respectively).

Conclusions and Clinical Relevance—The positive relationship between plasma leptin concentration and body fat content in dogs was similar to correlations reported for humans and rodents, suggesting that plasma leptin is a quantitative marker of adiposity in dogs. (*Am J Vet Res* 2002;63:7–10)

Obesity is the most common nutritional disorder encountered in small animal medicine.¹ Quantitative estimation of adiposity is essential for the accurate diagnosis of obesity and for evaluation of preventive and therapeutic challenges. Although there are various methods for diagnosis of obesity in humans, most of them are not successfully applicable to small animal practice. For example, body mass index calculated from body weight and height is the most simple and reliable variable in humans,² but it is not suitable in companion animals because of wide variations of somatoscopic variables among breeds. In fact, male adult Borzois and Newfoundlands, for instance, are

almost the same in standard height of approximately 70 cm, whereas their standard weights are considerably different (34 to 48 kg and 59 to 68 kg, respectively).^{3,4}

Leptin is a 16-kDa protein synthesized and secreted primarily by adipose tissue.⁵ Because mutations of leptin or its receptor induce hyperphagia, reduced energy expenditure, and obesity,^{6,8} leptin is recognized as 1 of the key molecules for the regulation of whole body energy balance, and thereby the cause of obesity.⁹

¹¹ In humans and rodents, the blood leptin concentration is known to positively correlate with body fat content and concentrations are higher in obese patients.^{12–}

¹⁷ It is thus hypothesized that blood leptin concentration is a quantitative index of adiposity in most mammals, including companion animals. In fact, Backus et al¹⁸ measured serum leptin immunoreactivity in cats, using a commercially available multispecies leptin radioimmunoassay kit and human leptin as a standard, and reported the positive relationship to body fat mass.

Recently, we cloned canine leptin cDNA and deduced its amino acid sequence.¹⁹ Furthermore, we produced recombinant canine leptin in *Escherichia coli* and established a sandwich ELISA method for detection of canine leptin, using a specific antibody.²⁰ The purpose of the study reported here was to assay plasma leptin (using our canine-specific ELISA method) of lean and obese Beagles and analyze the correlation between plasma leptin concentration and body fat content (estimated by use of the deuterium oxide [D₂O] dilution method) to assess the validity of using plasma leptin concentration as a diagnostic marker of adiposity and obesity in dogs.

Materials and Methods

Dogs and feeding conditions—Twenty female Beagles^a (10 months old) were spayed 4 weeks before the experiment. Dogs were housed individually indoors in 1.5 × 2-m concrete cages connected to an outdoor 22.5 × 1.5-m area. To obtain a wide range of body fat contents, dogs were separated into 3 groups and maintained for 21 weeks as follows: group A (3 dogs) was fed a standard dry food^b containing 14.2 kJ/g of metabolizable energy (Appendix). Groups B (7 dogs) and C (10 dogs) were fed a high-energy food (18.3 kJ/g). Group-A and-B dogs were allowed access to the outdoor area for 4 hours every day, whereas group-C dogs were allowed access for only 2 hours. Because food intake spontaneously decreased after 3 to 5 weeks in a considerable number of dogs in groups B and C, these dogs were fed the standard food for several days and then the high-energy food again. Animal care and procedure were in accordance with the guidelines for the Animal Care and Use Committee of Hokkaido University.

Measurement of somatoscopic and blood variables—After the 21-week period, blood was collected from the jugular vein at 9 to 10 AM after dogs were fasted overnight (16

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hours), and plasma samples were stored at -80 C until assays were performed. The body condition score (BCS) was also determined as the mean value assigned individually by 7 observers; a 5-point scale was used for determination of scores.²¹ The thickness of subcutaneous fat and muscle was measured approximately 3.0 cm laterally from the midline directly over the longissimus dorsi, using an ultrasonic probe device.^{22,c} Plasma concentrations of triglyceride (TG), cholesterol, and glucose were determined by use of an automatic analyzer.^d Nonesterified fatty acids (NEFA) were assayed at a commercial laboratory.^e

Estimation of body fat content by use of the D₂O dilution method—Body fat mass was estimated by use of the isotopic dilution method of Burkholder et al.,²³ with slight modifications. Briefly, after dogs were fasted for 16 hours, water was withheld, and dogs were injected with D₂O^f into the cephalic vein at a dose of 0.2 g/kg of body weight. Before and 90 minutes after the injection, blood (8 to 12 ml) was collected from the jugular vein into heparinized tubes. Plasma samples (4 ml) were purified by vacuum sublimation, and the concentration of deuterium was measured, using a gas-chromatography thermal conductivity detection system.^g Body fat content was calculated as follows:

$$\text{Body fat content (\%)} = \text{Body fat mass (kg)} / \text{body weight (kg)}$$

$$\text{Body fat mass (kg)} = \text{Total body water (L)}^{-4.7} \times \text{body weight (kg)}^{5.9} \times 0.019$$

$$\text{Total body water (ml)} = (\text{Injected D}_2\text{O [ml]} \times \text{purity of D}_2\text{O [= 0.999]}) / \text{plasma D}_2\text{O concentration (\mu g/g)} - \text{injected D}_2\text{O (ml)}$$

Assay of plasma leptin by use of a specific ELISA method—Plasma leptin concentration was determined by use of the previously reported method of sandwich ELISA, using an anticanine leptin antibody²⁰ with minor modifications. Briefly, each well of a 96-well microplate^h was coated with 100 μl of purified rabbit anticanine leptin antibody (2 $\mu\text{g/ml}$) by incubation for 2 hours at room temperature (23 C). After washing the wells twice with a buffer containing 10 mM Tris-HCl (pH 7.4), 150 mM NaCl, and 0.05% Tween 20, 300 μl of the buffer containing 0.1% bovine serum albumin was added to each well for blocking. After 2 hours incubation, wells were washed twice with buffer solution and incubated overnight at 4 C with 80 μl of 30% normal rabbit serum and 20 μl of the plasma sample or 0.25 to 16 ng/ml recombinant canine leptin. Then, the wells were washed 5 times with buffer solution, incubated with 100 μl of horseradish peroxidase-conjugated anti-canine leptin antibody (0.2 $\mu\text{g/ml}$) for 4 hours at 4 C. After washing 7 times, the wells were incubated with 100 μl of a peroxidase substrate solutionⁱ (100 μl) in the dark for 15 to 30 minutes at 23 C. After stopping the reaction by adding 50 μl of 0.5M H₂SO₄, the absorbance of each well was measured at 450 nm. Under this assay condition, 0.25 to 16 ng/ml of plasma leptin could be measured with intra- and interassay variations of less than 4%.

Statistical analyses—Linear regression and correlation analyses were performed by use of a computer software program^j for plasma leptin concentration, body fat content, body weight, BCS, subcutaneous thickness, and plasma concentrations of glucose, cholesterol, TG, and NEFA. The significance of the correlation coefficient was determined by use of the Pearson correlation coefficient test. Values of $P < 0.05$ were considered significant.

Results

We had preliminarily observed that few Beagles became obese when fed the standard food. To obtain a wide range of body fat contents, 20 young female

Beagles were spayed and maintained under 3 different conditions of feeding and exercise for 21 weeks (groups A, B, and C; Table 1). Although there were considerable differences among individual dogs, they were not obese at week 0, with BCS of 1 to 3. By week 21, all dogs in group A that were fed the standard food were still not obese, whereas 10 dogs in groups B and C that were fed the high energy food did become obese (BCS = 4 or 5), particularly 2 dogs in group C, with BCS of 5. In 11 dogs in groups B and C, food consumption spontaneously decreased during weeks 3 through 6, and 7 dogs remained at a BCS of 3.

The relationships between body fat content and other variables were determined (Table 2). As expected, a significant correlation was detected between body fat content and body weight ($r = 0.847$), BCS ($r = 0.766$), and subcutaneous tissue thickness ($r = 0.766$). Similarly, a good positive correlation was detected

Table 1—Body weight and body condition score of individual dogs at weeks 0 and 21. Dogs in group A were fed the standard food, whereas those in groups B and C were fed the high-energy food in addition to the standard food. In groups A and B, dogs were allowed access to an outdoor area for 4 hours every day, whereas in group C, it was for 2 hours. Body condition score was expressed on a 5-point scale: 1, thin; 2, lean; 3, optimum; 4, obese; 5, gross

Dogs	Week 0	Week 21
Group A (n = 3)		
Body weight (kg)	7.6 ± 0.6 (7.2–8.2)	9.8 ± 0.3 (9.6–10.1)
Body condition score	2 ± 1 (1–2)	2 ± 1 (2–3)
Group B (7)		
Body weight (kg)	7.9 ± 0.5 (7.3–8.6)	11.0 ± 0.7 (9.6–11.4)
Body condition score	2 ± 1 (2–3)	3 ± 1 (3–4)
Group C (10)		
Body weight (kg)	8.4 ± 0.5 (7.6–9.4)	12.4 ± 1.8 (10.5–16.4)
Body condition score	3 ± 0 (2–3)	4 ± 1 (3–5)

Table 2—Correlation of body fat content to plasma leptin concentration and other variables at week 21

Variable	n	r	P
Body weight	20	0.847	< 0.001
Body condition score	20	0.766	< 0.001
Subcutaneous thickness	20	0.766	< 0.001
Plasma leptin	20	0.920	< 0.001
Plasma cho	20	0.412	NS
Plasma NEFA	20	0.182	NS
Plasma glu	20	0.142	NS
Plasma TG	20	0.074	NS

Cho = Cholesterol. NS = Not significant. NEFA = Nonesterified fatty acids. Glu = Glucose. TG = Triglyceride.

Table 3—Correlation of plasma leptin concentration to other variables at week 21

Variable	n	r	P
Body fat content	20	0.920	< 0.001
Body weight	20	0.856	< 0.001
Body condition score	20	0.838	< 0.001
Subcutaneous thickness	20	0.823	< 0.001
Plasma cho	20	0.310	NS
Plasma NEFA	20	0.181	NS
Plasma TG	20	0.016	NS
Plasma glu	20	0.034	NS

See Table 2 for key.

between body fat content and plasma leptin concentration ($r = 0.920$). No significant correlation was detected between body fat content and plasma concentrations of cholesterol, NEFA, glucose, and TG. The correlation of plasma leptin concentration with other variables was also analyzed; plasma leptin concentration correlated well with body weight, BCS, and subcutaneous tissue thickness, but not with plasma concentrations of cholesterol, NEFA, glucose, and TG (Table 3).

Discussion

The primary objective of this study was to assess the validity of measuring plasma leptin concentration as a quantitative index of adiposity in dogs. Because plasma leptin concentration changes under various physiologic and pathologic conditions in rodents and humans,^{14,16,24,25} we spayed the Beagles in our study before the trial to exclude any possible hormonal effects of the estrous cycle. No significant change was detected in plasma leptin concentration before (0.59 ± 0.90 ng/ml) or after (0.50 ± 0.76 ng/ml) ovariectomy.

We found that body weight and body fat content correlated well with plasma leptin concentration. The correlation coefficient of plasma leptin concentration with body fat content was much higher than those of other variables measured in plasma such as cholesterol, glucose, TG, and NEFA concentrations. These results are similar to those reported in rodents and humans^{12,13,15} and indicate that plasma leptin concentration is a good index of body fat content in dogs, as it is in other species; in other words, the measurement of plasma leptin would be useful for assessment of adiposity and obesity. High correlations of body weight, BCS, and subcutaneous tissue thickness with body fat content were also found, but this was not surprising, because our dogs were rather homogeneous in breed, sex, and age. It should be stated that plasma leptin measurement would be particularly useful for assessment of adiposity and obesity in individual dogs of different breeds and ages, in which somatostatic characteristics vary considerably. Kitagawa et al²⁶ measured plasma leptin immunoreactivity of 28 lean and 45 obese dogs, using human leptin as a standard, and found that obese dogs had higher plasma concentrations by approximately 2.5-fold.

Measurement of plasma leptin concentration by use of ELISA testing may be a simple and practical method of evaluating body fat content, compared with other noninvasive and quantitative methods reported in dogs. For example, dual-energy x-ray absorptiometry (DEXA) was reported to be useful for estimation of body fat content in obese dogs.²⁷ However, the DEXA method is not practical and easy, because the DEXA units are not common to veterinary clinicians. Alternatively, subcutaneous fat measurement, using an ultrasonic probe device, appears to be more practical. The subcutaneous fat thickness measured near the last rib was reported to give a high correlation with total body fat mass in Beagles.²² This was also confirmed in the present study. However, in the authors' experience, the ultrasonographic method is not easy for routine use for 2 reasons: 1 is the difficulty in distinguishing fat from muscle tis-

sues, because commercial ultrasonographic systems for humans do not have sufficiently high resolution to distinguish these 2 tissues in small animals such as dogs. The other is that the method is best suited for sedated or anesthetized dogs, and it is troublesome in conscious animals to put the probe in an accurate position. The D₂O dilution method is known as the most reliable and noninvasive method for estimation of body fat content in various species, including dogs.^{23,28} Unfortunately, this method is time-consuming and, therefore, only practical for research purposes.

Synthesis and secretion of leptin are known to be regulated by various neuroendocrine factors such as insulin, glucocorticoids, and catecholamines.²⁹⁻³² In addition, plasma leptin concentration reflects diurnal changes in association with feeding-fasting cycles in rodents and humans.^{33,34} Therefore, for the best application of measuring plasma leptin concentration as an index of body fat content, it is important to collect blood samples from subjects maintained under steady conditions, particularly their feeding conditions. Because most companion dogs are fed regularly, blood sampling after overnight fasting (as in the present study) would give the most reliable results.

^aCrea Japan Inc, Tokyo, Japan.

^bVita-One, Nippon Pet Food Co, Ltd, Tokyo, Japan.

^cEUB-200V, Hitachi, Tokyo, Japan.

^dSpotchem, Arkray Inc, Kyoto, Japan.

^eHealth Science Research Institute Inc, Yokohama, Japan.

^fDeuterium oxide 99.9 atom %, Aldrich Chemical Co, Inc, Milwaukee, Wis.

^gAutomatic deuterium oxide analyzer HK-102, Shoko Co, Ltd, Tokyo, Japan.

^hNunc, Tokyo, Japan.

ⁱ3, 3', 5, 5'-Tetramethylbenzidine, Moss Inc, Pasadena, Md.

^jExcel, Microsoft Corp, Redmond, Wash.

Appendix

Composition of standard food and high-energy food.

Standard food*		High-energy food*	
Corn	420	Corn	420
Meat meal	180	Vegetable oil	250
Soybean cake	170	Casein	200
Bran	130	Soybean cake	100
Wheat	50	Meat meal	90
Animal tarrow	40	Wheat	50
Mineral mixture	7	Mineral mixture	7
Vitamin mixture	3	Vitamin mixture	3
Crude protein (%)	27.2	Crude protein (%)	31.7
Crude fat (%)	8.8	Crude fat (%)	27.9
Crude fiber (%)	2.2	Crude fiber (%)	0.6
Ash (%)	6.6	Ash (%)	5.7
Nitrogen free extract (%)	48.6	Nitrogen free extract (%)	25.4
Metabolizable energy (kJ/g)	14.2	Metabolizable energy (kJ/g)	18.2

Vitamin A content was 10,864 and 10,130 U/kg in standard food and high-energy food, respectively.
*All units in g/kg unless otherwise indicated.

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