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A Comparison of Metabolic Parameters Between Obese and Non-obese Healthy Domestic Dogs in Japan

Nobuko Mori, Hiroshi Takemitsu, Yuki Okada, Ichiro Yamamoto and Toshiro Arai
Department of Veterinary Biochemistry, School of Veterinary Medicine, Nippon Veterinary and Life Science University, 1-7-1 Kyonancho, Musashino, Tokyo, 180-8602, Japan

Corresponding Author: Toshiro Arai, Department of Veterinary Biochemistry, School of Veterinary Medicine, Nippon Veterinary and Life Science University, 1-7-1 Kyonancho, Musashino, Tokyo, 180-8602, Japan

ABSTRACT

Many veterinarians feel that obese dogs are increasing in prevalence worldwide. However, no data are available; thus, it is difficult to distinguish overweight from obesity as a sign of metabolic syndrome. The objectives are (1) To reveal the ratio of overweight and obese house dogs in Japan; and (2) To investigate differences in breed, gender, body condition and age of various purebreds on healthy dogs. Twelve metabolic parameters were assayed in 888 healthy dogs. The data were analyzed from various viewpoints of age, gender, Body Condition Score (BCS), castration and spay and breeds. The ratio of obese dogs was approximately 27% of all dogs examined. When the effects of aging were assessed, older females were found to be more affected by aging than males. In particular, lipid metabolism parameters, such as triglycerides (TG), Total Cholesterol (T-cho) and Free Fatty Acids (FFA) as well as insulin concentrations in female dogs >11 years old were the highest of all age groups. Because T-cho and insulin concentrations in BCS4 dogs were significantly high, these parameters could be useful as obesity markers in healthy dogs. Creatinine concentrations in small breed dogs, such as Beagles, Cavaliers, Chihuahuas, Miniature dachshunds, Pomeranians, Shih tzus and Yorkshire terriers, were lower than those in middle-sized and large-sized dogs. TG and FFA levels in neutered dogs were significantly higher than those in unaltered dogs. In future, it will be important to measure and assess metabolic parameters in healthy dogs to identify differences among different regions.

Key words: Purebred dogs, metabolic parameters, obesity, neutered, adiponectin

INTRODUCTION

Obesity, diabetes mellitus and hyperlipidemia, accompanied by elevated blood lipid concentrations (triglycerides, cholesterol, or both) (Johnson, 2005) are increasingly prevalent in dogs. Many veterinarians feel that obesity in dogs is increasing in prevalence in Japan. However, no data are available; thus, it is difficult to distinguish overweight from obesity as a sign of metabolic syndrome. In the US, dogs are diagnosed using a Body Condition Score (BCS) and scores of 4 and 5 indicate overweight and obesity, respectively which occurred in approximately 25% of dogs in US in 1996 (Armstrong and Lund, 1996). The American Veterinary Medical Association reported that 33,352 veterinarians were responsible for approximately 840 overweight or obese dogs and cats in their animal hospitals in 1998 (AVMA, 1999). The another recent studies showed that 29-34% of dogs and 19-29%of cats were classified as overweight and approximately from 5-8% in both species were considered obese (Lund *et al*., 2005, 2006). In addition, the People's

Dispensary for Sick Animals which is a charitable veterinarian organization, has reported that approximately 35% of domestic dogs in the UK were overweight or obese in 2009. The same source reported that in 2006, approximately 21% of dogs were overweight or obese in the UK. If obesity in dogs increases at the same rate as these 4 years (2006-2009), >50% of the dogs in the UK will be overweight or obese in 2013 (PDSA, 2010). Overweight and obese dogs have decreased life span (Kealy *et al*., 2002) and increased risk of associated diseases such as diabetes mellitus, orthopedic disease, neoplasia, respiratory and urinary tract diseases (German *et al*., 2010). Therefore it is important to establish reference values of metabolic parameters for dogs in order to prevent occurrence of the diseases. The objectives are (1) To reveal the ratio of overweight and obese house dogs in Japan and (2) To investigate differences in and effects of breed, gender, body condition and age of various purebreds of healthy dogs in order to establish reference values of metabolic parameters in blood. Unfortunately, there is a lack of fundamental data about purebreds and domestic dogs in Japan. Shetland sheep dogs and Miniature Schnauzers were found to have primary hyperlipidemia through previous studies (Sato *et al*., 2000; Xenoulis *et al*., 2007; Mori *et al*., 2010). Thus, in this study, Shetland sheepdogs and Miniature dachshund were removed from the subjects.

MATERIALS AND METHODS

Animals: Over 1,100 dogs that visited seven different veterinary clinics across Japan (Tokyo, Ibaraki, Chiba, Osaka and Fukuoka) from the spring of 2008-2012 which underwent filariasis screening were eligible for this study. About 212 canines of all breeds were excluded because of disorders, such as liver failure, kidney failure, cardiac disease and diabetes mellitus, based on examinations and clinical signs. Thus, 888 canines (419 females, 469 males; age, 1-13 years) were included. Purebred dogs represented Beagle (female (F) n = 9, male (M) n =18), Border collie (F-6, M-3), Cavalier (F-5, M-5), Chihuahua (F-11, M-9), Golden retriever (F-9, M-12), Labrador retriever (F-18, M-17), Miniature dachshund (F-45, M-44), Papillion (F-5, M-6), Pomeranian (F-6, M-5), Pug (F-5, M-6), Shiba (F-37, M-55), Shih tzu (F-24, M-20), Toy poodle (F-8, M-8), Welsh corgi (F-11, M-8), Yorkshire Terrier (F-8, M-12) and Mongrel (F-81, M-84) dogs. To investigate the effects of aging, the animals were divided into four age group categories such as 0-2, 3-5, 6-10 and >11 years. The subjects were diagnosed to be clinically healthy, according to evaluation and clinical signs assessed by a veterinarian who scored them with BCS of 1-5 (Laflamme, 1997) based on palpation and inspection. The BCS uses a 5-point scale: (1) Very thin, (2) Underweight, (3) Ideal, (4) overweight and (5) Obese. The 888 canines were classified according to BCS that BCS 4 dogs were as obese group and BCS 2 and 3 dogs were non-obese group.

Blood sampling: Postprandial blood samples (at least 4 h after the last meal) were collected from the forelimb vein into heparinized plastic tubes. The blood samples were immediately centrifuged at 1700 g for 10 min at 4°C. They were stored at -25°C until further use.

Analysis of plasma parameters: Plasma metabolites and hormone concentrations and enzyme activities were measured. Plasma glucose (Glu), triglycerides (TG), total cholesterol (T-cho), total protein(TP), blood urea nitrogen (BUN) and creatinine (Cre) concentrations as well as lactate dehydrogenase(LDH), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were measured using an Olympus AU680 autoanalyzer (Olympus Corp., Tokyo, Japan) using the manufacturer's reagents. Free Fatty Acids (FFA), adiponectin and insulin were determined with NEFA-C test Wako (Wako Pure Chemical Industries, Inc., Tokyo, Japan), Dog adiponectin ELISA kit (Circulex Co., Ltd, Nagano, Japan) and Lbis dog Insulin kit (Shibayagi Co., Gunma, Japan), respectively.

Statistical analysis: Values are expressed as Mean±SD. Statistical significance was determined by the Holm-Sidak one-way analysis of variance among groups and Student's t-test for paired groups. All tests were performed using Sigmaplot version.11.2 (Systat Software Inc., San Diego, CA, USA). The significance level was set at $p<0.05$.

RESULTS

Table 1 shows the effects of aging on the metabolic parameters. TG, T-cho, BUN and insulin concentrations and ALT activities in female dogs >11 years old were significantly higher than those in 3-5-year-old female control dogs. No significant difference in FFA concentration was observed, although FFA demonstrated an upward trend. Insulin concentrations in 6 -10-year-old female dogs were also higher than 3-5-year-old female control dogs. BUN concentrations in 0-2 year-old female dogs were higher than same female control dogs. In addition, T-cho concentration and ALT activity in male dogs >11 years old increased significantly compared to the male dogs of other ages. TG and T-cho concentrations were significantly higher in all female dogs compared to all other male dogs.

Table 2 classifies all dogs by the five scale BCS and shows the effects of body condition (normal or obese). The ratio of overweight (BCS 4) dogs was approximately 27% of all dogs. Table 2 also shows that there was no difference with respect to gender within the same BCS group. T-cho and insulin concentrations in BCS 4 group were significantly higher than those in BCS 2 and 3 groups. A significant decrease in BUN concentration was observed in the BCS 4 group compared to the other BCS groups.

The purebred groups were composed of an average age of 6-8 years (Table 3). The mongrel group was selected as a control group. Significantly decreased Cre concentrations were observed in the Beagle, Cavalier, Chihuahua, Miniature dachshund, Pomeranian, Shi tzu and Yorkshire terrier dogs. Glu concentrations in the Yorkshire terrier were significantly higher than those in mongrels. Miniature dachshunds had significantly increased adiponectin concentrations compared with those in mongrels.

Table 4 shows the effects of gender in the purebred dogs. Male Beagles, Golden retrievers, Labrador retrievers, Miniature dachshunds, Shih tzus and Yorkshire terriers were significantly more obese than females of the same breeds. T-cho concentration in male Labrador retrievers was significantly higher than those in female Labrador retrievers. Cre concentration in male Miniature dachshunds was significantly higher than that in female miniature dachshunds. The adiponectin concentrations in male Golden retrievers, Shih tzus and mongrels were significantly higher than that in female of the same breeds. AST and ALT activities in male Toy poodles were significantly higher than those in female Toy poodles. ALT activities in female Yorkshire terriers were significantly lower than those in males of the same breed. TG concentrations in female Welsh corgis and mongrels were higher than those in males of the same breeds.

Table 5 and 6 compare the metabolic parameters in dogs that were or were not sexually neutered. TG and FFA concentrations were significantly higher in neutered dogs than those in not neutered dogs, whereas adiponectin concentrations in not neutered dogs were significantly higher than those in neutered dogs.

Table 1: Comparison of metabolic parameters among healthy domestic dogs with gender and age in Japan

Sample size (n)	BCS (/5)	BW (kg)	Age (years)	Glu (mg dL^{-1})	TG (mg dL^{-1})	T-Cho (mg dL^{-1})	TP (g dL^{-1})	BUN (mg dL^{-1})	Cre (mg dL^{-1})	FFA (mEq dL^{-1})	Insulin (ng mL^{-})	Adiponectin (μg mL^{-1})	LDH (U L^{-1})	AST (U L^{-1})	ALT (U L^{-1})
Female															
0-2years (80)	3.1±0.5	8.0±5.5	1.4±0.6	98±15	68±70	194±59	6.0±0.8	20.5±7.9*	0.88±0.24	0.49±0.83	1.15±1.05	26.62±20.61	97±112	29±13	42±25
3-5years (98)	3.3±0.5	9.8±7.1	4.1±0.9	92±18	80±83	199±60	6.2±0.8	16.7±5.7	0.88±0.25	0.65±0.95	1.22±0.97	28.76±21.46	141±195	32±19	44±53
6-10 years (156)	3.3±0.6	12.0±8.4	7.7±1.5	94±22	94±91	192±63	6.3±0.9	17.0±8.1	0.88±0.30	0.63±0.91	1.94±1.82*	27.85±27.79	101±116	28±17	46±52
Over 11years (85)	3.1±0.7	12.4±8.1	12.6±1.3	89±21	135±152*	226±85*	6.4±0.9	22.4±14.3*	0.96±0.41	1.01±1.42	2.32±1.92*	22.29±23.11	120±120	32±25	67±58*
all female (419)	3.2±0.6	10.8±7.7*	6.7±4.0	93±20	94±104**	201±68**	6.2±0.9	18.7±9.5	0.90±0.31	0.68±1.04	1.70±1.61	26.77±24.18	113±139	30±19	49±50
Male															
0-2years (98)	3.1±0.5	9.2±7.3	1.5±0.6	98±18	61±58	165±53	6.0±0.7	19.8±6.9	0.87±0.24	0.53±0.82	1.40±1.22	31.96±21.92	92±137	31±12	49±26
3-5 years (117)	3.3±0.6	10.8±7.8	4.1±0.8	97±33	69±69	180±65	6.2±0.9	18.1±7.6	0.91±0.27	0.61±0.95	1.74±1.62	33.17±24.39	110±128	30±9	46±23
6-10 years (172)	3.4±0.5	13.9±9.7	7.8±1.4	97±32	87±93	190±65	6.3±0.8	16.4±5.9	0.91±0.29	0.79±1.14	1.88±1.61	31.04±26.41	95±104	27±10	55±60
Over 11 years (82)	3.1±0.6	13.1±7.0	12.7±1.5	91±15	98±122	206±63*	6.4±0.9	20.1±8.7	0.93±0.38	0.65 ±0.97	2.00±1.67	21.35±20.33	124±137	29±12	68±69*
All male (469)	3.2±0.5	12.0±8.5	6.4±3.9	96±27	79±88	185±63	6.2±0.8	18.2±7.2	0.91±0.29	0.66 ±1.00	1.76±1.55	30.17±24.23	103±124	29±10	54±50

Values are presented as Means±SD. *Significantly different from 3-5 years group of same gender (Holm-Sidak One Way ANOVA, $p<0.05$). **Significantly different from all male of dogs (Holm-Sidak One Way ANOVA, $p<0.05$). BCS: Body condition score, BW: Body weight, Glu: Glucose, TG: Triglyceride, T-cho: Total cholesterol, TP: Total protein, BUN: Blood urea nitrogen, Cre: Creatinine, FFA: Free fatty acids, LDH: Lactase dehydrogenase, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, BCS uses a 5-point scale: (1) Very thin, (2) Underweight, (3) Ideal, (4) Overweight and (5) Obese

Table 2: Changes of metabolic parameters in obese and non-obese dogs based on body condition score (BCS) with gender

Sample size (n)	BW (kg)	Age (years)	Glu (mg dL^{-1})	TG (mg dL^{-1})	T-Cho (mg dL^{-1})	TP (g dL^{-1})	BUN (mg dL^{-1})	Cre (mg dL^{-1})	FFA (mEq dL^{-1})	Insulin (ng mL^{-1})	Adiponectin (μg mL^{-1})	LDH (U L^{-1})	AST (U L^{-1})	ALT (U L^{-1})
BCS (2, 3)														
Female BCS 2, 3 (263)	9.7	6.5	94±22	85±93	193±63	6.2±0.9	19.5±10.7	0.9±0.3	0.60±0.93	1.60±1.50	27.01±21.48	125±144	31±19	50±48
Male BCS 2, 3 (289)	11.0	6.1	96±18	79±95	181±65	6.2±0.8	18.7±7.5	0.9±0.3	0.51±0.64	1.70±1.46	31.55±22.19	102±124	30±11	53±41
All (651)	10.4	6.3	95±20	82±94	187±64	6.2±0.8	19.1±9.2	0.9±0.3	0.55±0.79	1.65±1.48	29.38±21.94	113±134	30±15	52±44
BCS4														
Female BCS 4 (108)	10.7	6.7	94±18	110±127	218±77	6.4±0.8	17.6±7.5	0.9±0.3	0.58±0.49	2.09±1.84	26.09±20.32	125±246	31±21	49±55
Male BCS 4 (129)	12.8	6.9	94±20	76±61	190±60	6.3±0.8	17.3±7.0	0.9±0.3	0.53±0.68	2.15±1.79	29.58±27.63	119±140	29±10	58±48
All (237)	11.8	6.8	94±19	91±98	203±70**	6.3±0.8	17.4±7.2**	0.9±0.3	0.55±0.60	2.12±1.81**	27.99±24.56	122±195	30±16	53±51

Values are presented as Means±SD. *Significantly different from the other gender of the same BCS group (student's t-test, $p<0.05$). **Significantly different from all BCS2 and 3 groups (student's t-test, $p<0.05$). BCS: Body condition score, BW: Body weight, Glu: Glucose, TG: Triglyceride, T-cho: Total cholesterol, TP: Total protein, BUN: Blood urea nitrogen, Cre: Creatinine, FFA: Free fatty acids and LDH: Lactase dehydrogenase

Table 3: Changes of plasma metabolite and hormone levels and enzyme activities in young, middle aged 15 purebred house dogs in Japan

Breed (n)	Age (years)	BW (kg)	BCS (/5)	Glu (mg dL^{-1})	TG (mg dL^{-1})	T-Cho (mg dL^{-1})	TP (g dL^{-1})	BUN (mg dL^{-1})	Cre (mg dL^{-1})	FFA (mEq L^{-1})	Insulin (ng mL^{-1})	Adiponectin (µg mL^{-1})	LDH (U L^{-1})	AST (U L^{-1})	ALT (U L^{-1})
Beagle (27)	8.28	12.1±3.0	3.4±0.7	98±16	81±68	205±71	6.2±0.7	16.5±6.4	0.78±0.20*	0.64±0.81	1.53±1.16	21.4±16.2	68±50	24±7	53±42
Border collie (9)	6.37	16.2±2.0	3.2±0.4	96±12	59±35	251±47	6.3±0.5	20.2±5.4	1.10±0.21	0.47±0.40	1.66±2.14	20.8±10.2	220±210	30±5	35±16
Cavalier (10)	6.59	9.2±2.7	3.0±0.6	87±13	88±61	169±52	5.9±0.7	14.6±8.3	0.68±0.17*	0.24±0.13	1.65±1.49	20.2±14.2	88±66	28±9	51±39
Chihuahua (20)	6.20	3.2±0.8	3.4±0.6	93±24	84±39	185±62	6.0±0.8	18.6±5.3	0.76±0.22*	0.54±0.47	1.38±1.32	30.0±25.4	132±116	34±22	54±28
Golden retriever (21)	7.80	32.3±5.1	3.3±0.5	89±16	79±76	249±75	6.4±0.9	14.6±6.0	0.95±0.20	1.03±1.81	1.55±1.30	30.8±28.1	115±100	24±7	32±15
Labrador retriever (35)	7.00	27.8±5.5	3.3±0.5	92±16	72±59	182±43	6.2±0.9	16.7±5.7	1.03±0.24	0.76±1.24	1.72±1.79	23.3±15.1	66±62	26±6	62±46
Miniature dachshund (89)	6.18	5.9±1.7	3.4±0.6	85±22	68±61	170±49	6.2±0.8	15.1±5.6	0.70±0.20*	0.66±0.73	1.55±1.62	39.9±26.0*	113±142	32±15	53±57
Papillion (11)	6.64	4.7±1.6	3.2±0.4	82±17	77±45	169±66	5.6±1.0	20.4±6.8	0.77±0.18	0.26±0.20	0.94±0.47	15.7±5.8	181±144	30±7	50±19
Pomeranian (11)	7.73	4.5±1.8	3.4±0.5	100±20	84±62	235±84	6.0±1.0	16.3±4.8	0.64±0.15*	1.24±0.77	1.38±0.99	40.4±29.9	94±74	24±8	60±45
Pug (11)	7.32	9.1±2.7	3.5±0.5	89±17	56±35	198±72	6.3±0.9	16.8±6.4	0.86±0.30	0.57±0.30	1.74±1.30	20.9±11.3	223±300	40±37	32±12
Shiba (92)	7.00	11.2±2.6	3.3±0.5	93±15	74±70	172±58	6.2±0.9	18.6±6.9	1.08±0.30	0.59±0.81	1.90±1.94	22.8±20.1	100±149	31±17	41±33
Shi tzu (44)	7.16	6.2±1.6	3.3±0.5	95±18	105±89	242±72	6.6±0.8	19.1±8.2	0.80±0.18*	0.61±0.77	2.29±1.60	38.1±25.2	170±188	32±28	62±48
Toy poodle (16)	6.94	4.8±1.4	3.2±0.5	99±23	104±96	218±68	6.5±0.8	17.4±6.7	0.93±0.33	0.93±1.34	1.75±2.32	23.5±26.5	68±44	27±7	60±39
Welsh corgi (19)	6.26	12.4±2.6	3.4±0.5	93±30	55±34	226±55	6.7±0.8	16.5±7.2	0.87±0.23	0.80±0.54	1.63±1.46	33.8±24.5	142±118	32±11	49±13
Yorkshire trrier (20)	7.19	4.5±1.6	3.1±0.6	117±75*	106±70	207±73	6.6±0.8	22.3±12.0	0.76±0.17*	0.58±0.32	1.60±1.45	16.1±17.0	78±64	26±9	49±33
Mongrel (165)	8.14	14.7±6.0	3.2±0.5	96±18	79±75	185±58	6.3±0.8	17.9±8.1	0.99±0.33	0.67±1.08	2.00±1.53	25.2±22.4	103±124	28±16	46±39

Values are presented as Means±SD. *Significantly different from that in mongrels (Holm–Sidak One Way ANOVA, $p<0.05$). BW: Body weight, BCS: Body condition score, Glu: Glucose, TG: Triglyceride, T-cho: Total cholesterol, TP: Total protein, BUN: Blood urea nitrogen, Cre: Creatinine, FFA: Free fatty acids, LDH: Lactase dehydrogenase, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase. BCS uses a 5-point scale: (1) Very thin, (2) Underweight, (3) Ideal, (4) Overweight and (5) obese

Table 4: Comparison of plasma metabolite and hormone levels and enzyme activities with gender in 15 purebred house dogs

Breed (n)	Age (years)	BW (kg)	BCS (/5)	Glu (mg dL⁻¹)	TG (mg dL⁻¹)	T-Cho (mg dL⁻¹)	TP (g dL⁻¹)	BUN (mg dL⁻¹)	Cre (mg dL⁻¹)	FFA (mEq L⁻¹)	Insulin (ng mL⁻¹)	Adiponectin (μg mL)	LDH (U L⁻¹)	AST (U L⁻¹)	ALT (U L⁻¹)
Beagle															
Female (9)	8.2	10.1±2.3	3.1±1.4	104±8	74±66	213±102	6.2±0.8	18.2±6.3	0.84±0.18	0.61±0.43	1.14±0.79	26.08±18.68	92±70	27±8	47±31
Male (18)	8.3	13.1±2.9*	3.5±0.7	95±18	84±70	201±53	6.2±0.6	15.6±6.4	0.75±0.22	0.65±0.93	1.72±1.27	19.98±15.74	56±32	23±6	56±47
Border collie															
Female (6)	6.2	15.9±2.1	3.3±0.5	93±14	47±14	256±50	6.1±0.6	18.1±4.0	1.08±0.18	0.63±0.39	2.07±2.70	19.04±11.12	212±213	29±5	35±18
Male (3)	6.8	16.9±2.1	3.0±0.0	101±10	83±38	240±48	6.6±0.3	24.5±6.0	1.14±0.31	0.20±0.24	0.97±0.45	26.09±5.42	238±250	32±3	35±17
Cavalier															
Female (5)	6.1	9.2±2.6	3.3±0.6	86±11	87±51	153±51	5.8±0.7	10.7±2.3	0.68±0.18	0.27±0.18	1.10±1.46	25.67±19.84	84±74	28±8	46±22
Male (5)	7.1	9.2±3.2	2.8±0.5	88±15	89±80	184±53	5.9±0.8	17.7±10.4	0.68±0.19	0.22±0.07	1.98±1.56	15.83±7.50	92±65	29±11	57±54
Chihuahua															
Female (11)	6.4	3.3±0.9	3.6±0.5	89±22	126±135	206±62	5.9±0.8	16.8±6.1	0.78±0.20	0.54±0.49	1.22±0.83	30.36±22.85	90±68	35±28	44±27
Male (9)	6.0	2.9±0.7	3.1±0.5	97±28	82±40	160±55	6.1±0.9	20.6±3.6	0.74±0.25	0.55±0.48	1.54±1.73	29.76±29.22	184±143	32±13	65±27
Goldeu retriever															
Female (9)	7.5	28.7±3.9	3.3±0.5	81±20	88±93	259±96	6.0±0.9	15.0±4.7	0.89±0.16	0.86±1.66	1.44±1.19	15.80±11.81	111±111	23±7	30±16
Male (12)	8.0	34.9±4.3*	3.3±0.5	95±11	72±64	242±58	6.7±0.8	14.3±7.0	0.99±0.22	1.12±1.96	1.63±1.43	48.00±32.26*	118±96	26±7	34±14
Labrador retriever															
Female (18)	6.7	24.9±3.7	3.3±0.5	91±13	76±69	169±36	6.1±0.9	15.7±4.6	0.99±0.19	0.79±1.27	1.59±1.19	20.28±12.61	64±65	26±5	55±41
Male (17)	7.4	31.2±5.5*	3.3±0.6	92±19	69±50	197±46*	6.3±0.8	17.8±6.7	1.08±0.29	0.72±1.24	1.85±2.26	26.71±17.39	68±61	26±7	70±51
Miniature dachshund															
Female (45)	6.1	5.1±1.2	3.4±0.7	81±25	68±67	172±52	6.2±0.8	14.6±5.7	0.65±0.19	0.75±0.91	1.68±1.73	35.37±25.41	121±141	34±17	54±75
Male (44)	6.3	6.7±1.8*	3.5±0.6	90±19	68±55	169±47	6.2±0.8	15.7±5.4	0.76±0.19*	0.55±0.46	1.42±1.52	44.53±26.20	80±59	29±7	51±28
Papilliou															
Female (5)	6.4	4.3±1.4	3.0±0.0	85±22	84±52	193±47	5.8±1.2	22.3±8.1	0.77±0.21	0.29±0.21	0.98±0.51	13.01±8.62	150±106	31±4	45±25
Male (6)	6.9	5.0±1.9	3.4±0.5	79±12	71±42	149±76	5.4±0.9	18.9±5.8	0.78±0.17	0.19±0.24	0.86±0.58	18.30±0.36	206±176	30±9	54±15
Pomeranian															
Female (6)	8.0	4.6±1.9	3.5±0.5	105±19	90±67	256±84	6.1±1.1	15.0±2.5	0.59±0.10	0.95±0.52	1.45±1.25	45.00±30.78	104±83	21±8	39±30
Male(5)	7.4	4.4±1.8	3.3±0.5	93±21	77±64	209±86	5.8±0.8	17.5±6.5	0.69±0.18	1.54±0.94	1.32±0.85	32.81±32.99	82±69	27±8	86±48
Pug															
Female (5)	7.0	8.8±2.0	3.8±0.4	90±22	42±12	216±45	6.3±0.9	16.9±7.1	0.85±0.23	0.70±0.30	1.51±1.35	15.09±3.93	111±99	53±54	34±12
Male (6)	7.6	9.4±3.3	3.3±0.5	89±13	66±43	183±91	6.2±1.0	16.8±6.4	0.86±0.37	0.42±0.26	1.97±1.36	26.78±13.86	297±373	30±11	31±13
Shiba															
Female (37)	7.1	10.6±3.1	3.2±0.5	93±17	87±98	180±55	6.1±0.7	18.3±5.5	1.03±0.22	0.58±0.69	1.80±2.02	24.15±25.50	83±144	30±22	37±18
Male (55)	7.0	11.6±2.1	3.3±0.5	93±14	65±40	167±59	6.4±0.9	18.8±7.8	1.11±0.34	0.59±0.89	1.97±1.90	21.92±15.64	112±153	32±13	44±40

Table 4: Continue

Breed (n)	Age (years)	BW (kg)	BCS (/5)	Glu (mg dL^{-1})	TG (mg dL^{-1})	T-Cho (mg dL^{-1})	TP (g dL^{-1})	BUN (mg dL^{-1})	Cre (mg dL^{-1})	FFA (mEq L^{-1})	Insulin (ng mL^{-1})	Adiponectin (μg mL)	LDH (U L^{-1})	AST (U L^{-1})	ALT (U L^{-1})
Shi tzu															
Female (24)	7.2	5.4±1.3	3.2±0.4	99±18	104±91	246±74	6.5±0.9	20.1±5.9	0.80±0.19	0.49±0.51	2.22±1.75	31.25±23.30	208±225	36±36	67±58
Male (20)	7.2	7.1±1.4*	3.5±0.5	91±19	105±90	237±70	6.8±0.6	16.3±6.8	0.79±0.17	0.77±1.01	2.36±1.46	48.74±25.09*	123±121	28±9	56±31
Toy poodle															
Female (8)	7.3	4.7±1.7	3.0±0.5	104±28	100±113	223±76	6.5±1.0	19.2±7.6	0.88±0.34	1.07±1.32	1.03±1.47	32.44±34.42	79±50	24±7	40±17
Male(8)	6.6	4.9±1.1	3.4±0.5	94±17	109±86	252±128	6.5±0.5	15.5±5.6	0.98±0.35	0.80±1.46	2.57±2.93	14.60±12.36	56±37	31±5*	81±44*
Welsh corgi															
Female (11)	6.0	12.0±2.3	3.5±0.5	95±25	69±34	231±50	6.9±0.7	18.9±8.2	0.90±0.26	0.56±0.32	2.16±1.73	28.88±34.14	129±84	33±11	47±13
Male(8)	6.6	12.9±3.0	3.3±0.5	89±37	37±26*	219±64	6.6±0.9	13.0±3.6	0.84±0.19	1.02±0.63	1.16±1.07	38.81±12.84	160±157	32±12	51±14
Yorkshire terrier															
Female (8)	7.1	3.6±1.3	3.1±0.6	93±17	94±66	221±89	6.7±0.5	20.2±11.4	0.73±0.16	0.76±0.32	1.47±1.10	24.48±23.50	59±25	25±9	32±22
Male (12)	7.3	5.2±1.6*	3.0±0.5	133±94	116±76	198±62	6.5±1.0	23.5±12.7	0.79±0.18	0.46±0.27	1.68±1.69	10.79±9.87	92±79	27±10	61±35*
Mougrel															
Female (81)	8.0	13.9±4.8	3.2±0.6	96±17	92±83	192±63	6.4±0.9	18.5±9.3	1.01±0.36	0.56±0.98	2.01±1.47	20.95±19.43	119±160	30±20	47±43
Male (84)	8.3	15.6±6.9	3.1±0.5	95±18	67±65*	180±53	6.3±0.8	17.3±6.9	0.96±0.30	0.78±1.15	1.98±1.60	29.02±24.38*	89±76	26±11	46±34

Values are presented as Means±SD. *Significantly different from the other gender of the same breed (student's t-test, $p<0.05$). BW: Body weight, BCS: Body condition score, Glu: Glucose, TG: Triglyceride, T-cho: Total cholesterol, TP: Total protein, BUN: Blood urea nitrogen, Cre: Creatinine, FFA: Free fatty acids, LDH: Lactase dehydrogenase, AST: Aspartate aminotransferase, ALT: Alamine aminotransferase. BCS nses a 5-point scale: (1) Very thin, (2) Underweight, (3) Ideal, (4) Overweight and (5) Obese

Table 5: Comparison of biochemical parameters in neutered dogs with intact dogs

	Neutered dogs	Intact dogs
Glu (mg dL^{-1})	91±20	100±30
TG (mg dL^{-1})	185±210*	65±58
T-Cho (mg dL^{-1})	247±112	215±71
TP (g dL^{-1})	6.9±0.6	7.1±1.1
BUN (mg dL^{-1})	17±8	21±11
Cre (mg dL^{-1})	1.0±0.3	1.2±0.6
FFA (mEq L^{-1})	0.70±0.43*	0.47±0.27
Insulin (ng mL^{-1})	1.2±1	1.0±0.8
Adiponectin (μg mL^{-1})	9.1±5.2*	20.5±16.2
LDH (U L^{-1})	353±247	229±331
AST (U L^{-1})	68±24	41±25
ALT (U L^{-1})	138±169	60±35

Values are presented as Means±SD. *Significantly different from intact dogs (student's t-test, p<0.05). Glu: Glucose, TG: Triglyceride, T-cho: Total cholesterol, TP: Total protein, BUN: Blood urea nitrogen, Cre: Creatinine, FFA: Free fatty acids, LDH: Lactase dehydrogenase, AST: Aspartate aminotransferase and ALT: Alanine aminotransferase

Table 6: Comparison of biochemical parameters in neutered dogs and intact dogs with BCS groups

	BCS 2,3		BCS4	
	Neutered dogs	Intact dogs	Neutered dogs	Intact dogs
Glu (mg dL^{-1})	101±15	114±31	89±20	90±26
TG (mg dL^{-1})	128±65*	48±17	197±229*	80±75
T-Cho (mg dL^{-1})	220±31	226±61	253±123	206±80
TP (g dL^{-1})	6.8±0.8	7.3±1.5	6.9±0.5	6.9±0.7
BUN (mg dL^{-1})	17±4*	27±10	17±8	17±9
Cre (mg dL^{-1})	1.1±0.3	1.4±0.7	1.0±0.3	1.0±0.4
FFA (mEq L^{-1})	0.47±0.30	0.42±0.23	0.75±0.45	0.51±0.30
Insulin (ng mL^{-1})	0.9±0.9	1.1±0.7	1.3±1.4	1.0±0.8
Adiponectin (μg mL^{-1})	7.9±4.5	18.4±18.9	9.3±5.4*	22.2±14.5
LDH (U L^{-1})	110±112	186±221	191±123	267±416
AST (U L^{-1})	29±10	37±17	35±13	43±30
ALT (U L^{-1})	64±38	6.9±27	70±92	60±42

Values are presented as Means±SD. *Significantly different from intact dogs of the same BCS group (student's t-test, p<0.05). BCS: Body condition score, Glu; Glucose, TG: Triglyceride, T-cho: Total cholesterol, TP: Total protein, BUN: Blood urea nitrogen, Cre: Creatinine, FFA: Free fatty acids, LDH: Lactase dehydrogenase, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase. BCS uses a 5-point scale: (1) Very thin, (2) Underweight, (3) Ideal, (4) Overweight and (5) Obese. BCS2,3: non-obese group, BCS4: Obese group

TG concentrations in neutered BCS 2, 3 and 4 dogs were higher than those in the same BCS dogs that had not been neutered. BUN concentrations in neutered BCS 2 and 3 dogs were lower than those in the same BCS dogs that had not been neutered. Adiponectin concentrations in the BCS 4 dogs that had been neutered decreased significantly compared with those in BCS 4 dogs that had been not neutered. No significant difference was observed in adiponectin concentrations in the BCS 2 and 3 dogs.

DISCUSSION

The ratio of obese dogs in Japan was estimated to be approximately 27% of all dogs which was similar to the values reported in the US (Armstrong and Lund, 1996). As the dogs collected for this study lived in both city and rural areas, this result accurately reflects the current prevalence of obese dogs in Japan. Older female dogs seemed to be more affected when the effects of aging on metabolic parameters were studied. In particular, the lipid parameters (TG, T-cho and FFA) and insulin concentrations in female dogs >11 years old were significantly the highest of all age groups. A comparison of the weight change in males of 6-10 years of age with that of male dogs >11 years old showed that male weight decreased by approximately 0.8 kg, whereas that of female dogs of the same ages increased by approximately 0.4 kg. Thus, it appeared that female dogs accumulated more fat, resulting in increased weight gain more easily compared with male dogs. Alternatively, older male dogs may have decreased bone and muscle mass because of decreased androgen concentrations (Vandenput *et al*., 2004; Sinnesael *et al*., 2012). As T-cho and insluin concentrations in the BCS 4 group increased significantly, these parameters could emerge as obesity markers in healthy dogs. Specifically, the P value of insulin in a multiple linear regression with BCS was 0.012 ($p<0.05$), suggesting that BCS is affected by insulin. The other metabolic parameters did not have significant p-values.

The previous study showed that the Shetland sheepdog and Miniature schnauzer which are popular breeds in Japan, appear to have primary hyperlipidemia. Other popular purebred dogs of the same mean age (6-8 years) showed significant differences in Cre concentration. This result is consistent with that obtained by Braun *et al*. (2003). Cre concentration in Beagles, Cavaliers, Chihuahuas, Miniature dachshunds, Pomeranians, Shih tzus and Yorkshire terriers, as small-sized dogs, were lower than those of middle-sized and large-sized dogs. Interestingly, a previous study reported that Cre concentrations in large-sized dogs are higher (Braun *et al*., 2003; Kuhl *et al*., 2000). Greyhounds and some other hound breeds (Afghan hound, Saluki and Whippet) also have higher Cre concentrations (Freeman *et al*., 2003; Hillpo, 1986). Hounds dogs are not very popular in Japan, but large-sized dogs such as Labrador retrievers and Golden retrievers which are popular, revealed higher Cre concentrations. It may be that larger breeds are genetically predisposed to having higher Cre concentrations.

The effects of differences in gender on purebreds showed some interesting results. Significantly higher values were observed for many parameters in almost all purebred male dogs. Body weights in males of six breeds (Beagle, Golden retriever, Labrador retriever, Miniature dachshund, Shih tzu and Yorkshire terrier) were significantly higher than those of females of the same breed. This result is consistent with that obtained by Choi *et al*. (2011). Fifteen purebred male dogs, excluding Chihuahuas and Pomeranians, tended to have higher weights than females of the same breed.

Many Japanese owners prefer that their dogs do not reproduce; therefore, dogs usually undergo castration or spaying at a young age (≤1 year of age). In this study, the ratio of castrated and spayed dogs was about 60%. Lipid parameters (TG, FFA) in dogs that underwent castration or spaying were significantly higher than those of dogs that did not undergo these procedures. Previous studies have reported that spayed females are likely to become twice as fat as unspayed females (Edney and Smith, 1986). A reduction in metabolism (25%) occurs soon after spaying in cats (Root *et al*., 1996). Since, one of the roles of estrogens is to decrease appetite, spayed females develop an increasing appetite. As a result, they gain excess body weight (Flynn *et al*., 1996). In certain ways, castration and spaying are far more associated with body condition because they are closely related to the metabolic dysregulation of obesity.

Unfortunately, as with humans, periodic health check-up for healthy dogs in Japan is not established. If a dog does not show any abnormality, many owners do not take them to the animal hospital except for constitutionally imposed vaccinations. It is necessary to understand that castrated and spayed dogs have lipid metabolism issues. Quite a few veterinarians in Japan refer to old reference values from the US (Kaneko, 1991). There are obviously different housing conditions for popular breeds between the Japan and another region. Thus, it will be important for veterinarians to measure metabolic parameters in healthy purebred dogs to identify differences among different regions.

CONCLUSION

The ratio of obese dogs was approximately 27% of all dogs examined in Japan. Older females were found to be more affected by aging than males. In particular, lipid metabolism parameters, such as TG, T-cho and FFA as well as insulin in female dogs >11 years old were significantly the highest of all age groups. Cre concentrations in small-sized dogs were lower than those in middle-sized and large-sized dogs in Japan. Lipid metabolism parameters (TG, FFA) in castrated and spayed dogs were significantly higher than those in unaltered dogs.

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