Subclinical Myocardial Injury in Clinically Healthy Obese Dogs

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ABSTRACT

The aim of this study was to evaluate the relationship between obesity and adipokines and also to determine subclinical myocardial injury by cardiac biomarkers. Twenty-five healthy obese (HO) and 15 healthy normal weight (HN) mixed breed dogs were enrolled in the study. Blood sampling and cardiologic examination were performed in all dogs in order to exclude any diseases. The HO group had higher levels of triglycerides (P<0.005), glucose (P<0.025), and lower levels of creatinine (P<0.010) compared to HN dogs. HO dogs had higher serum leptin (P<0.001) but lower adiponectin concentrations (P<0.009) than HN dogs. Systolic blood pressure (P<0.004) and serum concentration of cardiac troponin T was significantly higher in HO dogs compared to HN dogs (P<0.038). There was no statistical significance in concentrations of blood urea nitrogen, alanine aminotransferase, alkaline phosphatase, albumin, total protein, cholesterol, cardiac troponin I, and N-terminal pro brain natriuretic peptide between HO and HN dogs. The bivariate analysis revealed that triglyceride, leptin and systolic blood pressure had positive (r=0.45, P<0.01; r=0.78, P<0.001; r=0.46, P<0.05, respectively) and adiponectin had negative correlation with body condition score (r=-0.39, P<0.05). Cardiac troponin T had a positive correlation with systolic blood pressure (r=0.56, P<0.01). The findings indicate that unfavorable metabolic and lipid changes in obese dogs and systemic hypertension have harmful effects on the myocardium. Elevation of serum cardiac troponin T in obese dogs compared to healthy dogs could represent a probable subclinical myocardial damage in obese dogs.

Keywords: Cardiac Biomarkers; Hypertension; Metabolic Disorder; Troponin.

INTRODUCTION

Obesity is a common nutritional disorder in dogs and the incidence has been reported to range from 24% to 44% (1). Obesity typically occurs when an animal consumes more calories than expends and has decreased physical activity. As in humans, obesity in dogs is commonly associated with increased risks of diseases, such as diabetes (2), pancreatitis (3), hypothyroidism (4), dyslipidemia (5), osteoarthritis (6), hypertension (7), altered kidney function (8) and respiratory distress (1). In humans, obesity is an independent risk factor for the development of obesity-related cardiac dysfunctions. Various adaptations and changes in the structure and the function of the heart are observed in cases of obesity. In the case of overweight or obesity, various cardiovascular system diseases including coronary heart disease, heart failure, venous insufficiency, venous thrombosis, pulmonary embolism, hypertension, arrhythmia and sudden death can be observed (9, 10). In dogs, obesity can adversely affect the cardiovascular system as reported in humans. Rocchini *et al.* (11) reported that weight gain in dogs causes an increase in heart rate, blood pressure, cardiac volume, plasma volume and fasting insulin concentration. Weight gain causes heart rhythm deterioration and an increase in left ventricular volume, plasma volume and blood pressure (12). In obesity, changes in the morphological

structure of the heart are similar to those of hypertension and eccentric hypertrophy arises as the first morphological change in the left ventricle (13). However, there are a limited number of studies of left ventricular hypertrophy and cardiac functions in obese dogs (14, 15), and no biomarker study of possible cardiac damage has been conducted among clinically healthy obese dogs.

The hypothesis of the present study was to propose that obesity may lead to subclinical myocardial injury in clinically healthy obese dogs. For this purpose, the objective of this study was to determine the relationship between obesity and adipokines, and also hypertension and cardiac biomarkers in obese dogs.

MATERIALS AND METHODS

The study was approved by the ethics committee of the Faculty of Veterinary Medicine, Selcuk University (Permit number: 2017/59).

Study population

Twenty-five clinically healthy obese (HO) and 15 healthy normal weight (HN) client-owned dogs were included in the study among the referral population of the Selcuk University, Veterinary Faculty, Department of Veterinary Internal Medicine, Small Animals Hospital from October 2017 to November 2018. The dogs from different breeds included into the HO (12 Golden Retriever, 3 Beagle, 2 Boxer, 2 Corgi, 1 English Cocker, 1 Labrador Retriever, 1 Pug, 1 Rottweiler, 1 German shepherd dog, 1 Anatolian shepherd dog) and HN (5 Golden Retriever, 5 Anatolian shepherd dogs, 1 Terrier, 1 Rottweiler, 1 Pointer, 1 Arab hound and 1 mixed) groups.

Animals with evidence of pre-existing endocrine diseases (e.g. diabetes mellitus, hypothyroidism, hyperadrenocorticism), hepatic and/or renal failure, congenital or acquired cardiac diseases or any inflammatory/infectious diseases were excluded from the study.

Evaluation of obesity

A nine-point body condition score (BCS) system (16) was utilized and scores were allocated by the same investigator. Dogs with a BCS≥7/9 were considered obese (17). The dogs, which included in the normal weight (HN) group had a BCS of 4-5/9 and were considered to be healthy based on clinical examination and laboratory analyzes.

Systemic blood pressure measurement

Three consecutive measurements of systemic arterial blood pressure were performed using an automated oscillometric system (Compact 7, Medical Econet, Germany) on the left forelimb of conscious dogs in right lateral recumbency. The systolic blood pressure (SBP) and diastolic arterial pressure (DAP) were recorded.

Cardiologic examination

A standard six-lead electrocardiogram (VE-300, Edan, China) was performed in right lateral recumbency and the electrocardiography (ECG) traces were recorded (paper speed: 50 millimeters second (mm/s); calibration at 1 millivolt (mV)=1 centimeter (cm). Transthoracic echocardiography (Apogee 3500V, SIUI, China) was performed only in dogs with suspected heart disease and, excluded from the study when any heart problem was detected.

Sample collection

The animals remained fasting for 12 hours prior to the collection of blood samples. Ten milliliters of blood was collected by vena cephalica venipuncture and placed in tubes without anticoagulant, allowed to clot, and centrifuged at 2000x *g* for 5 minutes at 4°C. After extraction, half of the serum samples were stored at -80°C and defrosted immediately before the enzyme-linked immunosorbent assay (ELISA) analysis. The remaining half used for biochemical analyzes.

Biochemical analyzes

Glucose, blood urea nitrogen (BUN), creatinine, triglycerides, total cholesterol, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), albumin and total protein (TP) concentrations were measured by a semi-automatic biochemical analyzer (BT 3000 plus, Italy).

Adipokines and cardiac biomarkers analyze

Serum leptin, adiponectin, cardiac troponin I (cTnI), cardiac troponin T (cTnT) and N-terminal pro-brain natriuretic peptide (NT-proBNP) levels were measured according to the manufacturer's protocol using a canine leptin commercial sandwich ELISA kit (Lep, MyBioSource, USA), canine adiponectin commercial ELISA kit (Acrp30, MyBioSource, USA), canine cTnI commercial ELISA kit (cTnI, MyBioSource, USA), canine cTnT commercial ELISA kit

(cTnT, MyBioSource, USA) and canine NT-proBNP commercial ELISA kit (NT-proBNP, MyBioSource, USA).

Statistical analysis

One sample Kolmogorov-Smirnov test was used to determine whether the data were parametric or nonparametric. Parametric data were evaluated with Student t-test as mean ± standard deviation (SD) and non-parametric data were evaluated by Mann Whitney U test as median (min/max). For detection of correlation between variables, Spearman correlation test was used. Linear regression analysis was also conducted for cTnT and SBP (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.). Statistical significance was considered as significant at $P<0.05$.

RESULTS

The mean age, body weight (BW) and BCS recorded in the HO dogs (12 males and 13 females) were 4.25±2.51 years, 34.45±13.31 kg and 8.33±0.48, respectively; compared with 4.69±2.01 years, 26.50±6.50 kg and 4.46±0.51, respectively for the HN dogs (5 males; 10 females). The two groups showed a significant difference in BW ($P<0.05$).

Although, SBP was significantly higher in HO than HN (173.81±10.76 mmHg and 130.08±12.24mmHg, respectively) ($P<0.004$), there was no significant difference in DBP (104.09±11.17mmHg and 93.91±26.85mmHg) and heart rate (HR) (146.42±25.30 and 132.72±25.33) between HO and HN groups, respectively ($P>0.05$).

Totally 19 obese dogs were decided not to be considered for further investigation because of mild mitral regurgitation (6=dogs), various types of LV diastolic dysfunction (n=4), pulmonary hypertension (n=1), LV asynchrony (n=1), atrial fibrillation (n=2), supra-ventricular premature complex (n=4) and second degree atrioventricular block (n=1). Although some HO dogs which were included in the study had various types of physiologic dysrhythmias (sinus tachycardia (n=3), sinus arrhythmia (n=8), and sinus bradycardia (n=2)), the echocardiographic indices of the systolic and diastolic functions were within the reference ranges.

The results of serum biochemistry, levels of adipokines, and cardiac biomarkers summarized in (Table 1). The HO group had higher levels of triglycerides ($P<0.005$), glucose ($P<0.025$), and lower levels of creatinine ($P<0.010$) compared to HN dogs. There was no statistically significant difference in the concentration of BUN, ALT, ALP, albumin, TP and total cholesterol among study groups. On the other hand, the higher levels of leptin ($P<0.001$), cTnT ($P<0.038$) and, lower levels of adiponectin ($P<0.009$) were found in HO dogs compared to HN dogs. There was no significant alteration in cTnI and NT-proBNP between the study groups.

The bivariate analysis revealed that triglyceride, leptin and SBP had a positive correlation (r=0.45, $P<0.01$; r=0.78, $P<0.001$; r=0.46, $P<0.05$, respectively) and adiponectin had a negative correlation with the BCS (r=-0.39, $P<0.05$). Cardiac troponin T had a positive correlation with SBP (r=0.56, $P<0.01$) (Table 2). Linear regression analysis revealed a significant equation ($F(1,21)=7.885$, $P<0.011$) with an $R^2=0.273$ between cTnT and SBP (Figure 1).

Linear regression analysis showed a significant slope ($F(1,21)=7.885$, $P<0.011$) with an $R^2=0.273$ between cTnT and SBP. This simple scatter with fit line of cTnT by SBP demonstrated positive significant association between serum levels of cTnT and measured SBP in healthy obese dogs.

DISCUSSION

Obesity in dogs is associated with many diseases such as dyslipidemia, cardiovascular diseases, diabetes mellitus, orthopedic and urinary disorders (12), and most of the time these are insidious. Biochemical analysis and biomarker measurements are beneficial tools for the diagnosis and management of these concurrent diseases with obesity. In this study, some biochemical parameters, adipokines, and cardiac biomarkers of the obese and normal-weight dogs were evaluated.

Hyperlipidemia is one of the most common metabolic disorders observed in obese people and animals (18, 19). In a study conducted on overweight and obese dogs, serum triglycerides, and cholesterol concentrations have been reported to be higher than normal-weight dogs (19). In the present study, serum triglyceride concentration were found to be significantly higher in HO dogs compared to HN and positively correlated with BCS (7). Hypertriglyceridemia observed in our study has also expressed by several researchers (20, 21). Weight gain in dogs is characterized by an increase in serum glucose concentration (22). In dogs, overweight and obesity are considered as a cause of insulin resistance. However, insulin resistance can be seen in dogs without the presence of diabetes mellitus (23). In the present study, serum

Table 1: Results of blood variables. Comparison of selected biochemical, and cardiac biomarker variables in 25 clinically healthy obese dogs and 15 healthy normal weight dogs, with data being expressed as mean ± SD and median (range) in parentheses.

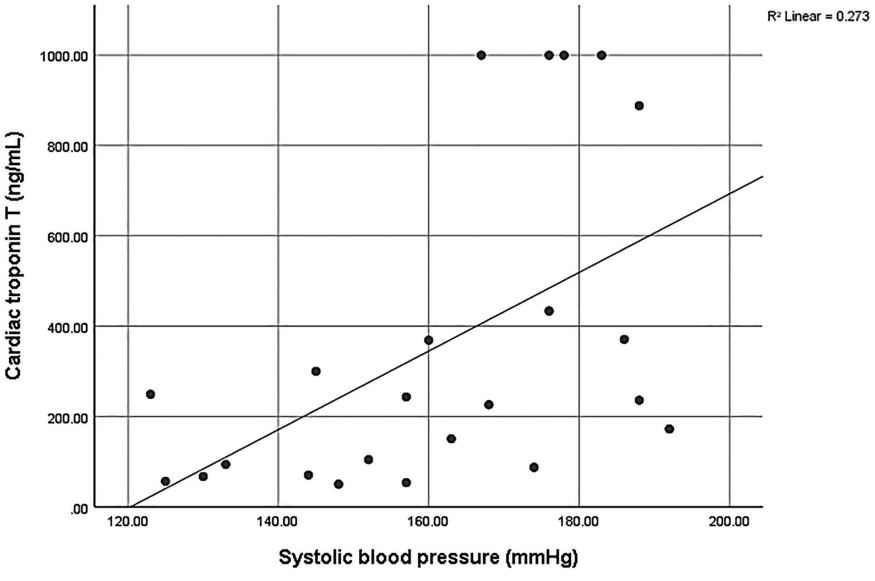
Variables	HN group	HO group	*P* value
BUN (mg/dL)	15.06±6.35	12.12±5.56	0.132
Creatinine (mg/dL)	1.23±0.30	0.99±0.24	0.010
ALT (U/l)	31.26±14.16	41.08±21.73	0.130
ALP (U/l)	65±27.35	58.76±35.55	0.563
Glucose (mg/dL)	90.26±13.44	100.92±14.22	0.025
Albumin (g/dL)	3.48±0.27	3.50±0.35	0.822
Total protein (g/dL)	7±0.63	6.94±0.52	0.765
Cholesterol (mg/dL)	195.20±52.83	210.48±48.38	0.356
Triglyceride (mg/dL)	210±123.8	384±206.63	0.005
Adiponectin (ηg/mL)	12.3 (0.2/55)	3.1 (0/31)	0.009
Leptin (ng/mL)	0.7 (0.1/1.6)	3 (1/8)	0.000
Cardiac troponin T (ηg/mL)	104 (45/172)	236 (23/1000)	0.038
Cardiac troponin I (ηg/mL)	0 (0/6)	0 (0/37)	0.581
NT-proBNP (ηg/mL)	0 (0/40)	0 (0/18)	0.069

Table 2: Correlation statistics. Spearman correlation coefficients of selected metabolic and cardiovascular variables in 25 clinically healthy obese dogs.

Variables	SBP	Triglyceride	Adiponectin	Leptin	cTnT	NT-proBNP	BCS
SBP	1	0.34	-0.14	0.63***	0.56**	-0.32	0.46*
Triglyceride		1	0.13	0.49**	-0.05	-0.19	0.45**
Adiponectin			1	-0.33*	-0.17	0.40**	-0.39*
Leptin				1	0.20	-0.28	0.78***
cTnT					1	-0.08	0.17
NT-proBNP						1	-0.39*
BCS							1

* $P<0.05$. ** $P<0.01$. *** $P<0.001$.

Table 3: Linear regression analysis showed a significant slope (F(1.21)=7.885, $P<0.011$) with an R squared=0.273 between cTnT and SBP. This simple scatter with fit line of cTnT by SBP demonstrated positive significant association between serum levels of cTnT and measured SBP in healthy obese dogs.



glucose concentration was found to be statistically higher in obese dogs compared to normal weight dogs. However, the high glucose concentration in obese dogs was within normal reference values. Increased serum glucose concentration in obese dogs is consistent with the findings of many investigators (19, 22).

Raffa *et al.* (19) showed that creatinine concentration to be low only in obese dogs. In the present study, creatinine was significantly lower in the HO group compared to the HN group. It is widely recognized that creatinine concentrations are related to muscle mass (24). The reduction of creatinine levels in obese dogs may be a result of decreased muscle mass due to increased adipose tissue (25). It has been reported that obesity may lead to an increase in systemic hypertension, glomerular intracapillary pressure, hyperfiltration, glomerular filtration rate in humans and an increase in creatinine clearance (26). This latter finding may be an early indication of renal dysfunction.

Adiponectin and leptin have an important role in the pathophysiology of obesity (27). Park *et al.* (28) and Park *et al.* (29) showed that obese dogs have high levels of serum leptin and low levels of serum adiponectin. Our results showed that in HO dogs, serum leptin levels were higher and serum adiponectin levels were lower compared to HN dogs (Table 1). On the other hand, there was a positive correlation between leptin, triglycerides, and BCS and negative correlation of adiponectin and BCS (Table 2). Previous studies proposed the association of high levels of leptin with adipose tissue mass (30), leptin resistance (28, 29) and blockage of lipogenic effects of insulin (30) and also, lower levels of adiponectin to be associated with adipose tissue mass (27, 29), insulin resistance (31) and reduction in adiponectin gene expression due to release inflammatory mediators from adipose tissue (27).

Systemic hypertension in one of the important complications of obesity in humans (32). Studies in obese dogs clearly show that dogs with higher BCS had a higher risk of systemic hypertension (7, 11). Our results showed that SBP in HO dogs is significantly higher than HN dogs (Table 1). Moreover, there was a positive correlation between leptin and SBP. Piantedosi *et al.* (33) concluded that systemic hypertension in obese dogs is related to higher leptin levels and lower adiponectin levels in circulation.

Other studies demonstrated that some mechanisms such as renin-angiotensin-aldosterone system activation could lead to systemic hypertension in obese and overweight dogs (9). In the cases of obesity-related hypertension, the most affected organ is the heart (34). Morphological changes (eccentric and concentric hypertrophy) as well as microscopic changes (e.g., oxidative stress, fibrosis, and apoptosis) has been reported in both humans and animals (15, 35).

Cardiovascular diseases are commonly encountered in both human and veterinary medicine (36). Cardiac biomarkers are required as parameters that diagnose a cardiac disease with as high sensitivity and specificity as possible whether it is primary or secondary. The reason for the increasing interest in cardiac biomarkers is due to their are convenience, low cost, non-invasive, risk-free, fast turn-around time, efficient and highly specificity and sensitivity (37).

The most specific and commonly used cardiac biomarkers in the veterinary field are cTnT, I, and NT-proBNP (38). Cardiac troponins are specific indicators of cardiomyocyte damage (39). Cardiac damage causes destruction in myocytes resulting in cardiac troponins, a component of the heart muscle, passing into the bloodstream. These biomarkers are undetectable in the blood of healthy animals (36). Our results showed that the serum concentration of cTnT is significantly higher in HO dogs compared to HN dogs. There was no significant change in cTnI and NT-proBNP between HO and HN dogs (Table 1). In addition, there was a positive correlation between cTnT and SBP (Table 2) (Figure 1). Setsuta *et al.* (32) in a study of 176 patients with hypertension showed that most of the patients with hypertension had higher cTnT levels than those with normal blood pressure. Researchers have explained this condition resulting from left ventricular hypertrophy, interstitial fibrosis and apoptosis of the contractile apparatus. Changes in the morphological structure of the heart in obesity (cardiac remodeling) were found to be similar to that of cases of hypertension (13). Piantedosi *et al.* (33) suggested that obesity may be associated with systemic hypertension and left ventricular hypertrophy in dogs.

Cardiac troponin T is known as a specific biomarker of the heart muscle (98% myofibril bound and 2% cytosolic) and released at minimal levels from the skeletal muscles (38, 43). Burgener *et al.* (40) showed that in acute myocardial damage in dogs, high concentrations of serum cTnT demonstrated myocardial injury. Another study of dilated cardiomyopathy (DCM) in dogs also showed that 63% of dogs with DCM had higher levels of cTnT in comparison to dogs in the control group (41). And finally, O'Brien *et al.* (42) demonstrated

that cardiac troponin T is a sensitive and specific biomarker of cardiac injury in laboratory animals. In the present study, the high levels of cTnT in obese dogs can be interpreted by myocardial damage due to systemic hypertension which possibly may be a result of structural damage to the myocardial contractile apparatus and accompanied with releasing cTnT. Ndumele *et al.* (44) showed that obese individuals without any cardiovascular disease history but with high body mass index had significantly higher cTnT than non-obese individuals. They concluded that hypertension and obesity can lead to chronic myocardial damage, myocardial oxidative injury, myocardial apoptosis, and fibrosis, and also, they play important role in the pathogenesis of subclinical myocardial injury and heart failure in the decompensated stage. On the other hand, the cross-reaction between cardiac and skeletal muscular troponin T cannot be ignored (45) and some literature in human medicine suggest that systemic hypertension can lead to myostatin dysregulation and skeletal muscle injury (46). Although cardiac troponin T can be released from skeletal muscles of obese dogs, it seems that those same mechanisms which have been described in the human obesity studies (44) may have a role in obese dogs resulting in the elevation of serum concentration of cTnT, possibly indicating myocardial damage and subclinical myocardial injury in healthy obese dogs.

The results of the present study suggest that obesity and high systemic blood pressure are the most important risk factors of cardiovascular dysfunction in dogs. In conclusion, the results of our study revealed that due to changes in the metabolic and lipid status of obese dogs, high systemic blood pressure may be present. The high systemic blood pressure has harmful effects on myocardium and elevation of serum cardiac troponin T due to myocardial damage in obese dogs if not diagnosed and treated may lead to the heart failure in the future.

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REFERENCES

1. Markwell, P.J., Butterwick, R.F., Wills, J.M. and Raiha, M.: Clinical studies in the management of obesity in dogs and cats. Int. J. Obes. Relat. Metab. Disord. 18: 39–43, 1994.
2. Gayet, C., Baihache, E., Dumon, H., Martin, L., Siliart, B. and Nguyen, P.: Insulin resistance and changes in plasma concentrations of TNFa, IGF1, and NEFA in dogs during weight gain and obesity. J. Anim. Physiol. Anim. Nutr. 88: 157-165, 2004.
3. Hess, R.S., Kass, P.H., Shofer, F.S., Van, T.W.and Washabau, R.J.: Evaluation of risk factors for fatal acute pancreatitis in dogs. J. Am. Vet. Med. Assoc. 214: 46-51, 1999.
4. David, L.P.: Conditions associated with canine hypothyroidism. Vet. Clin. North. Am. Small. Anim. Pract. 31: 935-950, 2001.
5. Jeusette, I.C., Lhoest, E.T., Istasse, L.P. and Diez, M.O.: Influence of obesity on plasma lipid and lipoprotein concentrations in dogs. Am. J. Vet. Res. 66: 81-86, 2005.
6. Impellizeri, J.A., Tetrick, M.A. and Muir, P.: Effect of weight reduction on clinical signs of lameness in dogs with hip osteoarthritis. J. Am. Vet. Med. Assoc. 216: 1089-1091, 2000.
7. José Lahm Cardoso, M., Fagnani, R., Zaghi Cavalcante, C., de Souza Zanutto, M., Júnior, A.Z., Holsback da Silveira Fertonani, L., Calesso, J.R., Melussi, M., Pinheiro Costa, H. and Yudi Hashizume, E.: Blood pressure, serum glucose, cholesterol, and triglycerides in dogs with different body scores. Vet. Med. Int. 1: 7, 2016.
8. Henegar, J.R., Bigler, S.A., Henegar, L.K., Tyagi, S.C. and Hall, J.E.: Functional and structural changes in the kidney in the early stages of obesity. J. Am. Soc. Nephrol. 12: 1211-1217, 2001.
9. Rahmouni, K., Correia, M.L., Haynes, W.G. and Mark, A.L.: Obesity-associated hypertension: new insights into mechanisms. Hypertension. 45: 9-14, 2005.
10. Poirier, P., Giles, T.D., Bray, G.A., Hong, Y., Stern, J.S., Pi-Sunyer, F.X. and Eckel, R.H.: Obesity and cardiovascular disease: pathophysiology, evaluation, and effect of weight loss. Circulation. 113: 898-918, 2006.
11. Rocchini, A.P., Moorehead, C., Wentz, E. and Deremer, S.: Obesity-induced hypertension in the dog. Hypertension., 9: III64, 1987.
12. German, A.J.: The Growing Problem of Obesity in Dogs and Cats. The WALTHAM International Nutritional Sciences Symposia. 136: 1940-1946, 2006.
13. Ganau, A., Devereux, R.B., Roman, M.J., De Simone, G., Pickering, T.G., Saba, P.S., Vargiu, P., Simongini, I. and Laragh, J.H.: Patterns of left ventricular hypertrophy and geometric remodeling in essential hypertension. J. Am. Coll. Cardio. 19: 1550-1558, 1992.
14. Tropf, M., Nelson, O.L., Lee, P.M. and Weng, H.Y.: Cardiac and metabolic variables in obese dogs. J. Vet. Intern. Med. 31: 1000-1007, 2017.
15. Mehlman, E., Bright, J.M., Jeckel, K., Porsche, C., Veeramachaneni, D.N.R. and Frye, M.: Echocardiographic evidence of left ventricular hypertrophy in obese dogs. J. Vet. Intern. Med. 27: 62-68, 2013.
16. Laflamme, D.R.P.C.: Development and validation of a body condition score system for dogs: a clinical tool. Canine. Pract. 22: 10-15, 1997.
17. Tvarijonaviciute, A., Ceron, J.J., Holden, S.L., Cuthbertson, D.J., Biourge, V., Morris, P.J. and German, A.J.: Obesity-related metabolic dysfunction in dogs: a comparison with human metabolic syndrome. BMC. Vet. Res. 8: 147, 2012.
18. De Godoy, M.R.C. and Swanson, K.S.: Companion Animals Symposium: Nutrigenomics: Using gene expression and molecular biology data to understand pet obesity 1. J. Anim. Sci. 91: 2949-2964, 2013.

19. Rafaj, R.B., Kuleš, J., Marinculić, A., Tvarijonaviciute, A., Ceron, J., Mihaljević, Ž. and Mrljak, V.: Plasma markers of inflammation and hemostatic and endothelial activity in naturally overweight and obese dogs. BMC. Vet. Res. 13: 13, 2016.
20. Comazzi, S., Pieralisi, C. and Bertazzolo, W.: Haematological and biochemical abnormalities in canine blood: frequency and associations in 1022 samples. J. Small. Anim. Pract. 45: 343-349, 2004.
21. Xenoulis, P.G. and Steiner, J.M.: Lipid metabolism and hyperlipidemia in dogs. Vet. J. 183: 12-21, 2010.
22. Peña, C., Suarez, L., Bautista-Castaño, I., Juste, M.C., Carretón, E. and Montoya-Alonso, J.A.: Effects of low-fat high-fibre diet and mitratapide on body weight reduction, blood pressure and metabolic parameters in obese dogs. J. Vet. Med. Sci. 76: 1305-1308, 2014.
23. Verkest, K.R., Rose, F.J. and Fleeman, L.M.: Adiposity and adiponectin in dogs: Investigation of causes of discrepant results between two studies. Domest. Anim. Endocrinol. 41: 35–41, 2011.
24. Hjelmesæth, J., Røislien, J., Nordstrand, N., Hofsø, D., Hager, H. and Hartmann, A.: Low serum creatinine is associated with type 2 diabetes in morbidly obese women and men: a cross-sectional study. BMC. Endocr. Disord. 10: 6, 2010.
25. Thomas, G., Sehgal, A.R., Kashyap, S.R., Srinivas, T.R. and Kirwan, J.P., Navaneethan, S.D.: Metabolic syndrome and kidney disease: A systematic review and meta-analysis. Clin. J. Am. Soc. Nephrol. 6: 2364–2373, 2011.
26. Gerchman, F., Tong, J., Utzschneider, K.M., Zraika, S., Udayasankar, J., McNeely, M.J. and Boyko, E.J.: Body mass index is associated with increased creatinine clearance by a mechanism independent of body fat distribution. J. Clin. Endocrinol. Metab. 94: 3781-3788, 2009.
27. Radin, M.J., Sharkey, L.C. and Holycross, B.J.: Adipokines: a review of biological and analytical principles and an update in dogs, cats, and horses. Vet. Clin. Pathol. 38: 136–156, 2009.
28. Park, H.J., Lee, S.E., Oh, J.H., Seo, K.W. and Song, K.H.: Leptin, adiponectin and serotonin levels in lean and obese dogs. BMC. Vet. Res. 10: 113, 2014.
29. Park, H.J., Lee, S.E., Kim, H.B., Isaacson, R.E., Seo, K.W. and Song, K.H.: Association of obesity with serum leptin, adiponectin, and serotonin and gut microflora in beagle dogs. J. Vet. Intern. Med. 29: 43-50, 2015.
30. Lago, F., Gómez, R., Gómez-Reino, J.J., Dieguez, C. and Gualillo, O.: Adipokines as novel modulators of lipid metabolism. Trends. Biochem. Sci. 34: 500-510, 2009.
31. Hotta, K., Funahashi, T. and Bodkin, N.L.: Circulating concentrations of the adipocyte protein adiponectin are decreased in parallel with reduced insulin sensitivity during the progression to type 2 diabetes in rhesus monkeys. Diabetes. 50: 1126–1133, 2001.
32. Setsuta, K., Kitahara, Y., Arae, M., Ohbayashi, T., Seino, Y. and Mizuno, K.: Elevated cardiac troponin T predicts adverse outcomes in hypertensive patients. Int. Heart. J. 52: 164-169, 2016.
33. Piantedosi, D., Di Loria, A., Guccione, J., De Rosa, A., Fabbri, S., Cortese, L., Carta, S. and Ciaramella, P.: Serum biochemistry profile, inflammatory cytokines, adipokines and cardiovascular findings in obese dogs. Vet. J. 216: 72-78, 2016.
34. Lawler, P.R., Hiremath, P. and Cheng, S.: Cardiac target organ damage in hypertension: Insights from epidemiology. Curr. Hypertens. Rep. 16: 446, 2014.
35. Abel, E.D., Litwin, S.E. and Sweeney, G.: Cardiac remodeling in obesity. Physiol. Rev. 88: 389-419, 2008.
36. Wells, S.M. and Sleeper, M.: Cardiac troponins. J. Vet. Emerg. Crit. Care. 18: 235-245, 2008.
37. Drobatz, K.J.: Measures of accuracy and performance of diagnostic tests. J. Vet. Cardiol. 11: 33-40, 2009.
38. Langhorn, R. and Willesen, J.L.: Cardiac troponins in dogs and cats. J. Vet. Intern. Med. 30: 36-50, 2016.
39. Liquori, M.E., Christenson, R.H., Collinson, P.O. and Defilippi, C.R.: Cardiac biomarkers in heart failure. Clin. Chem. 47: 327-337, 2014.
40. Burgener, I.A., Kovacevic, A., Mauldin, G.N. and Lombard, C.W.: Cardiac troponins as indicators of acute myocardial damage in dogs. J. Vet. Intern. Med. 20: 277-283, 2006.
41. Cakiroglu, D., Meral, Y., Bakirel, U. and Kazanci, D.: Cardiac troponin levels in dogs with dilate cardiomyopathy. Kafkas. Univ. Vet. Fak. Derg. 15: 13-17, 2009.
42. O'brien, P.J., Dameron, G.W., Beck, M.L., Kang, Y.J., Erickson, B.K., Di Battista, T.H., Miller, K.E., Jackson, K.N. and Mittelstadt, S.: Cardiac troponin T is a sensitive, specific biomarker of cardiac injury in laboratory animals. Comp. Med. 47: 486-495, 1997.
43. Voss, E.M., Sharkey, S.W., Gernert, A.E., Murakami, M.M., Johnston, R.B., Hsieh, C.C. and Apple, F.S.: Human and canine cardiac troponin T and creatine kinase-MB distribution in normal and diseased myocardium. Infarct sizing using serum profiles. Arch. Pathol. Lab. Med. 119: 799-806, 1995.
44. Ndumele, C.E., Coresh, J., Lazo, M., Hoogeveen, R.C., Blumenthal, R.S., Folsom, A.R., Selvin, E., Ballantyne, C.M. and Nambi, V.: Obesity, subclinical myocardial injury, and incident heart failure. JACC. Heart. Fail. 2: 600-607, 2014.
45. Tarkowska, A. and Furmaga-Jabłońska, W.: The Evaluation of Cardiac Troponin T in Newborns. Biomed. Hub. 2: 1-7, 2017.
46. Butcher, J.T., Mintz, J.D., Larion, S., Qiu, S., Ruan, L., Fulton, D.J. and Stepp, D.W.: Increased muscle mass protects against hypertension and renal injury in obesity. J. Am. Heart. Assoc. 7: e009358, 2018.