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Serum biochemistry profile, inflammatory cytokines, adipokines and cardiovascular findings in obese dogs

Diego Piantedosi [a], Antonio Di Loria [b], Jacopo Guccione [a,*], Angela De Rosa [a], Silvia Fabbri [a], Laura Cortese [a], Sergio Carta [a], Paolo Ciaramella [a]

[a] Department of Veterinary Medicine and Animal Productions, University of Naples 'Federico II', via F. Delpino 1, 80137 Naples, Italy
[b] Department of Health Sciences, University Magna Græcia of Catanzaro, viale Europa-Germaneto, 88100 Catanzaro, Italy

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ABSTRACT

The aim of this study was to evaluate the serum biochemistry profile, inflammatory cytokines, adipokines and cardiovascular findings in obese dogs. Twenty obese and 20 normal weight healthy pet dogs were recruited into the study, where they underwent blood testing and assessment of cardiovascular function (blood pressure analysis, electrocardiography and echocardiography). Higher concentrations of total cholesterol, triglycerides, lactate dehydrogenase, total serum proteins, α-globulins, total bilirubin, insulin, insulin:glucose ratio, alkaline phosphate and alanine aminotransferase were observed in obese dogs than dogs of normal weight. There were no differences in concentrations of tumour necrosis factor (TNF)-α or interleukin (IL)-6 between the two groups. Obese dogs had higher serum leptin but lower adiponectin concentrations than dogs of normal weight. Systolic arterial blood pressure was higher in obese dogs than dogs of normal weight. The values for the thickness of the free wall of the left ventricle and interventricular septal thickness were greater at end-diastole in obese dogs compared to dogs of normal weight. Four of 20 obese dogs were determined to have obesity-related metabolic dysfunction (ORMD). The findings indicate that a chronic inflammatory state is not necessarily evident in obese dogs, as has been described in human beings, and the criteria used for ORMD can be used to define this syndrome in dogs. In this study, canine obesity was associated with cardiac and vascular dysfunction.

Introduction

Obesity is the most common nutritional disorder in the pet dog population, whereby animals are considered to be clinically obese when their body weight (BW) exceeds the optimum by at least 15% (Laflamme, 2001). A high prevalence of canine obesity (25–52%) has been reported in developed countries, where lifestyle, diet and lack of exercise can contribute to dogs becoming overweight (Robertson, 2003; McGreevy et al., 2005; Colliard et al., 2006; Gossellin et al., 2007).

Obesity is a metabolic disorder, representing an imbalance between energy intake and energy expenditure. In human beings, this is typically associated with the presence of low grade chronic inflammation, characterised by dysregulation of a network of inflammatory signalling pathways, abnormal cytokine production and increased concentrations of circulating acute phase proteins (Maury and Brichard, 2010). This persistent inflammatory state, co-existing with insulin resistance, is associated with a number of adverse outcomes, including type 2 diabetes, cardiovascular disease and increased susceptibility to neoplastic disease (Matarese et al., 2010).

Similar to the situation in humans, canine obesity can predispose to or exacerbate several clinical conditions, such as osteoarthritis and respiratory disease, as well as contribute to reduced longevity, increased surgical risk and exercise intolerance (Impellizeri et al., 2000; Kealy et al., 2002; Bach et al., 2007). Although the relationship between obesity and some of these conditions might be explained by the mechanical/physical effects of the deposition of excess fat tissue, associations with metabolic dysfunction, chronic inflammation and cardiovascular disease are less clear in veterinary medicine. Whereas German et al. (2009) described an increase in insulin resistance in obese animals and a reduction of plasma inflammatory cytokines and adipokines following initiation of a weight loss programme, Tvarijonaviciute et al. (2012a) reported that inflammatory biomarkers (such as acute phase proteins) remained within the reference range in obese dogs before and after weight loss. On the basis of a modification to the diagnostic criteria specified by the International Diabetes Federation, Tvarijonaviciute et al. (2012b) classified around 20% of obese dogs with obesity-related metabolic dysfunction (ORMD), with hyperinsulinaemia and reduced concentrations of adiponectin. In a study of obese and lean dogs, leptin, triglycerides and cholesterol were positively correlated with

* Corresponding author.
E-mail address: jacopo.guccione@unina.it (J. Guccione).

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body condition score (BCS), whilst a negative correlation was observed for adiponectin and serotonin (Park et al., 2014).

There is limited published literature regarding the effects of obesity on the cardiovascular system in dogs. Mehlman et al. (2013) reported an increased systolic blood pressure and left ventricular free wall thickness at end-diastole and end-systole in a small number of obese dogs. In a retrospective study, hypertension was not related to obesity in dogs (Pérez-Sánchez et al., 2015). The aim of the present study was to evaluate the biochemical profile, inflammatory cytokines and adipokines in the blood, as well as assessing cardiovascular parameters in obese dogs.

Materials and methods

Study population

Twenty obese (OB) and 20 normal weight (NW) healthy dogs were recruited into the study from the client-owned referral population of the Veterinary Teaching Hospital, Department of Veterinary Medicine and Animal Productions, University of Naples 'Federico II', Naples, from October 2014 to February 2015 (approved by the Ethical Animal Care and Use Committee; date of approval: 26 June 2014). All dogs were of mixed breed and medium size. Animals with evidence of pre-existing endocrine diseases (e.g. diabetes mellitus, hypothyroidism, hyperadrenocorticism), hepatic and/or renal failure, congenital or acquired cardiac diseases, inflammatory/infectious diseases, systemic hypertension or pulmonary arterial hypertension, as well as physiological conditions (e.g. pregnancy or lactation) were excluded from the study. A nine-point body condition score (BCS) system (Laflamme, 1997) was employed and scores were allocated by the same investigator. Dogs with a BCS ≥7/9 were considered to be obese (Tvarijonaviciute et al., 2012b). The dogs enrolled into the NW group had a BCS of 5/9 and were considered to be healthy on the basis of clinical examination, complete blood count (CBC) and serum biochemistry.

Sample collection

Ten millilitres of blood were collected by jugular venepuncture after 12 h of fasting. The first drops of blood were immediately taken for blood gas analysis, whilst the remaining amount was divided into three fractions. The first fraction was placed in tubes containing potassium ethylene diamine tetra-acetic acid (EDTA) for a CBC; the second was placed in tubes without anticoagulant, allowed to clot and centrifuged at 908 *g* for 15 min at 4 °C, while the third fraction was placed in tubes containing lithium heparin anticoagulant and centrifuged at 327 *g* for 10 min at 4 °C. Serum and plasma samples were stored at –80 °C and defrosted immediately before batch analysis. Urine samples were collected by cystocentesis and stored at –80 °C until analysis.

Complete blood count and serum biochemistry

CBCs were performed using a semi-automatic cell counter (Genius S, SEAC Radom Group). Venous blood gas analysis, including pH, partial pressure of carbon dioxide (pCO_2), partial pressure of oxygen (pO_2), base excess of the extracellular fluid (BEecf), bicarbonate or alkaline reserve (HCO_3), total content of carbon dioxide (TCO_2) and total oxygen saturation (SaO_2), was obtained using an automatic blood gas-analyser (i-STAT, Abbott).

A semi-automatic chemical chemistry analyser (OLOT, Spinreact) was used to analyse concentrations or activities of glucose, blood urea nitrogen (BUN), creatinine, triglycerides, total cholesterol, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphate (ALP), total bilirubin (T-Bil), lactate dehydrogenase (LDH), sodium, potassium, albumin and total serum proteins (TP). Serum protein electrophoresis was also performed. Plasma insulin measurements were carried out in duplicate for each sample using a commercial ELISA kit (Canine insulin ELISA, Mercodia AB), validated by Öberg et al. (2011). The insulin:glucose ratio (I:G) was calculated as the plasma insulin (μU/mL) × 100/plasma glucose (mg/dL), according to Bailhache et al. (2003). The urinary protein:creatinine ratio (UP:C) was calculated after their spectrophotometric determination (OLOT, Spinreact).

Inflammatory cytokines and adipokines

Tumour necrosis factor (TNF)-α and interleukin (IL)-6 concentrations were measured in serum samples in duplicate using commercial canine cytokine ELISA kits (Quantikine HS, R&D Systems) (Hofer et al., 2011; Paim et al., 2013; Park et al., 2015). Serum adiponectin and leptin concentrations were measured in duplicate using commercial canine ELISA kits (Millipore), validated by Tvarijonaviciute et al. (2010, 2011).

Systemic blood pressure

Five consecutive measurements of systemic arterial blood pressure were performed using an automated oscillometric system (HDO, S + B MedVet) on the right forelimb of conscious dogs, performed by the same operator. The highest and lowest values of systolic, mean and diastolic arterial blood pressure were excluded, and the mean of the remaining three measurements was recorded. Dogs with systolic arterial blood pressure (SABP) >160 mmHg were considered to be hypertensive (Brown et al., 2007).

Electrocardiography and echocardiography

A standard six-lead electrocardiogram (ECG model 08SD, BTL Italia) was performed with dogs in right lateral recumbency. For each dog, a 2 min strip (paper speed: 50 mm/s; calibration at 1 mV = 1 cm) was recorded. An echocardiographic examination was undertaken for each dog, including transthoracic two-dimensional, M-mode, spectral and colour flow Doppler (Mylab 50, Esaote). The echocardiographic study was performed by the same investigator according to methodologies and reference ranges reported previously (Sahn et al., 1978; Bonagura, 1983; Thomas, 1984; Shober and Fuentes, 2001; Hansson et al., 2002). For each measurement, mean values derived from three consecutive cardiac cycles were considered. In the OB group, monodimensional measurements of the left ventricle were compared with those of the NW group and with the reference ranges, based on the actual weight in dogs (Goncalves et al., 2002).

Criteria for obesity-related metabolic dysfunction (ORMD)

On the basis of the guidelines suggested by Tvarijonaviciute et al. (2012b), dogs were considered to be affected by ORMD when BCS was ≥7/9 and at least two of the following parameters were present: triglycerides >200 mg/dL, total cholesterol >300 mg/dL, glucose >100 mg/dL and SABP >160 mmHg.

Statistical analysis

The data were assessed by the Shapiro–Wilk test to test for normality and where necessary logarithmic transformation was performed to compare the OB and NW groups using Student's *t* test (SPSS version 20.0, IBM). *P* values < 0.05 were considered to be significant.

Results

The mean age, BW and BCS recorded in the OB group (four males, two of which were spayed and 16 females, 15 of which were spayed) were 8 ± 2.6 years, 20.8 ± 10.5 kg and 7.4 ± 0.7, respectively; compared with 7.5 ± 0.5 years, 13.6 ± 6.4 kg and 5 ± 0, respectively for the NW group (six entire males; 14 females, 10 of which were spayed). The two groups showed a significant difference in BW ($P < 0.01$). No significant difference for BW was seen with respect to sex. In the OB group, 18 dogs were fed a mixed diet (commercial and home-made food), while two dogs were fed a commercial diet, with 17 dogs in this group also receiving snacks or treats; in this group, 14 dogs had a BCS of 7, three had a BCS of 8 and three had a BCS of 9. In the NW group, six dogs were fed a mixed diet, 13 were receiving a commercial diet and one dog a home-made diet. Three NW dogs also received snacks or treats. The amount of daily food was divided into two meals for the majority of dogs in both groups.

The results of metabolic profiling of blood samples are summarised in Table 1. The OB group had higher values than the NW group for total cholesterol, triglycerides, LDH, TP and α2-globulins ($P < 0.01$), as well as total bilirubin, insulin, I:G, ALP, ALT and α1-globulins ($P < 0.05$). The values for ALT (5/20 dogs), ALP (7/20 dogs), LDH (12/20 dogs) and T-Bil (2/20 dogs) were above the upper limit of the reference range in the OB group. There were no significant differences in haematological values, venous blood gas results and UP:C values between groups.

There were no significant differences between groups in serum TNF-α concentrations (mean ± SD: OB group 0.11 ± 0.01 pg/mL; NW group 0.10 ± 0.01 pg/mL; Fig. 1A) or serum IL-6 concentrations (mean ± SD: OB group 0.10 ± 0.12 pg/mL; NW group 0.08 ± 0.03 pg/mL; Fig. 1B). Serum TNF-α was undetectable in three NW dogs, while IL-6 was below the limit of detection in three OB dogs. Elevated serum leptin concentrations were significantly higher in the OB group (12.19 ± 6.36 ng/mL) than the NW group (5.80 ± 2.88 ng/mL; $P < 0.01$; Fig. 2A), whereas adiponectin values