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Assessment of asymmetric dimethyl arginine, cardiac troponin I, thyroxine, cholesterol, and triglyceride levels in obese dogs and dogs with normal body condition

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Abstract: The aim of this study was to assess serum asymmetric dimethyl arginine (ADMA), cardiac troponin I (cTnI), thyroxine (T4), cholesterol and triglyceride (TG) levels in dogs with normal body condition and obese dogs. A total of 40 dogs (n = 40) were included in the study and dogs were divided into two groups; 20 dogs with normal body condition and 20 obese dogs. The dogs were grouped according to the five-point body-condition scoring (BCS) system. Serum ADMA, cTnI, T4, cholesterol, and TG levels were measured in addition to routine biochemical parameters. Serum ADMA, cTnI, TG, and cholesterol levels were statistically significant between the two groups ($P < 0.001$, $P < 0.001$, $P = 0.027$, $P = 0.006$, respectively). There was a positive correlation between the serum cholesterol levels and both TG and cTnI levels among obese dogs. It is thought that the findings of this study may help future studies in determining novel biomarkers for obesity in dogs.

Key words: Obesity, dog, asymmetric dimethylarginine, cardiac troponin I, cholesterol, triglyceride

1. Introduction

Obesity is one of the most common malnutrition disorder among pet animals and humans in recent years [1,2]. In veterinary medicine, obesity is defined as 15%–20% over the ideal body weight [3,4]. Obesity is the imbalance between basal energy intake and consumption [5]. Body condition scoring (BCS) system is the most common method for determining the degree of obesity in dogs [6]. According to the five-point body condition system, obese dogs have a body condition score of 5, while dogs with normal body condition have a body condition score of 3. Obese dogs suffer from respiratory, cardiovascular, and gastrointestinal system diseases, endocrine and reproductive disorders, heat intolerance, coagulopathy, tumors, dyslipidemia, and dermatological diseases [7,8].

Asymmetric dimethyl arginine (ADMA) is an endogenous inhibitor of nitric oxide synthase, which is a primary stimulating molecule in cellular functions [9]. Nitric oxide bioavailability is effective in various physiological mechanisms, such as vasodilatation, platelet aggregation, low-density lipid oxidation, smooth muscle cell proliferation, superoxide radical regulator, and monocyte adhesion [10,11].

Cardiovascular diseases are one of the major complications of obesity [12]. Cardiac troponin I (cTnI) is a specific and sensitive biomarker in cardiac disease in dogs, cats, laboratory animals and humans [13-16]. Moreover, cTnI was evaluated as a marker in dogs with gastric dilatation volvulus, babesiosis, and cardiac damage [14,17,18].

Thyroxine (T4) hormone is secreted from thyroid gland and is either bound to proteins or in the free state [19]. Thyroid hormones are strong regulators of basal metabolism, and also have various functions in the metabolism [20,21].

Dyslipidemia, which is commonly observed in dogs, is characterized by an increase in serum triglyceride (TG) or serum cholesterol levels or even both [22,23]. Triglycerides consist of fatty acids and glycerol which are synthesized in the liver. They are an important source of energy. When the blood glucose level falls, they are broken down to fatty acids, and fatty acids are released into the circulatory system for energy metabolism [19]. Cholesterol is an important material for cell membranes [24]. The required amount of cholesterol is synthesized in the body; however, the excess amount of cholesterol is accumulated in the veins when it is taken extensively by nutrition. Obesity-induced lipid metabolism disorders play a significant role in the formation of obesity-related diseases [25]. According to Cardoso et al. [26], an increase

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in body condition score results in an increase in systolic hypertension and total cholesterol.

The aim of this study was to assess the levels of biochemical parameters, such as ADMA, cTnI, T4, TG, and cholesterol, in dogs with normal body condition and in obese dogs.

2. Materials and methods

This study was performed between February 2017 and December 2017 at Bursa Uludağ University, Veterinary Faculty, Internal Medicine Clinics of Animal Teaching Hospital. The study groups consisted of 20 dogs with normal body condition and 20 obese dogs (n = 40). Dogs with no clinical signs of any disease and not receiving any treatment were included in the study after completing the routine clinical examination and obtaining the consent of their owner. According to the five-point body condition scale of the American Animal Hospital Association (AAHA), dogs with a body condition score of 5 were defined as obese and those with a body condition score of 3 were defined as normal.

Blood samples were collected from the cephalic vein into a silicone-coated sterile serum tube containing 10 mL of clotting activator (Hema & Lab Products Medical Co. Ltd., Turkey). The serum samples were separated by centrifugation (1200 rpm, 10 min) and stored at −80 °C until analysis.

MyBio Source test kit was used to analyze ADMA (MyBio Source Inc., USA) and SunLong Biotech test kit was used to analyze cTnI by ELISA method (SunLong Biotech Co. Ltd. China) via Biotek ELx808 Absorbance Microplate Reader (BioTek Instruments Inc., Winooski, VT, USA). The levels of cholesterol and T4 were measured using VetScan Thyroxine (T4)/Cholesterol (Chol) Panel (Abaxis Inc., Union City, CA, USA). TG and routine biochemical parameters were measured using VetScan Comprehensive Profile (Abaxis Inc).

Statistical analyses were performed via SigmaPlot 14 (Systat Software Inc. CA, USA). Mann–Whitney U and t-test were used to determine the difference between the groups. Spearman's correlation test was performed to analyze correlation. The P-value <0.05 was accepted as statistically significant.

This study was approved by the Ethical Committee of Bursa Uludağ University Animal Experiments (No. 2016-08/01).

3. Results

Serum ADMA, cTnI, T4, TG, and cholesterol levels were compared among the groups. The levels of serum ADMA, cTnI, TG, and cholesterol levels were found to be lower in the dogs with a body condition score of 3 than the dogs with a body condition score of 5 ($P < 0.001$, $P < 0.001$, $P = 0.027$, $P = 0.006$, respectively) (Table 1). Serum T4 levels did not differ significantly between the two groups ($P = 0.642$).

The correlations between serum ADMA, cTnI, T4, cholesterol, and TG levels in the obese dogs are shown in Table 2. Correlations were performed using the Spearman test. A positive correlation was determined between serum cTnI and cholesterol levels, and between serum TG and cholesterol levels in obese dogs ($P = 0.0476$, $P = 0.00281$, respectively).

No statistical difference was found in the correlation analysis performed between serum ADMA, cTnI, T4, and cholesterol levels of the dogs with body condition score 3. There was a negative correlation between serum ADMA and TG levels ($P = 0.0195$).

4. Discussion

Several studies have revealed that the levels of certain biochemical parameters varied in obesity and related diseases [12,20,27–31]. It is stated that there is a positive relationship between obesity and ADMA in studies conducted with obese people [12,28]. In this study, there was a significant difference between the ADMA levels of obese dogs and dogs with normal body condition ($P < 0.05$). However, no study has been performed regarding the relationship between obesity and ADMA in dogs.

In a study conducted in mice, obesity was experimentally induced with a high-fat diet and increased ADMA levels were observed [32]. In this study, the levels of dimethyl arginine dimethyl amino hydrolase (DDAH) enzyme, which is involved in ADMA metabolism and expressed in liver, showed a decrease due to fatty liver. Hence, fatty liver resulted in an increase in the ADMA level [32].

Adipocytokines secreted physiologically by adipocyte tissue provide hemostasis of lipid metabolism. The disorders of lipid metabolism are occurred in obesity that is induced by disorders in adipose tissue metabolism [33]. In the study, serum TG and cholesterol levels, which were found to be high in obese dogs when compared to those with normal body condition, supported the obesity-related changes in lipid metabolism. Meanwhile, the serum cholesterol and TG levels in the obese group also showed a positive correlation with dyslipidemia in obesity.

Hypercholesterolemia, which results in impaired lipid metabolism, increases the risk of cardiovascular disease because it causes both atherosclerosis and decreased aggregation and distortion of erythrocyte membrane flexibility in the circulation [34–37]. The negative effect of obesity on lipid metabolism increases cholesterol levels, which can lead to cardiovascular disorders [25,38–40]. cTnI is a frequently used biomarker in the identification of cardiovascular diseases in dogs [16]. In the study, serum cholesterol level, which was higher in the obese dogs than those with normal body condition, showed

Table 1. According to BCS (3 and 5) mean ± SEM, maximum - minimum value of blood serum parameters (ADMA, cTnI, T4, TG, cholesterol, TP, and GLOB) in dogs.

Parameter	BCS = 3 Mean ± SEM/ Min - Max (n = 20)	BCS = 5 Mean ± SEM/ Min - Max (n = 20)	P-value
ADMA (µmol/L)	0.759 ± 0.109 0.051 - 2.412	1.179 ± 0.107 0.899 - 2.451	P < 0.001
cTnI (pg/mL)	304.700 ± 5.628 274.000 - 398.000	481.800 ± 7.600 389.000 - 516.000	P < 0.001
T4 (µg/dL)	1.615 ± 0.145 0.500 - 2.600	1.730 ± 0.198 0 - 3.600	P = 0.642
TG (mg/dL)	99.400 ± 5.367 70.000 - 153.000	145.390 ± 2 0.011 70.000 - 465.000	P = 0.027
Cholesterol (mg/dl)	176.750 ± 8.314 101.000 - 253.000	248.450 ± 21.058 139.000 - 520.000	P = 0.006
TP (g/dL)	7.010 ± 0.183 5.900 - 8.800	7.065 ± 0.212 6.100 - 9.800	P = 0.978
GLOB (g/dL)	3.445 ± 0.205 2.200 - 5.200	3.270 ± 0.245 2.400 - 6.700	P = 0.336

Table 2. Correlations between serum ADMA, cTnI, T4, cholesterol and TG in 20 obese dogs.

Parameter	cTnI (pg/mL) Correlation coefficient P-value	ADMA (µmol/L) Correlation coefficient P-value	T4 (µg/dL) Correlation coefficient P-value	Cholesterol (mg/dL) Correlation coefficient P-value
TG (mg/dL)	0.439 0.0519	0.154 0.513	−0.144 0.538	0.632 0.00281
Cholesterol (mg/dL)	0.446 0.0476	0.191 0.414	0.234 0.314	
T4 (µg/dL)	−0.0889 0.705	0.177 0.448		
ADMA (µmol/L)	−0.0459 0.841			

a positive correlation with the cTnI level. This finding supports the association between the cholesterol levels and cardiovascular markers which are increased as a result of the negative effects of obesity on lipid metabolism.

In the present study, serum total T4 levels did not differ between dogs with normal body condition and obese dogs. In a study conducted in obese pigs, T4 levels were reported to be at normal levels [41]. In the circulatory system, 75% of T4 hormone is bound to alpha globulin physiologically [19]. According to the findings of this study, there was no difference in serum total protein (TP) and globulin (GLOB) levels between the two groups. Therefore, the lack of difference in serum T4 levels between the groups can be related to the lack of difference in serum total protein and globulin levels.

In conclusion, the results obtained from this study should be taken into consideration for the evaluation of obese dogs and the related risk factors. In this study, findings revealed that serum the ADMA levels in obese dogs were higher than dogs with normal body condition. Moreover, the serum cTnI, cholesterol, and TG levels were also higher in obese dogs than those with normal body condition. However, there was a positive correlation between the serum TG and cholesterol levels, and between the cTnI and cholesterol levels in the group consisted of obese dogs. The serum T4 levels did not show variation between the two study groups.

This study will contribute to the future studies that will be performed to reveal novel biomarkers of obesity in dogs.

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