



Comparison of Asymmetric Dimethyl Arginine and Oxidative Stress Parameters in Obese Dogs and Dogs with Clinically Normal Body Condition Based on Routine Hemogram and Serum Biochemicals

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ABSTRACT

Background: Obesity, which has recently created many problems in humans and animals, is stated as being above 15-20% of normal body weight. This condition is commonly classified using body condition scoring in dogs. This study compared oxidative stress (malondialdehyde, catalase, superoxide dismutase), asymmetric dimethylarginine (ADMA), routine hemograms and biochemicals in obese dogs and dogs with normal body conditions.

Methods: Blood samples were taken from 20 normal body condition and 20 obese dogs. ADMA, oxidative stress and routine hematologic and biochemical parameters were evaluated.

Result: Platelet count ($p = 0.009$), blood urea nitrogen ($p < 0.001$), cholesterol ($p = 0.006$), ADMA ($p < 0.05$) and malondialdehyde ($p < 0.001$) were statistically high, while superoxide dismutase and catalase ($p < 0.001$ and $p < 0.001$, respectively) were statistically low in the obese group than the normal body condition group. As a result, we think the interpretation of the indicated biomarkers may help evaluate obesity and related pathologies in dogs, monitor treatment, or help prevent potential problems associated with obesity.

Key words: Asymmetrical dimethyl arginine, Dog, Obesity, Oxidative stress.

INTRODUCTION

Obesity is dogs' most common and vital problem (Barko *et al.*, 2017). More than 30 per cent of dogs in wealthy countries are overweight or obese, with rates expected to range from 33% to 58%, according to research data. In animals, obesity may be associated with many factors, such as genetics, excessive consumption of high-energy foods and a sedentary lifestyle (Kawauchi *et al.*, 2017). The usability of diagnosis and treatment tools in preventing and treating obesity is thought-provoking (Barko *et al.*, 2017). Some studies have evaluated the awareness of pet owners regarding care and feeding. It has been determined that the owners of obese animals are overweight and struggle with their body weight (Candellone *et al.*, 2017).

Asymmetric dimethyl arginine (ADMA) has increased in adipose tissue and serum levels in obese people due to primary or underlying causes of obesity (Holguin and Fitzpatrick, 2010). ADMA is an arginine-like amino acid found in plasma, urine and tissues. ADMA, naturally formed by the methylation of arginine residues in proteins, is an endogenous nitric oxide (NO) synthetase inhibitor and was excreted in the urine as methylated arginine (Aydin *et al.*, 2013). In the following years, methylated arginines were detected in the immune system cells and neurons of animals and the endothelial cells of humans (Surmeli, 2013). ADMA is in the group of methylarginines. Methylarginines are found in ADMA, monomethyl-L-arginine (L-NMMA) and symmetrical dimethyl arginine (SDMA). Only L-NMMA and ADMA are nitric

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oxide synthetase (NOS) inhibitors. The plasma ADMA level is ten times higher than the L-NMMA level. ADMA is the major inhibitor of NO biosynthesis.

Oxidative stress also increases ADMA in the blood by decreasing the level of DDHA (Nabity *et al.*, 2015). Oxidative stress, disrupting the balance between the oxidant and the antioxidant systems toward the oxidant system, is the formation of cellular damage in the organism because of lipid peroxidation and the release of free radical/reactive oxygen products. ADMA levels are high in many diseases that increase oxidative stress (Aydin *et al.*, 2013). When the organism's defense mechanisms (antioxidant mechanisms) against oxidative stress are insufficient, oxidative damage

develops in the cells and functions are disrupted significantly. This situation is critically important in the pathogenesis of many diseases and the severity of the disease increases. The enzyme systems in the cells are primarily effective in the defense system against free radicals. Catalase (CAT), glutathione peroxidase (GSH-Px) and Superoxide dismutase (SOD) are the most important enzymatic antioxidants that prevent the accumulation of free radicals and the initiation of lipid peroxidation (Isiklar and Mutaf, 2010).

Malondialdehyde (MDA), a parameter of oxidative stress and one of the most critical indicators of lipid peroxidation, causes changes in membrane properties such as enzyme activity, ion transport and aggregation of cell surface components (Tabakoglu and Durgut, 2013). Obesity can be detected together with disease states, including hyperlipidemia, hypertension, insulin resistance, diabetes mellitus and hypothyroidism. Each disease listed alone can increase the oxidative stress load, so in obesity cases, oxidative stress parameters may increase in the serum either with these diseases or alone (with the formation of fat accumulation) (Tvarijonaviciute *et al.*, 2012). It has been reported that obesity in dogs is also associated with a decrease in plasma antioxidant levels (Tvarijonaviciute *et al.*, 2012).

Although it is thought that the underlying cause of oxidative stress in animals is the disorder in metabolic functions (Webb and Falkowski, 2009; Zoran, 2010), there are very few studies on obese dogs on this subject (Tvarijonaviciute *et al.*, 2012).

Therefore, this study aimed to evaluate whether ADMA and oxidative stress parameters are diagnostic and prognostic biomarkers in addition to routine hematologic and biochemical markers in obese dogs.

MATERIALS AND METHODS

Study materials

The experiment was conducted between 2017-2018 at the Internal Medicine Department of the Faculty of Veterinary Medicine, Bursa Uludag University, Türkiye. The study material consisted of 20 clinically healthy dogs with normal body condition and 20 obese dogs brought to our Animal Teaching Hospital. The dogs that formed the study were between 3 and 5 years old. Dogs that were clinically healthy and did not receive any treatment were included in the study after their routine clinical examinations were completed, as stated in the previous study (Cihan and Tural, 2019). The study group consisted of regularly vaccinated dogs and antiparasitic applications were made. All dogs were fed quality dry commercial food once daily without any treatment and/or supplementation, consuming tap water. The owners' information about their feeding interval starts from one year old.

Dogs with a score of 3 (normal body condition) or 5 (obese) according to the clinical definition of BCS of the

American Animal Hospitals Association (AAHA) according to a 5-point score were added to the study groups. Among these dogs, ten female and ten male dogs were in the group with BCS 3 and nine female and 11 male dogs had a score of 5.

Sample collection and methodology

For hemogram analysis from dogs in the study, blood samples were taken into 2 ml sterile vacuum tubes containing ethylenediamine tetraacetic acid (EDTA) three to four hours after feeding. Hemogram analysis was performed using an Abacus Junior Vet (3240 Whipple Road Union City, CA 94587, USA) device in the Bursa Uludag University Faculty of Veterinary Medicine, Department of Internal Medicine Laboratory.

For biochemical analysis [alanine aminotransferase (ALT), alkaline phosphatase (ALP), amylase (AMY), blood urea nitrogen (BUN), total bilirubin (TBIL), phosphorus (PHOS), glucose (GLU), creatinine (CRE), sodium (Na), potassium (K), globulin (GLOB), total protein (TP), cholesterol (CHOL), asymmetric dimethyl arginine (ADMA), malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT)], blood was taken into 9 ml sterile vacuum serum tubes. Samples of the study material were collected between February and December 2017. The blood samples were centrifuged after coagulation at 1200 rpm for 10 min using Elektro-mag Laboratory Centrifuge M4812P (Elektro-mag Lab. Alt. San. Tic. A.Ş. İkitelli O.S.B. Demirciler Sitesi B7/153 İkitelli, İstanbul, Türkiye). Then, the separated serum samples were transferred into capped cryo tubes and WISD Ultra Low-Temperature Freezer SWUF-300 at -80°C. (DAIHAN Scientific Co., Ltd., Korea) instrument until the time of analysis. Biochemical analyses were performed in the Central Laboratory of our faculty; VetScan Abaxis Comprehensive Diagnostic Profile (3240 Whipple Road Union City CA 94587, USA) and Reflotron Plus Clinical Chemistry Analyser (Roche Diagnostics GmbH Mannheim, Germany) device were used. ADMA, MDA, SOD and CAT were analyzed using the enzyme-linked immunosorbent assay (ELISA) method using the ELx808 Absorbance Microplate Reader-BioTek (BioTek Instruments, Inc., Winooski, VT, USA).

Ethical approval

This study was approved by Bursa Uludag University Animal Experiments Local Ethics Committee with the decision numbered 2016-08/01 on 07.06.2016.

Informed consent

Informed consent has been obtained for client-owned animals included in this study.

Statistical analysis

The findings obtained from the cases mentioned in the study were evaluated with Student's t, Mann-Whitney U, Pearson and Spearman correlation tests in the SigmaPlot 14

statistical program. Those with a probability value (p) of 0.05 or were statistically significant.

RESULTS AND DISCUSSION

Hematologic and biochemical profiles

Platelet count was significantly higher in the obese dogs than in normal body condition group (355.2 ± 22.568 ; 245.2 ± 28.723 $10^9/L$, $p=0.009$, respectively), as shown in Table 1. Also, CHOL (248.450 ± 21.058 vs. 176.750 ± 8.314 mg/dL, $p=0.006$), BUN (17.950 ± 2.139 vs. 10.500 ± 0.925 mg/dL, $p<0.001$), ADMA (1.179 ± 0.107 vs. 0.759 ± 0.109 $\mu\text{mol/L}$, $p<0.001$) and MDA (198.250 ± 4.070 vs. 125.100 ± 3.797 pg/mL, $p<0.001$) were significantly higher; SOD (474.400 ± 14.377 vs. 565.350 ± 5.987 pg/mL, $p<0.001$) and CAT (0.937 ± 0.010 vs. 1.691 ± 0.035 pg/mL, $p<0.001$) values were significantly lower in the obese than in dogs with normal body condition, as shown in Table 2.

It has been reported that obesity is a low-grade inflammatory condition (Chmelar *et al.*, 2013). In a study (Rafaj *et al.*, 2016), the hypothesis of finding high WBC, RBC, HCT and HGB values in subjects in the obese group was proposed. The researchers determined no difference between obese dogs and those with normal body condition in terms of the specified parameters. Our study found WBC and NEU values to be higher in the obese group, although not statistically. Radakovich *et al.* (2017) obtained results from their study in parallel with ours and researchers based this result on the response to stress and low-grade inflammatory reaction. The short lifespan of the NEU indicates continued low-grade chronic infection and immune system activation in overweight and obese dogs. Also, higher PLT counts found in the obese group in the present study might be secondary to chronic inflammation. In addition to this inflammatory condition, it has been reported that one of the possible reasons for the high

Table 1: Comparison of blood parameters of dogs with normal body condition and obese groups.

Parameter	Normal body condition group (Mean±Standard error)	Obese group (Mean±Standard error)	P value
WBC ($10^9/L$)	12.889±1.403	13.381±0.566	0.185
LYM ($10^9/L$)	3.261±0.555	2.470±0.298	0.441
MONO ($10^9/L$)	0.626±0.0891	0.529±0.0527	0.635
NEUT ($10^9/L$)	8.488±1.063	10.131±0.488	0.133
EOS ($10^9/L$)	0.383±0.073	0.309±0.0472	0.705
BASO ($10^9/L$)	0.0845±0.0102	0.123±0.0159	0.052
RBC ($10^{12}/L$)	7.220±0.211	6.768±0.216	0.142
HGB (g/dL)	16.065±0.624	15.980±0.559	0.920
HCT (%)	49.170±1.818	48.109±1.584	0.662
MCV (fL)	68.145±1.096	71.185±0.869	0.056
MCHC (g/dL)	32.585±0.307	33.265±0.526	0.674
RDWC (%)	16.050±0.595	14.650±0.218	0.155
PLT ($10^9/L$)	245.2±28.723	355.2±22.568	0.009
PCT (%)	0.228±0.0265	0.291±0.0193	0.061

Table 2: Pairwise comparisons of serum biochemical parameters between normal body condition and obese dogs.

Parameter	Normal body condition group (Mean±Standard error)	Obese group (Mean±Standard error)	P value
ALP (U/L)	63.000±5.846	60.050±11.371	0.208
ALT (U/L)	34.000±1.672	39.100±4.284	0.534
AMY (U/L)	638.050±28.199	715.150±58.195	0.507
TBIL (mg/dL)	0.285±0.0109	0.315±0.0293	0.642
BUN (mg/dL)	10.500±0.925	17.950±2.139	<0.001
PHOS (mg/dL)	4.810±0.273	4.215±0.224	0.100
CRE (mg/dL)	0.830±0.0417	0.920±0.0511	0.181
GLU (mg/dL)	77.100±4.474	86.650 ± 4.981	0.162
NA (mmol/L)	141.350±0.595	142.800±0.635	0.104
K (mmol/L)	4.935±0.0838	4.985±0.0930	0.786
TP (g/dL)	7.010±0.183	7.065±0.212	0.978
GLOB (g/dL)	3.445±0.205	3.270±0.245	0.336
CHOL (mg/dL)	176.750±8.314	248.450±21.058	0.006
ADMA ($\mu\text{mol/L}$)	0.759±0.109	1.179±0.107	<0.001
SOD (pg/ml)	565.350±5.987	474.400±14.377	<0.001
MDA (pg/mL)	125.100 ±3.797	198.250±4.070	<0.001
CAT (pg/mL)	1.691±0.035	0.937±0.010	<0.001

NEU may be that the subjects in the obese and overweight groups are hypercholesterolemic. Based on this situation, this study's increased total cholesterol amount increases the NEU level, as reported in a study (Yvan-Charvet *et al.*, 2008). In our research, the CHOL level of dogs in the obese group was higher and hypercholesterolemia and/or low-grade inflammatory reaction might cause higher NEU levels in the obese group, as indicated in the previous studies. Additionally, high ADMA levels have been found in humans and dogs with hypercholesterolemia and inflammatory diseases (Gori *et al.*, 2020; Korkmaz *et al.*, 2021). In our study, ADMA levels were higher in the obese group than in the normal body condition group (1.179 ± 0.107 , 0.759 ± 0.109 $\mu\text{mol/L}$, respectively) ($p < 0.05$). It may indicate an inflammatory response and the high levels of neutrophils support our argument.

Many studies have detected changes in some biochemical parameters in obesity and related diseases. Studies on obese people and dogs have reported a positive relationship between obesity and ADMA (Marliss *et al.*, 2006; Kocak *et al.*, 2011; Huang *et al.*, 2017; Cihan and Tural, 2019). In our study, ADMA levels in obese dogs were significantly higher. However, as a result of a search in scientific article resources, no publications related to obesity and ADMA were found in dogs, except in our previous study (Cihan and Tural, 2019). In an experimental study, mice were given a high-fat diet to induce obesity and their ADMA levels were evaluated (Li *et al.*, 2017). The DDAH enzyme, which is involved in ADMA metabolism and expressed in the liver, is decreased due to fatty liver. Therefore, fatty liver has been reported to cause an increase in ADMA levels (Li *et al.*, 2017).

SOD and CAT are important antioxidant enzymes that are activated to act against oxidative stress in tissues and prevent the accumulation of free radicals and the initiation of lipid peroxidation (Isiklar and Mutaf, 2010; Torkanlou *et al.*, 2016). In a study, it was determined that the level of SOD in obese people was lower than in the control group (Torkanlou *et al.*, 2016). Moreover, in a study conducted on mice, SOD and CAT values were significantly lower in obese mice compared to the control group (Manna and Jain, 2015). In another study on cats, no change was found in the antioxidant activity of CAT and SOD during the development of obesity (Hoenig *et al.*, 2013). To the authors' knowledge, there are no studies on SOD and CAT antioxidant activity in obese dogs. However, antioxidant mechanisms are stimulated in the formation process of obesity and high levels of antioxidants can be determined in the metabolism at the beginning. In late obesity, a decrease in antioxidant levels indicates uncompensated oxidative stress (Sabitha *et al.*, 2014). In our study, serum SOD and CAT levels were found to be significantly lower in the obese group. Considering the studies above, we can evaluate the reason for the decrease in SOD and CAT levels in the obese group in our study as a response to the long-term obesity of these dogs.

Since it is costly and difficult to determine the increase in ROS, MDA measurement is made; therefore, oxidative

stress can be determined (Holguin and Fitzpatrick, 2010). MDA is a product of lipid peroxidation reaction and MDA levels increase in parallel with increased oxidative stress in obesity (Holguin and Fitzpatrick, 2010; Tabakoglu and Durgut, 2013; Amirkhizi *et al.*, 2010). In a study conducted on dogs with hyperlipidemia, serum MDA levels were measured before and after treatment and it was determined that the MDA level decreased after treatment (Li *et al.*, 2014). In another study by Kawasumi *et al.* (2018), MDA levels were found to increase, although not statistically, in obese dogs fed high-fat diets for a long period. In our study, serum MDA levels were significantly higher in obese dogs than in dogs with normal body condition ($p < 0.001$). With the formation of fat accumulation, obesity can alone increase oxidative stress in dogs, resulting in an increase in biomarker levels such as MDA. Chronic postprandial change may affect oxidative stress and ADMA metabolism (Mah *et al.*, 2011). This study suggested that increases in ADMA and MDA values might also be associated with chronic postprandial increase.

In vivo and *in vitro* studies have reported that bilirubin is an antioxidant that can physiologically inhibit lipid and lipoprotein oxidation (Tomaro and Battle, 2002; Liu *et al.*, 2006). It is thought that bilirubin constitutes one-tenth of all antioxidant effects in healthy adult humans and bilirubin levels are negatively correlated with serum oxidative biomarker levels (Kalousova *et al.*, 2005; Demir *et al.*, 2013). It has been reported that antioxidant levels increase due to increased oxidative stress during the development of obesity (Sabitha *et al.*, 2014). Previous studies determined higher bilirubin levels in obese or hyperlipidemic dogs and humans (Yamka *et al.*, 2006; Li *et al.*, 2014; Piantedosi *et al.*, 2016; Bosco *et al.*, 2018). The bilirubin level determined in obesity increases to reduce the increasing oxidative stress load in metabolism and creates an antioxidant effect (Zhong *et al.*, 2017). In our study, the serum total bilirubin level was higher in obese dogs compared to dogs with normal body condition, but it was within the reference range in both groups. Thus, it was concluded that bilirubin could not have a negative effect on MDA, which is a secondary product produced during lipid peroxidation and is considered a biomarker for oxidative stress due to its insufficient antioxidant effect.

Some studies have reported high serum BUN values in obese dogs (Tribuddharatana *et al.*, 2011; Kawasumi *et al.*, 2018). The reasons for the increased serum BUN levels in obesity can be counted as an increased excretory load of the kidney, increased renal sodium retention and lipotoxicity (Bagby, 2014). Our study determined that obese dogs had higher serum BUN levels than those with normal body conditions. The fact that the serum BUN values we obtained were in the reference range showed parallelism with the study by Abinaya *et al.* (2018). These researchers evaluated age-related biochemical parameters in obese dogs and found serum BUN values in the reference range of all obese dogs. In addition, the presented study found a positive correlation between BUN and MDA levels. This situation

raises the opinion that it may explain the possible increases in BUN levels with the increase in oxidative stress.

CONCLUSION

In conclusion, serum ADMA levels in obese dogs were higher than normal body condition group. This difference supports that ADMA may be a diagnostic biomarker in obese dogs. Statistically significant correlations between bilirubin, BUN and MDA will shed light on future studies. Examining routine hemograms and biochemicals with oxidative stress parameters and ADMA in evaluating the health status of obese dogs will provide us with prognostically essential data. However, it may be helpful to assess these parameters in more dogs with optimum conditions, which are obese and in normal body condition. We believe this study will form the basis for future studies on obesity in dogs.

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Conflict of interest

All authors declare that they have no conflict of interest.

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