UNIVERSITI PUTRA MALAYSIA

PREVALENCE, PLASMA LIPID AND ADIPONECTIN CONCENTRATIONS OF OBESE DOGS IN KLANG VALLEY, MALAYSIA

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PREVALENCE, PLASMA LIPID AND ADIPONECTIN CONCENTRATIONS OF OBESE DOGS IN KLANG VALLEY, MALAYSIA

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It is hereby certified that we have read this project paper entitled "Prevalence, Plasma Lipid, and Adiponectin Concentration of Obese Dogs in Klang Valley, Malaysia," by Riyouko Lim and in our opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfillment of the requirement for the course VPD 4901-Project.

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PREVALENS, KEPEKATAN LIPID DAN ADIPONEKTIN DALAM ANJING OBES DI LEMBANG KLANG, MALAYSIA

Oleh
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2015
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Dengan meningkatnya standard kehidupan rakyat Malaysia, keobesan di kalangan haiwan kesayangan, terutama sekali anjing, semakin nyata. Kini, belum ada kajian terhadap ciri keobesan anjing di Malaysia. Kajian ini bertujuan untuk menentukan prevalens anjing obes di Lembah Klang, Malaysia. Bioelectric impedance device (BID) telah diguna untuk menentukan lemak badan pada anjing tegap kurus and obes untuk perbandingan. Juga, biopenanda keobesan, kepekatan adiponektin plasma, ditentukan bersama kepekatan alanina aminotranferase (ALT), kolesterol sepenuh and trigliserida ditentukan untuk pembezaan di antara anjing tegap kurus dan obes. Satu ratus tujuh-puluh-dua ekor anjing yang nampak sihat telah dipilih untuk kajian ini; daripadanya 12 ekor anjing tegap kurus dan 13 ekor anjing obes dipilih untuk penentuan kepekatan parameter plasma. Skor keadaan badan skala 5-mata (BCS) dan sukatan peratus lemak juga diguna kepada semua anjing ini. Kajian ini menunjukkan yang prevalens anjing berat badan lampau dan obes melalui sukatan BSC adalah 46.5% dan melalui BID adalah 56%. Ada korelasi tererti statistik di antara BCS dengan peratus lemak (r = 0.70, N = 172, p < 0.01) di kalangan anjing. Berasaskan BCS dan peratus lemak, ada korelasi positif di antara umur dan keobesan (BCS: r = 0.22, N = 172, p < 0.01), peratus lemak (r = 0.38, N = 172, p < 0.01), dan di antara status jantina (p < 0.01). Walaupun tiada kelainan tererti stastistik dalam kepekatan ALT dan kolesterol sepenuh di kalangan anjing, peratus lemak (p < 0.01), kepekaan trigliserida (p < 0.01) and adiponektin (p < 0.01) adalah lebih tinggi tererti dalam anjing obes daripada yang tegap kurus. Kajian ini menunjukkan yang hasil analisis BCS dan BID ada korelasi dengan kepekatan adiponektin dan lemak plasma. Analisis BCS dan BID adalah kaedah yang baik dalam penentuan keobesan pada anjing.

Kata kunci: prevalens, anjing, keobesan, kolesterol, trigliserida, adiponektin.

ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfillment of the course VPD 4901-Project.

PREVALENCE, PLASMA LIPID AND ADIPONECTIN CONCENTRATIONS OF OBESE DOGS IN KLANG VALLEY, MALAYSIA

By

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2015

Supervisor: Professor. Dr. Rasedee Abdullah

With the improvement of living standards of the Malaysian society, obesity among pets, particularly dogs appears to become more prevalent. Currently, no study has been done on the characteristics of obesity in dogs in Malaysia. This study aims to determine the prevalence of obese dogs in Klang Valley, Malaysia. A portable Bioelectric Impedance Device (BID) was used to determine the body fat of lean and obese dogs for comparison. In addition, an obesity biomarker, plasma adiponectin, together with alanine aminotransferase (ALT), total cholesterol and triglyceride concentrations were also estimated to determine differences between lean and obese dogs. One hundred seventy-two apparently healthy dogs were selected for the study; of which 12 lean and 13 obese dogs were selected for estimation of plasma parameter concentrations. A 5-point scale body condition score (BCS) and fat percentage measurements were employed for all dogs. The study showed that the prevalence of overweight and obese dogs based on BCS was 46.5% and based on BID was 56.0%. There was statistically significant correlation ($r = 0.70$, $N = 172$, $p < 0.01$) between BCS and fat percentage among dogs. Based on BCS and fat percentage, there were positive correlations between age and obesity (BCS: $r = 0.22$, $N = 172$, $p < 0.01$, fat percentage: $r = 0.38$, $N = 172$, $p < 0.01$), and between sex status ($p < 0.01$). Although there was no statistical significant difference in plasma ALT and total cholesterol concentrations among dogs, the fat percentage ($p < 0.01$) and triglyceride ($p < 0.01$) were significantly higher, and adiponectin concentrations ($p < 0.01$) was significantly lower in obese than lean dogs. This study shows that the results from BCS and the BID analyses correlate with plasma adiponectin and lipid concentrations. The BCS and BID analyses are good methods for the determination of obesity in dogs.

Keywords: prevalence, dog, obesity, cholesterol, triglyceride, adiponectin

1.0 INTRODUCTION

Obesity, defined as excessive adipose tissue and weight of 20% more than ideal weight (Stone *et al.,* 2009), is becoming common in dogs in Malaysia. This is partly due to the improvement in the Malaysian economy and income of its citizens in recent years, allowing them feed the pets under their care, well. Obesity in dogs, like in humans, poses a health risk, predisposing them to diseases like diabetes mellitus, cardiovascular disease, respiratory disorder, certain neoplasias, reproductive disorder, and early onset of degenerative disorders. Accumulation of fat has an association with important metabolic and hormonal changes in the body (Zoran, 2010).

Increase in body weight, which is measurable, could be due muscle mass gain instead of excessive adipose tissue. Currently, there is no really good standard for determining obesity in dogs. In veterinary practice, distinguishing obese from non-obese dogs is by body condition score (BCS), which is subjective. Since obesity is related to body fat, determination of blood and tissue fat content would be a more direct method for the determination. There is a portable and non-invasive device, Bioelectric Impedance Device (BID), which is used to estimate the body fat percentage in dogs. The data obtain from this machine can be correlated with the lipid content in plasma of obese dogs. At present, there has been no report on the prevalence of obesity in dogs in Malaysia. The information from this study would be useful for owners in the management of their pets. Therefore, the objectives of this project are as follows;

1. To determine the prevalence of obesity in dogs in Klang Valley, Malaysia
2. To determine the association between 5-point body condition score and body fat percentage.
3. To determine the factors associated with obesity in dogs.
4. To estimate the concentration of plasma lipids and adiponectin in obese and lean dogs.

The hypotheses of this project are;

1. The Bioelectric Impedance Device is effective in determining obesity in dogs.
2. There are associations between age, sex status, and obesity in dogs.
3. There is correlation between plasma alanine aminotransferase, total cholestrol, triglyceride and adiponectin concentration with obesity in dogs.

2.0 LITERATURE REVIEW

2.1 Obese dogs in the world

Prevalence studies of obese dogs were carried out in multiple countries based on BCS. In Australia, among 2,661 dogs in 209 clinics, there were 33.5% overweight and 7.6% obese dogs (Mcgreevy *et al*., 2005). Similarly, there were 29 to 34% overweight and 5 to 8% obese dogs among 21,754 dogs in the United States of America (Lund *et al*., 2005, 2006) and 38.8% overweight and 5% obese dogs among 616 dogs in France (Colliard *et al*., 2006). In contrast, 25.6% of dogs are obese in Japan and this was identified using the BID (Okawa, 2006). In fact, there appeared to be more obese dogs detected when BID was used.

In France, when owners were first interviewed whether they noticed their dogs were overweight or obese, only 20% thought their pets were overweight or obese, indicating that owners' perception usually underestimate the condition of their dogs (Colliard *et al*., 2006).

2.2 Methods of fat measurement

There are several methods of measuring fat composition in dogs and some require general anesthesia. Firstly, Deuterium oxide (D_2O) dilution is a method where D_2O solution is administered intravenously. The D_2O solution is a stable, nontoxic isotope, and freely exchange with water (Ishioka *et al*., 2005). This method, with the use of an equation, is an indirect measurement of body mass. Secondly, dual energy x-ray absorptiometry is a method which requires general anesthesia to assess bone mineral density and whole body composition including lean and soft tissues by attenuation of x-rays. Both of these methods require laboratory settings and are costly. Thirdly, BID analysis is a method in which a low voltage with high frequency current is employed and this method estimates fat free mass and body composition. Adipose tissue, a dehydrated compartment diminishes electrical current flow, causing a voltage drop, which is then translated into fat percentage (Stone *et al*., 2009). Kao Corporation has invented this handy device for fat percentage determination without the use of anesthesia or sedation. There is another method that uses the Bioelectrical Impedance Analysis (BIA) method in which the dog has to be on sternal recumbency and the electrodes of the BIA are attached on the limbs of the dog under anesthesia. Fourthly, body condition score is a physical examination and visual observation assessment by the veterinarian based on their ribs and other body prominences, abdominal tuck, and tail base bony structure using either a 5- or 9-point scale. Using this method, the dog are categorized as very thin or emaciated, thin or under weight, ideal, overweight, or obese (Laflamme, 1997). Other methods in use for the determination of obesity are computed tomography (CT), magnetic resonance imaging (MRI) and ultrasonography.

2.3 Fat deposition

Previous work done by Richey *et al*. (2009) revealed that dogs have typical abdominal fat compartments similar to humans, which are defined as visceral and subcutaneous fat depots. In that study, a hypercaloric high-fat diet was provide to 20 mongrel dogs and fat accumulation observed for 16 weeks. Then the amount of fat and nonfat tissue were quantified by MRI for 6 sessions. Fat accumulations were observed at intra- and retro-peritoneal region are known as visceral adipose tissue and at subcutaneous region located bilaterally known as subcutaneous adipose tissue. The BID, which was used in this experiment, measures subcutaneous adipose tissue at the lumbar region immediately caudal to the last rib; however, it was suggested that the most suitable location for body fat CT analysis is at L3 (to L5) location, but not cranial to that (Ishioka *et al*., 2005).

2.4 Plasma Parameters

Hepatocytes produce bile acids and deliver to the intestine via bile duct and aid in lipid metabolism; therefore, liver is an important organ for lipid metabolism. Hepatocyte contains alanine aminotransferase (ALT), a cytosolic enzyme is associated with hepatocellular injury or necrosis.. Cholesterol and triglycerides are the two major types of lipid in plasma and these parameters are in close relation with obesity, endocrine, hepatic, pancreatic, and renal diseases. According to Johnson (2005), obese dogs have high prevalence of hyperlipidemia with elevation of either plasma triglycerides or cholesterol concentrations, or both.

Mature adipocytes produce an anti-inflammatory cytokine, adiponectin, to increase insulin sensitivity, reduce plasma glucose concentrations, increase fatty acid oxidation, and decrease tissue triglycerides content in the liver and muscle (Fruebis *et al*., 2001; Yamauchi *et al.*, 2001, 2002; Radin *et al*., 2009). Adiponectin is currently used as a biomarker for obesity with negative correlation (Radin *et al*., 2009). Because increased fat masses inhibit adiponectin gene expression by increasing production of the pro-inflammatory cytokines, TNF- α and IL-6, (Radin *et al*., 2009), obese dogs are expected to show low plasma adiponectin concentrations.

Thus, there should be differences in plasma adiponectin concentrations between lean and obese dogs.

This study was undertaken to determine the association between BID measurements, body condition scores, plasma parameter concentrations with obesity in dogs of the Klang Valley, Malaysia.

3.0 MATERIAL AND METHODS

3.1 Animals

One hundred and seventy-two healthy client-owned dogs of any breed and more than 1 year old, from three private clinics in Klang Valley, Malaysia were selected for the study. The study was conducted over a 3-week period. The body condition of the dogs was determined, upon palpation and inspection, according to a 5-point Body Condition Score (BCS) described in Laflamme (1997). Measurement of fat percentage was done using the Bioelectric Impedance Device (BID) for dogs (IBF-D02, Kao Corporation, Haga, Tochigi, Japan; Appendix 1). The score is based on the following; BCS ≤ 3 = lean, BCS ≥ 3.5 = obese. Lean and obese group were classified based on the Kao Corporation referral parameters. The dogs were divided into 3 groups, based on breed types. Group 1 is “Higher Ideal Body fat % Breed” that included Shetland Sheep dog, Pomeranian, female Cavalier. Fat percentage of ≥ 33 % in intact males, and ≥ 36 % in females and castrated males were considered obese. Group 2 is “Lower Ideal Body fat % Breed” that included Jack Russell Terrier, French Bulldog, and Bulldog. Fat percentage of ≥ 23 % in intact males, and ≥ 25 % in females and castrated males were considered obese. Group 3 is “Typical Breed” that included other types of breed which did not fall under the first two groups. Fat percentage of ≥ 26 % in intact males, and ≥ 30 % in females and castrated males were considered obese. Other parameters taken into consideration were sex status including neutered or intact, and types of diet.

3.2 Blood sampling

A minimum of 3 mL postprandial blood samples (at least 12 hours after the last meal) from 12 lean dogs that were categorized as BCS ≤ 3 and 13 obese dogs that categorized as BCS ≥3.5 were chosen for blood collection via jugular, saphenous, or cephalic venipuncture. The blood was collected into EDTA tube, centrifuged with 300×g for 8 minutes and plasma obtained. The plasmas were stored into -20 °C until analysis.

3.3 Plasma biochemical parameters

Plasma alanine aminotransferase (ALT), total cholesterol (T-Chol), and triglycerides (TG) concentration were measured using a Hitachi 902 Automatic Analyzer (Boehringer Mannhein Diagnostica Inc, Indianapolis, IN, USA) according to manufacturer's instructions.

3.4 Adiponectin

Plasma adiponection concentration was determined using a commercial sandwich enzyme-linked immunosorbent assay (ELISA) kit (Millipore, Billerica, MA, USA) and fluorescent spectrophotometer (Infinite M200 Pro Tecan). The absorbances were obtained at 450 nm and 590 nm.

3.5 Statistical analysis

Statistical analysis was performed using a computer-based software program (IBM SPSS Statistics 20.0.0, SPSS Inc., Chicago, IL, USA). Pearson correlation was used to determine the correlation of between age and BCS, and fat percentage and BCS. Kruskal Wallis and Mann-Whitney test was used to determine differences among sex status groups (male, castrated male, female, and spayed female) in relation with BCS and fat percentage. Independent t-test was used to compare mean differences in ALT, TG, T-Chol and adiponectin concentrations between lean and obese dogs. *P*-values < 0.05 was used for determination of significant differences.

4.0 RESULTS

4.1 Prevalence of obesity in dogs

Eighty dogs were classified as obese with BCS ≥ 3.5 with a prevalence of 46.5 %. Based on the fat percentage referral parameters of the Kao Corporation, 96 were overweight and obese dogs with a prevalence of 56.0 %. The most common breeds were local (16%) and Shih Tzu (13%) (Appendix 2). Table 1 describes the demography of the samples for 172 dogs. Obese dogs had BCS ≥ 3.5.

Table 1. Demography of 172 dogs used in the study.

	Mean	SD	Range
Age (years)	5.18	3.38	1 - 16
Weight (kg)	15.93	11.58	1.82 - 49
BCS	3.38	0.74	2 - 5
Lean dogs Fat %	24.34	6.45	13 - 42
Obese dogs Fat %	34.65	6.81	18 - 54

4.2 Body condition score versus age

The most frequent age group in the study was 6 to 10 years old dogs (36%) (Appendix 3). Based on BCS, there was a positive correlation between age and obesity (r = 0.22, N = 172, p < 0.01; Figure 1). As the dogs were older, there was higher prevalence of obesity.

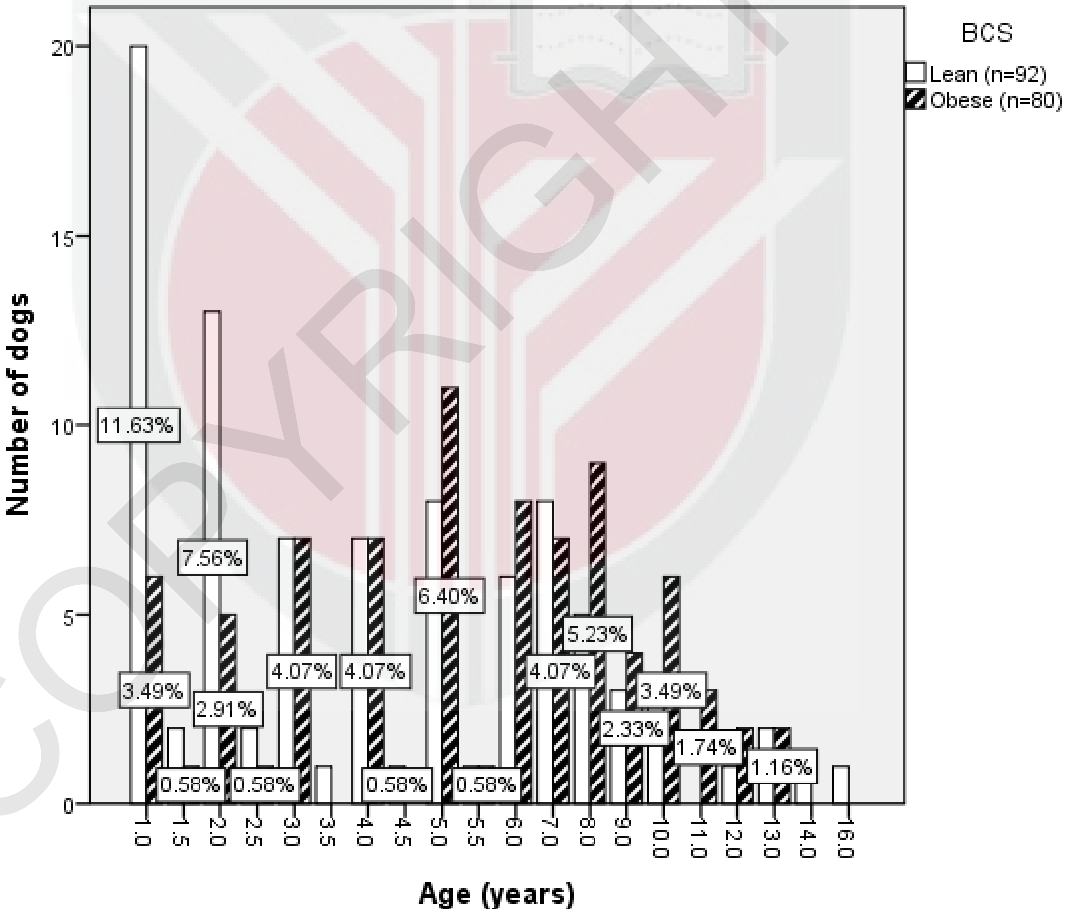

Figure 1. Prevalence of obesity by age based on body condition score.

4.3 Body condition score versus sex status

The distribution of sex status in the sample population of the study was 55 males, 36 castrated males, 27 females, and 54 spayed females. The prevalence of dogs that were considered in obese category for intact males were 9.3%, castrated males 13.95%, intact female 4.65%, and spayed females 18.6% (Figure 2).There were significant ($p < 0.05$) differences in BSC between among sex status, between male, castrated male, and spayed female group and between female and spayed female (Figure 3).

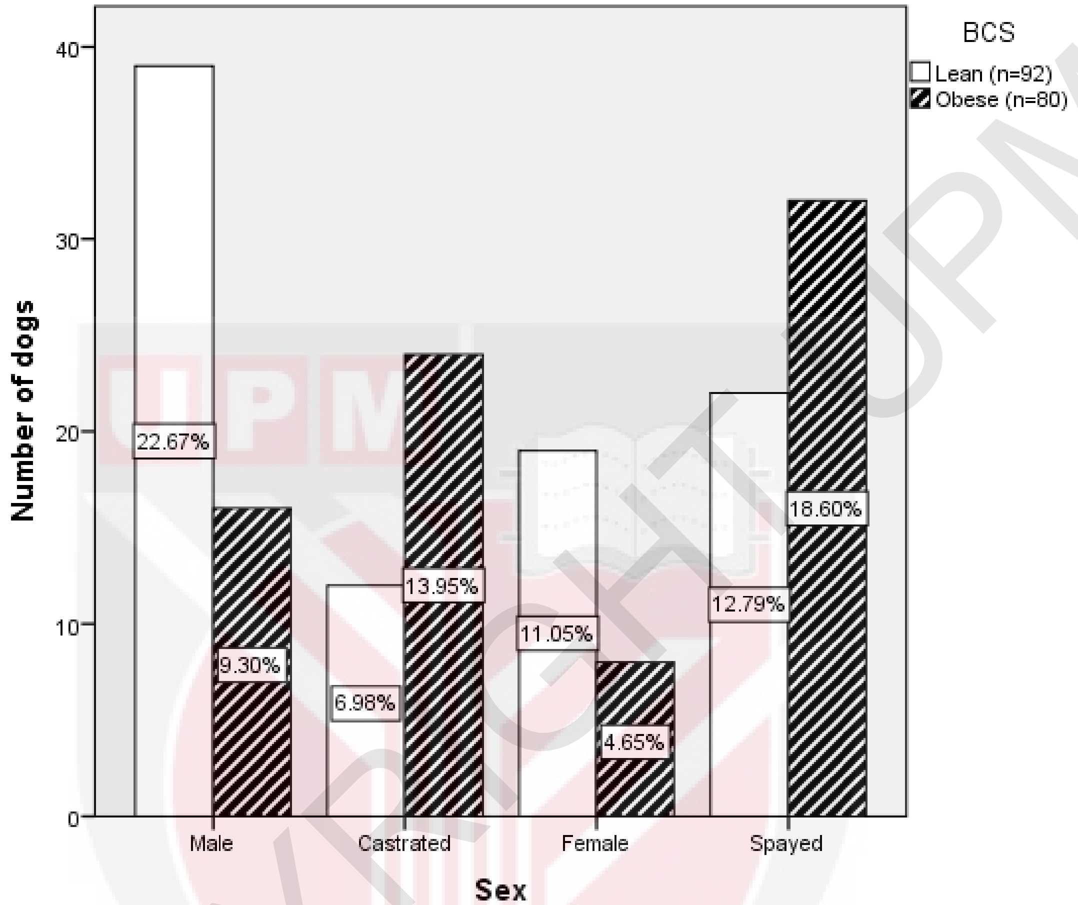

Figure 2. Prevalence of obesity by sex status in dogs based on body condition score.

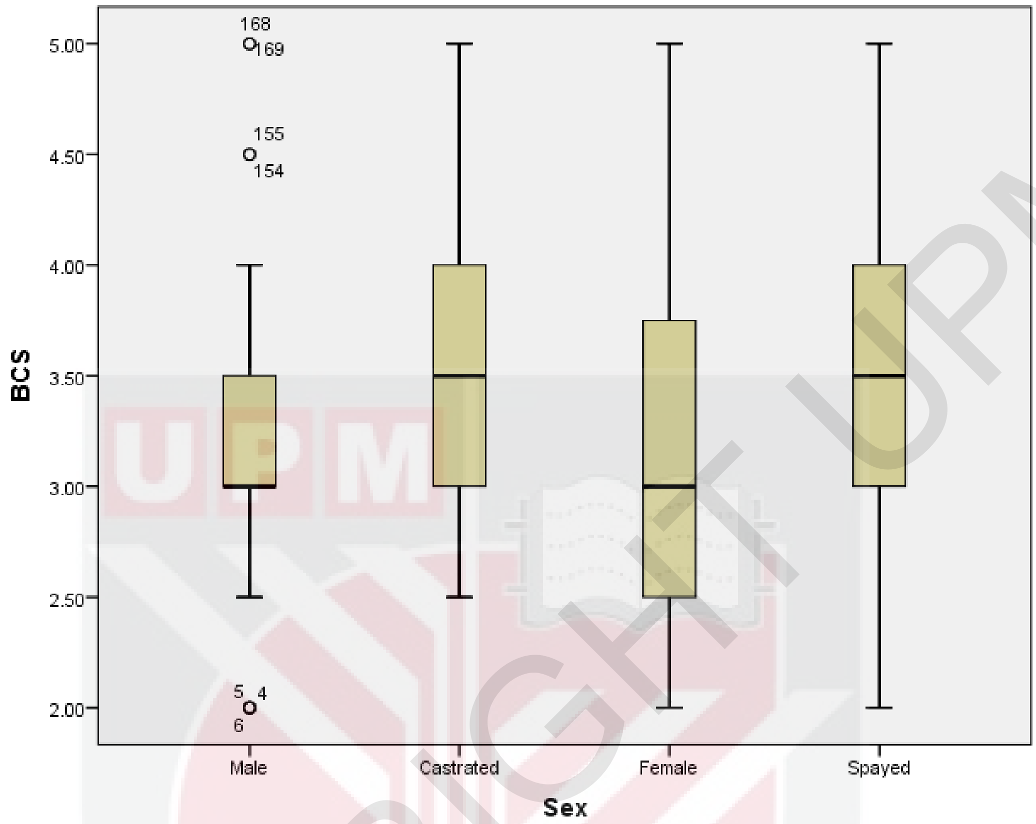

Figure 3. Comparison of body condition score of dogs among sex status.

4.4 Fat percentage versus body condition score.

There was a positive correlation between BCS and fat percentage ($r = 0.70$, $N = 172$, $p < 0.01$). The mean fat percentage between the lean ($M = 24.34$, $SD = 6.45$) and obese dogs ($M = 34.65$, $SD = 6.81$) were significantly ($p < 0.01$) different (Figure 4).

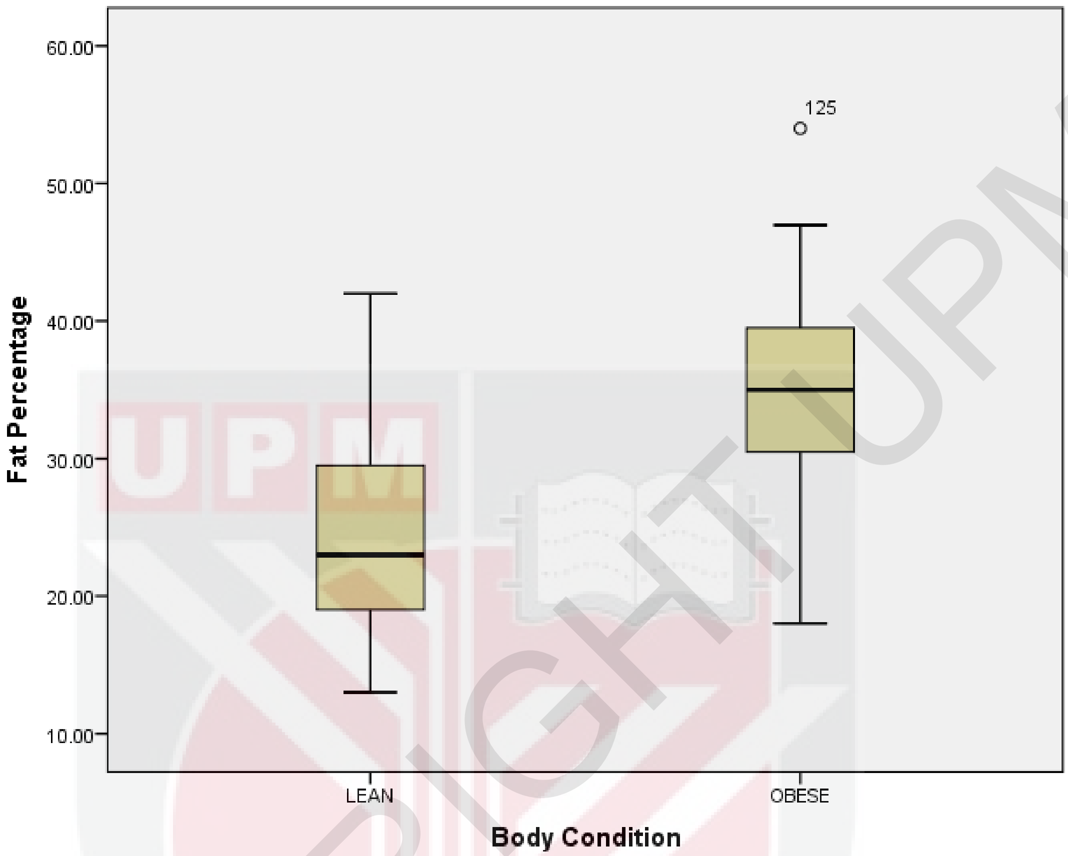

Figure 4. Mean fat percentage comparison between lean and obese dogs.

4.5 Fat percentage versus age

In the study, 76 dogs were lean and 96 were obese. The correlation between age and fat percentage was positive (r = 0.38, N = 172, p < 0.01) (Figure 5).

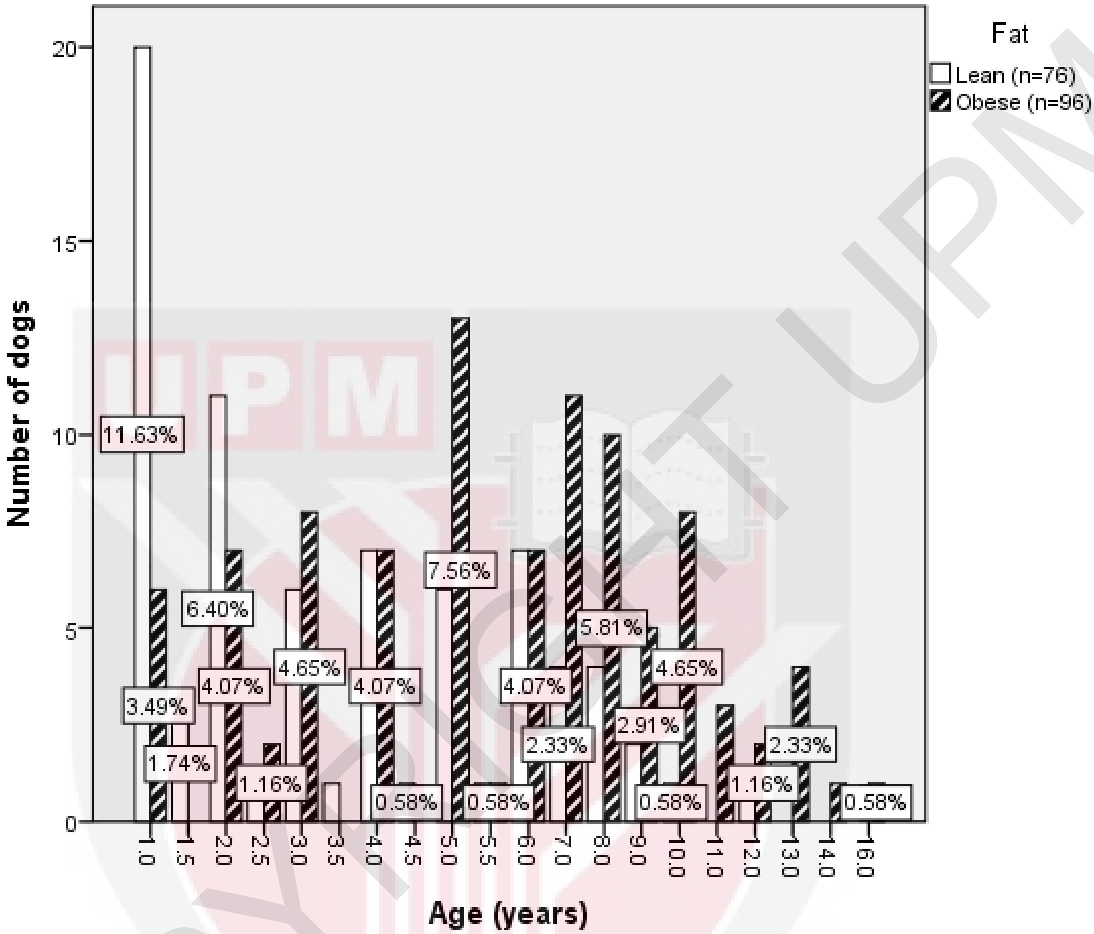

Figure 5. Prevalence of obesity based on fat percentage of dogs in different ages.

4.6 Fat percentage versus sex status

The prevalence of dogs that were considered obese was 13.37%, intact males, 14.53% castrated males, 4.65%, intact female and 23.26% spayed females (Figure 6). There were significant (p < 0.01) differences in fat percentage between sex status, males, castrated males and spayed females (Figure 7).

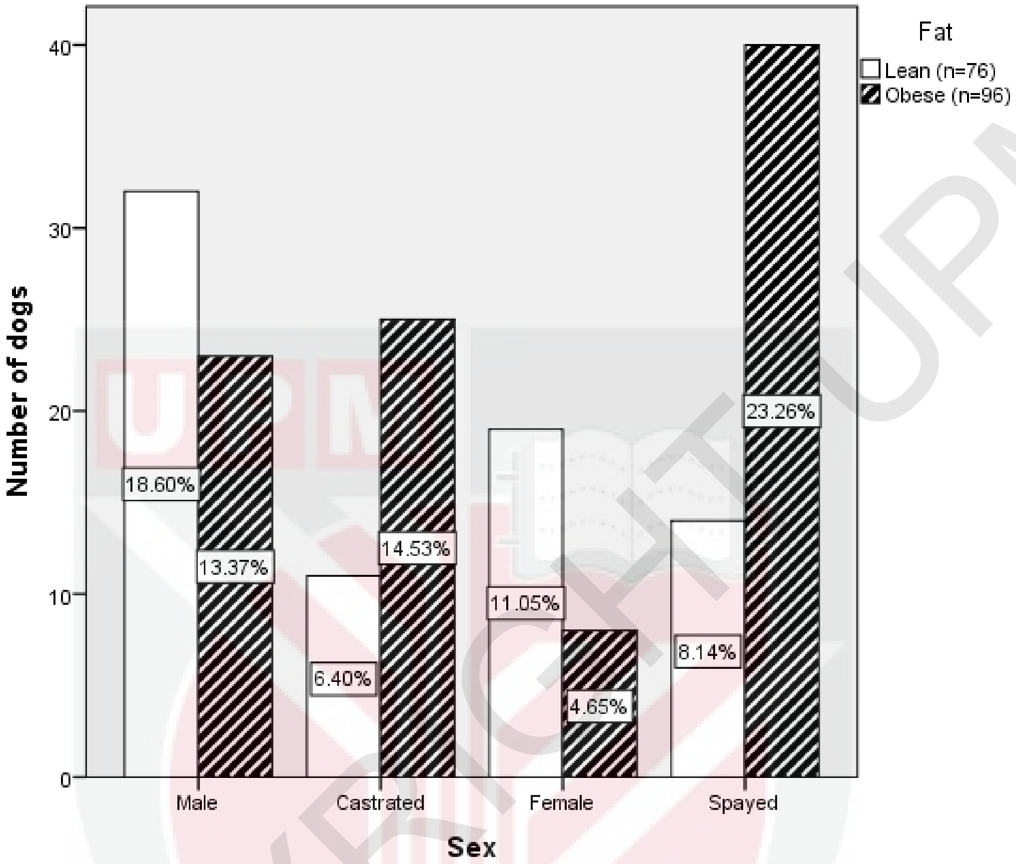

Figure 6. Prevalence of obesity in dogs by sex status based on fat percentage

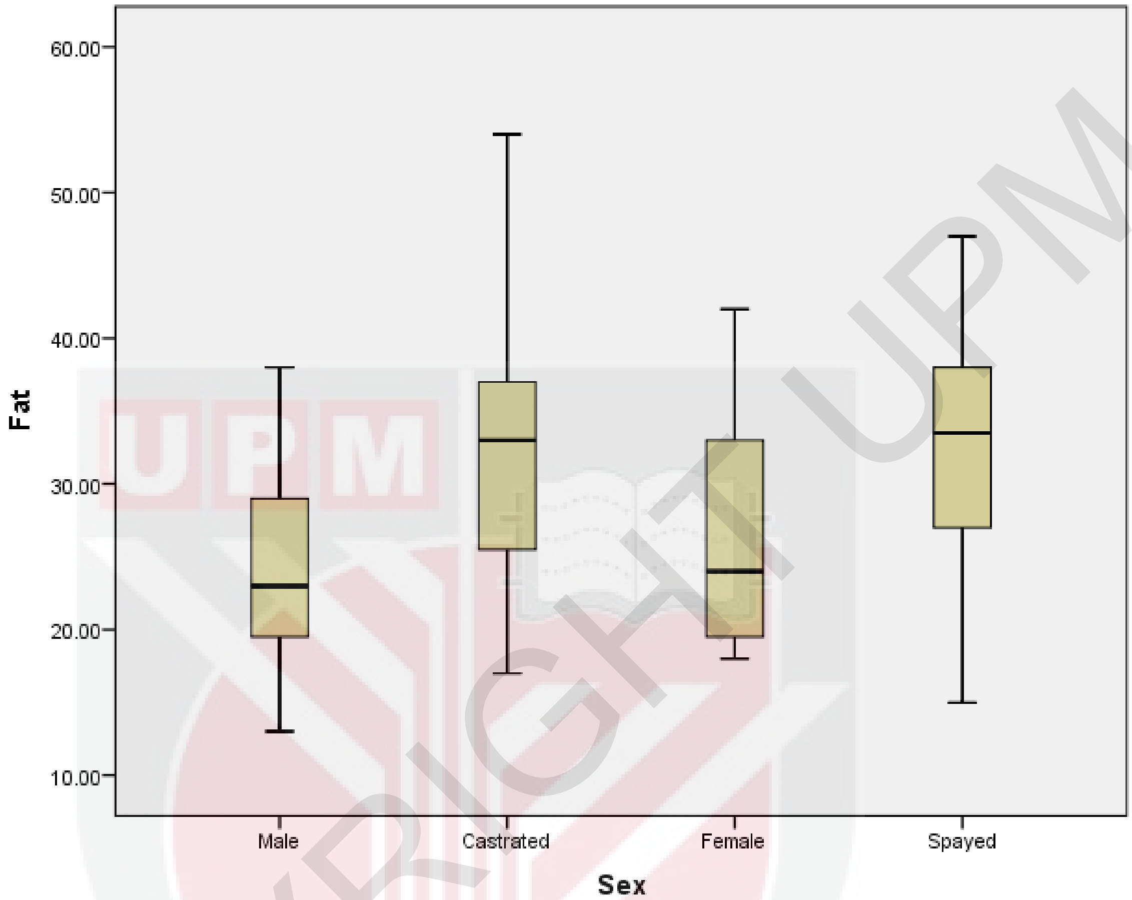

Figure 7. Comparison of fat percentage among sex status of dogs.

4.7 Plasma parameter and body condition score of lean and obese dogs

The blood lipid profile and adiponectin concentration of 12 lean and 13 obese dogs were compared against BCS (Table 2). There were 7 intact males, 3 intact females, 4 castrated males, and 11 spayed females in the sample population. Obese dogs showed significantly (*p < 0.01*) higher TG and adiponectin concentrations than lean dogs (Table 3). The ALT and T-Chol did not differ between these groups of dogs.

Table 2. Physical parameters of lean and obese dogs.

	Parameters	Mean	SD	Range
Lean (N=12)	Age (years)	3.96	2.78	1-10
	Weight (kg)	18.08	13.07	1.82 – 39
	BCS	2.79	0.33	2.0-3.0
	Fat %	22.83	6.01	15 – 36
Obese (N=13)	Age (years)	5.61	2.73	2-12
	Weight (kg)	23.03	13.27	5.00-40
	BCS	4.19	0.48	3.5-5
	Fat %	36.3	2.5	33 - 41

Table 3. Plasma biochemical parameters of lean and obese dogs.

	Parameters	Mean	SD	Range
Lean (N=12)	ALT (U/L)	28.13	6.92	16.60-37.90
	T-Chol (mmol/L)	4.56	0.90	3.31-5.57
	TG (mmol/L)	0.48	0.06	0.40-0.56
	Adiponectin (ng/mL)	15.88	3.27	9.47-21.10
Obese (N=13)	ALT(U/L)	29.34	7.14	15.90-40.70
	T-Chol (mmol/L)	4.86	1.09	3.72-7.14
	TG (mmol/L)	0.92[a]	0.28	0.42-1.45
	Adiponectin (ng/mL)	9.60[b]	6.55	1.40-21.49

[a,b]For each parameter, mean with different superscripts are significantly different ($p < 0.01$).

4.7.1 Fat percentage and body condition score of lean and obese dogs

Independent t-test showed significant ($p < 0.01$) difference in fat percentage between lean (22.83 ± 6.01 %) and obese dogs (36.30 ± 2.50 %) (Figure 8).

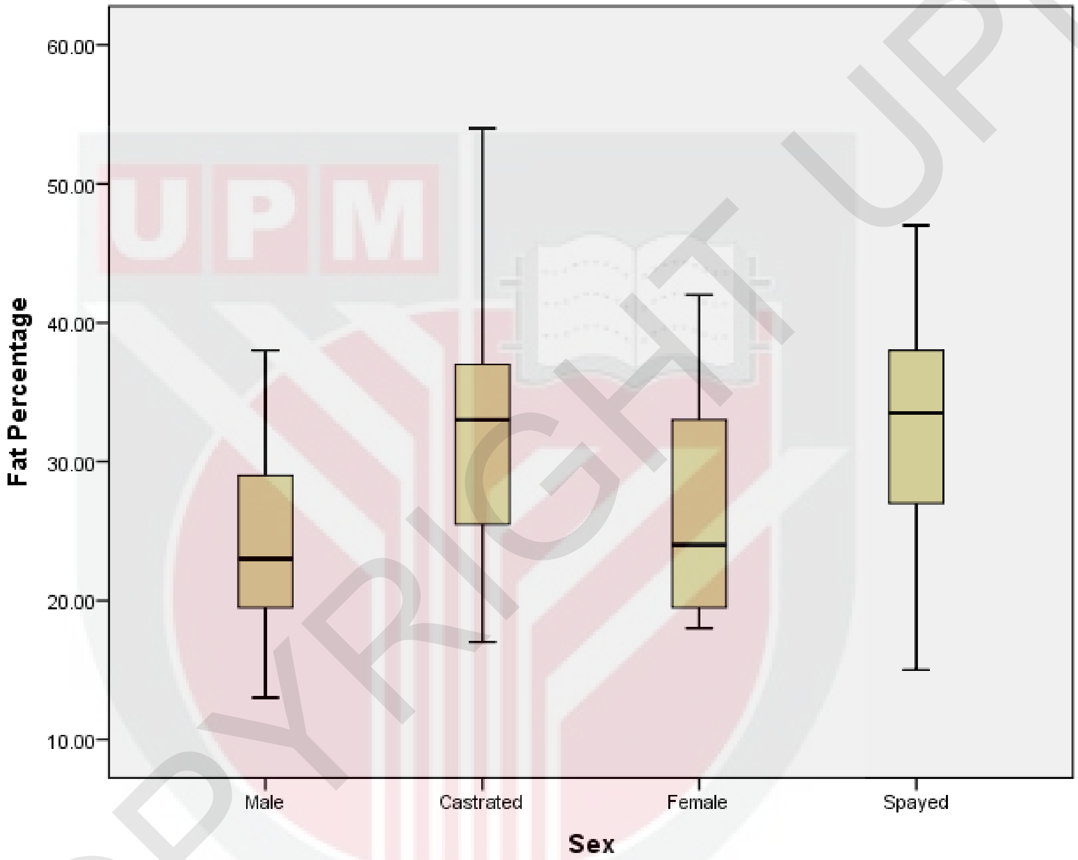

Figure 8. Fat percentage of lean and obese dogs.

4.7.2 Plasma alanine aminotransferase concentration of lean and obese dogs.

The result did not significant ($p > 0.05$) difference in plasma ALT concentration between lean (28.13 ± 6.92 U/L) and obese dogs (29.34 ±7.14 U/L) (Figure 9).

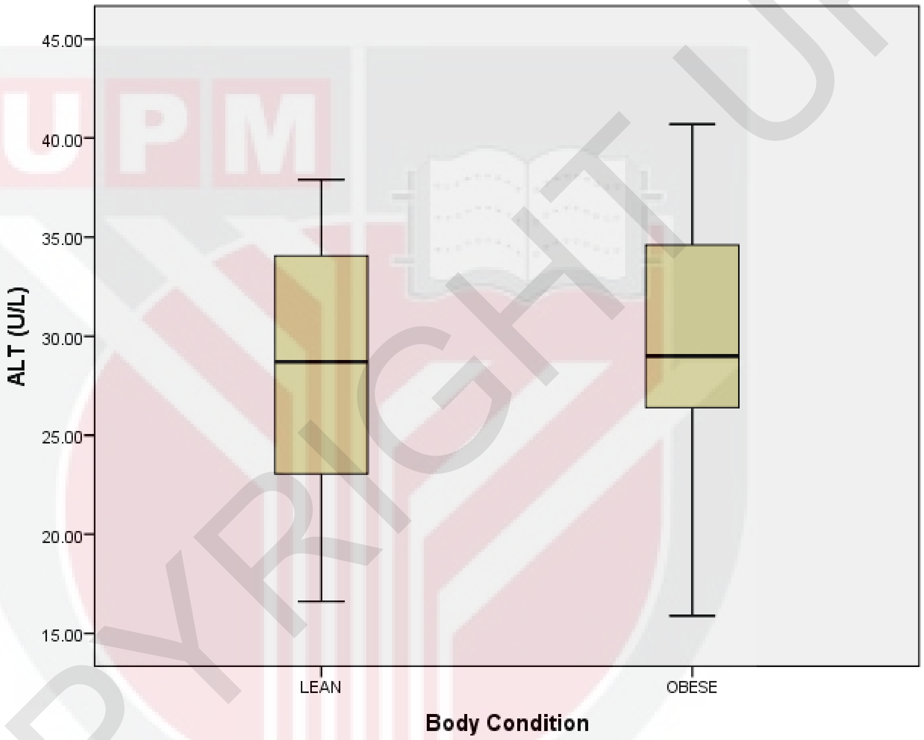

Figure 9. Plasma alanine transaminase concentrations of lean and obese dogs.

4.7.3 Plasma total cholesterol concentrations in lean and obese dogs.

There result did not show significant ($p > 0.05$) difference in plasma T-Chol concentrations between lean (4.54 ± 0.90 mmol/L) and obese (4.86 ± 1.09 mmol/L) (Figure 10).

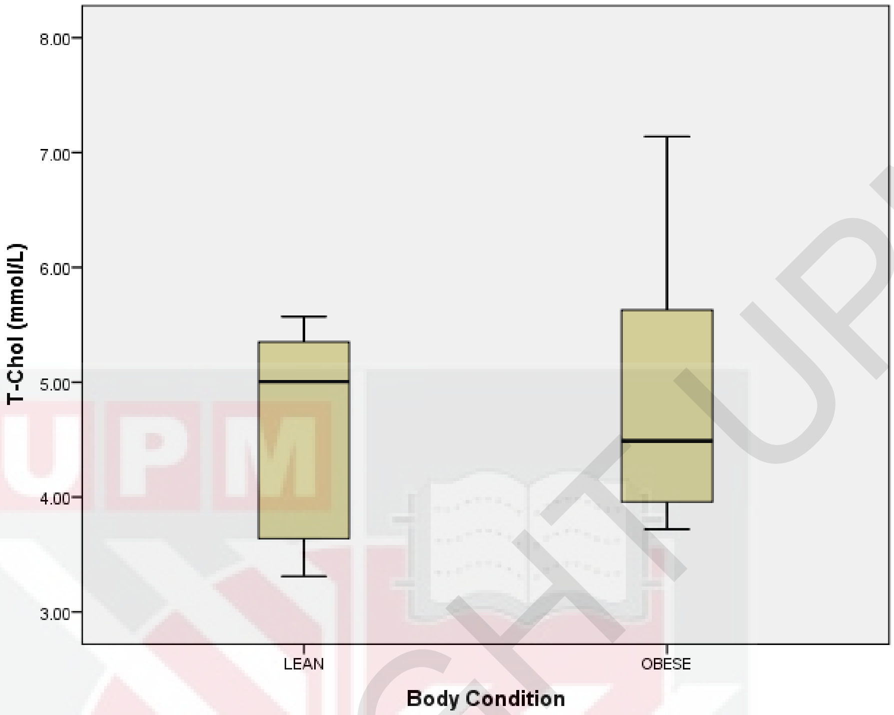

Figure 10. Plasma total cholesterol concentration in lean and obese dogs.

4.7.4 Plasma triglyceride concentrations of lean and obese dogs.

Obese dogs showed significantly (p < 0.01) higher TG concentrations in obese (0.92 ± 0.28 mmol/L) than lean (0.48 ± 0.06 mmol/L) dogs (Figure 11).

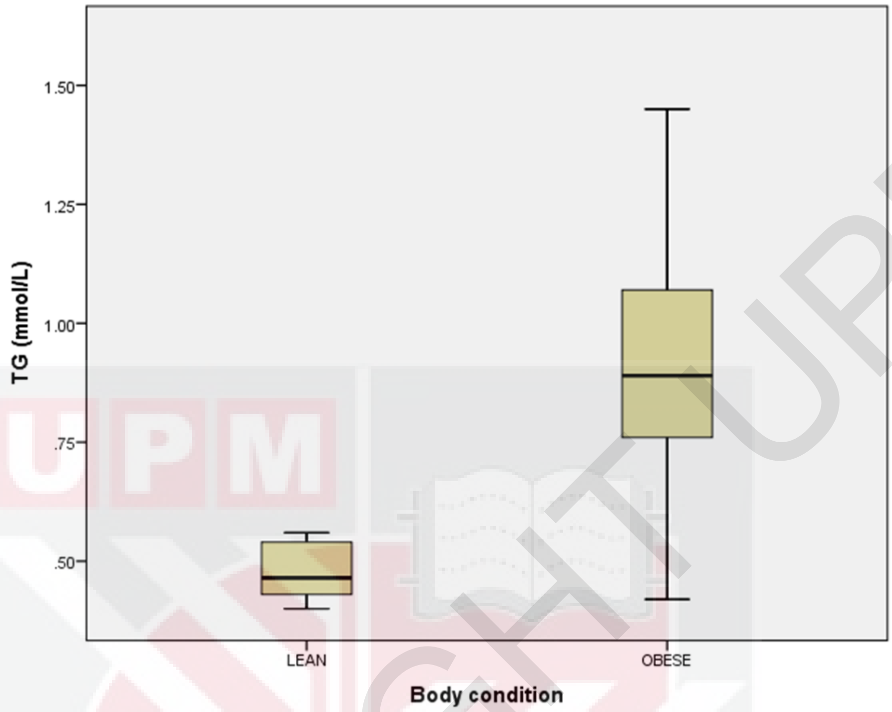

Figure 11. Plasma triglyceride concentrations of lean and obese dogs.

4.7.5 Plasma adiponectin concentration of lean and obese dogs.

The plasma adiponectin concentration was significantly (p < 0.01) higher in lean (15.88 ± 3.27 ng/mL) than obese (9.60 ± 6.55 ng/mL) dogs (Figure 12).

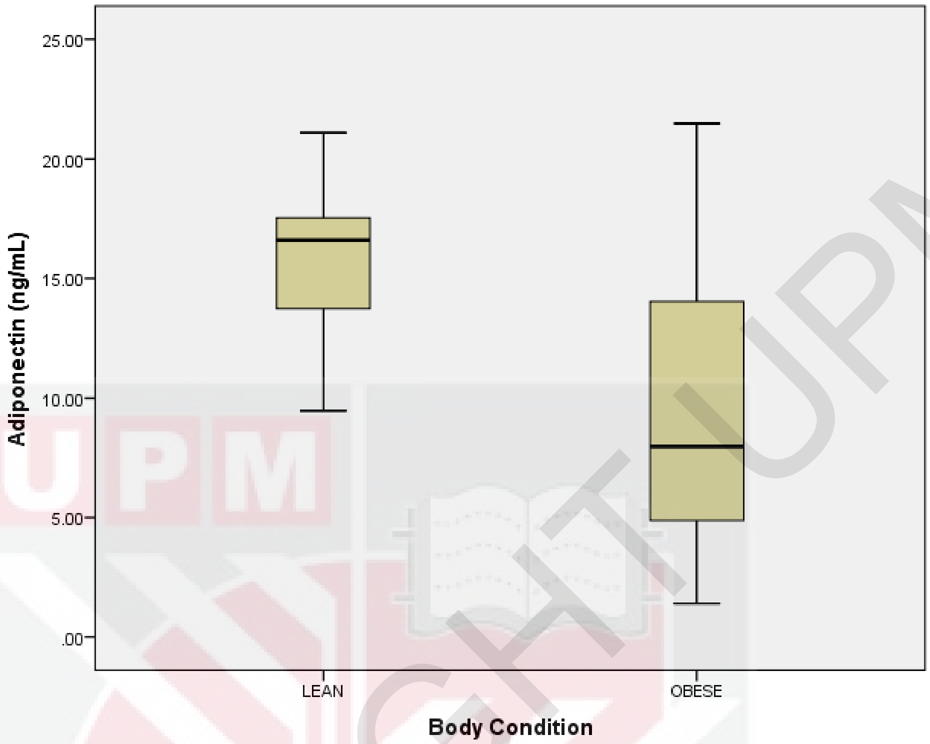

Figure 12. Plasma adiponectin concentrations of lean and obese dogs.

5.0 DISCUSSION

The prevalence of obese dogs was 46.5% based on 5-point BCS and 56.0% based on fat percentage using BID. There was correlation between 5-point BCS and fat percentage using BID. The BID was able to identify more obese dogs than the 5-point BCS; therefore, it is a useful device to be use together with the BCS for the quantification of body fat percentage (Stone *et al*., 2009).

The study showed a trend where sex status and age had correlation with obesity. It seems that there are more obese neutered dogs than obese intact dogs in Klang Valley. This corresponds with the finding of a previous study in France, which showed that neutered dogs had 2.23 times more likely to become obese (Colliard *et al*., 2006). This phenomenon was suggested to be due to the decrease in metabolic rate of the animal after neutering (Harper *et al*., 2001; Flynn *et al*., 1996; Hoenig and Ferguson, 2002). Loss of sex hormone, especially estrogen which inhibits lipogenesis, influences the brain centers affecting satiety and metabolism, and indirectly affecting cell metabolism and hormonal regulators of food (Cook and Naaz, 2004; Diez and Nguyen, 2006; Houpt and Hintz 1978; Jeusette *et al*., 2005); therefore, the veterinarian should advice the owners on the nutrient intake for their neutered dogs to prevent obesity. Aging was another factor that influences occurrence of obesity. Based on BCS and fat percentage, although the correlation factor was relative low, the study showed that age was positively correlated with obesity. Older dogs had greater chance in becoming obese because of lack of physical activity that increase fat accumulation; hence, reducing basal metabolic rate (Robertson, 2003; Harper, 1998). In addition, progressive sarcopenia which is a reduction of muscle occurs in aging dogs leading to reduction in physical strength and stamina for physical activity (Harper, 1998).

There were significant differences in plasma TG and adiponectin concentrations between lean and obese dogs, with obese dogs showing higher TG and lower adiponectin concentrations. Increase in plasma TG concentration is a characteristic of liver lipid accumulation (Hsiao *et al.*, 2007), which is reflected by increased in circulating lipid in obesity. The low adiponectin concentration is also characteristic of obesity because increase in fat mass would result in decrease adiponectin gene expression and consequently lowering circulating adiponectin concentration (Radin, 2009).

6.0 CONCLUSION

Body fat percentage measured by BID is practical and a useful parameter to be used together with the 5-point body condition score for determination of obesity.

In addition, the plasma triglyceride and adiponectin concentrations are valuable parameters in the detection of obesity. Neuter status and age of dogs are among factors associated with obesity. Based on sample population in this study, the prevalence of obesity in dogs in Klang Valley was almost 50%, which is high. Precautions should be taken by the owners in management of pets to prevent obesity. It is the role and responsibility of veterinarian practitioners to advice the owners, although challenging, on the provision of nutrition with appropriate energy balance to ensure their pet dogs do not become obese.

7.0 FURTHER STUDIES

The current study was conducted in three private clinics only in Klang Valley. The sample population in this study is small and may not represent the Klang Valley dog population. It is recommended for further studies to be conducted in more districts of Malaysia to determine whether the lifestyle of the owner is contributory to their pet dogs becoming obese. Future studies can also include more parameters such as plasma glucose, non-esterified fatty acid, and leptin besides ALT, T-Chol, TG, and adiponectin concentration. Studies that compare breed differences in development of obesity can also be conducted in the future.

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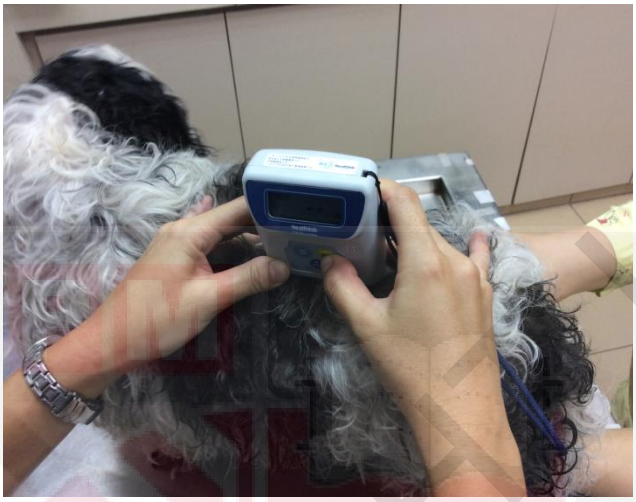
APPENDICES

Appendix 1: Fat measurement using Bioelectric Impedance Device (BID)



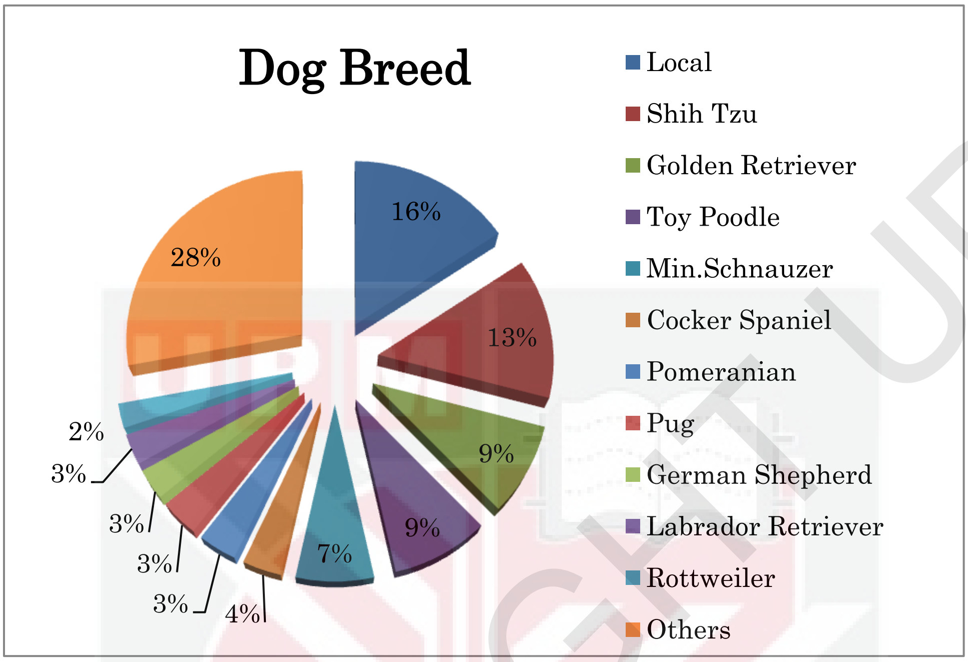
1. Palpate the spinous process and the last rib to ensure the proper measurement the location.

2. Fur should be well dabbed using cotton soaked with 70 % alcohol to separate the fur and expose the skin on the proper location for measurement. The skin as well as the root of the surrounding fur should be wet.

3. The probe should be placed immediately caudal to the last rib and 2cm lateral from the spine. Sufficient separation of the fur is necessary and increase contact of the probe. Both hands were used and place all four probes on to the skin directly after the alcohol dabbed and with right angle from the lateral side.

4. Measurement button was pressed and average of three recordings was determined as fat percentage of the dog.

Appendix 2: Dog Breeds in 172 dogs' samples



*Others include Chow Chow, Corgi, Dachund, Greatdane, Min, Pincher, Siberian Husky, Spitz cross, Terrier cross, poodle cross, German Shepherd Doberman Cross, and White Highland Terrier.

Appendix 3: Age Distribution (N=172)