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Canine obesity, overweight, and adipokine serum concentration are associated with hematological, biochemical, hormonal, and cardiovascular markers

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ABSTRACT

Background: Canine obesity is an increasingly concerning issue that negatively impacts dogs' health, quality of life, and lifespan.

Aim: This study aimed to evaluate the adipokine profiles of overweight (OW) and obese (OB) dogs and their associations with various hematological, biochemical, hormonal, and cardiovascular markers.

Methods: A total of 84 dogs were classified according to their body condition scores as normal weight (NW), OW, or OB, and were subsequently subjected to blood pressure measurement, blood testing, and urine sampling.

Results: The findings revealed that OB dogs had higher serum concentrations of leptin and resistin while exhibiting lower levels of adiponectin when compared to NW dogs. Additionally, they showed higher systolic blood pressure. Serum creatinine levels were lower in OB dogs, and urinary specific gravity was reduced in both OW and OB dogs compared to their NW counterparts. Furthermore, total leukocyte counts and neutrophil counts were elevated in OW and OB dogs. The study also found that serum insulin levels were positively correlated with triglycerides, cholesterol, and C-reactive protein.

Conclusion: Canine obesity is reflected in altered adipokine concentrations and is associated with insulin resistance, as well as changes in renal function, protein metabolism, and hematological markers.

Keywords: Adipokines, Dog, Insulin resistance, Obesity, Resistin.

Introduction

Obesity is defined as the excessive accumulation of adipose tissue, influenced by environmental, neuroendocrine, hereditary, and individual factors such as: age, sex, breed, neutering status, and comorbidities (Mao *et al.*, 2013; Muñoz-Prieto *et al.*, 2019). A dog is classified as obese (OB) or overweight (OW) when its body fat percentage is 40% or higher, or between 20% and 40%, respectively (Pérez-Sánchez *et al.*, 2015). Similarly to human medicine, obesity is an increasingly common disorder in dogs, with a global prevalence estimated at between 32% and 59% (McGreevy *et al.*, 2005; Lund *et al*., 2006; Courcier *et al.*, 2010; Mao *et al.*, 2013; Usui *et al.*, 2016; Montoya-Alonso *et al.*, 2017; Porsani *et al.*, 2020).

The excessive accumulation of adipose tissue is associated with various pathological conditions, including cardiovascular, respiratory, orthopedic, reproductive, and neoplastic diseases (Zoran, 2010), as well as obesity-related metabolic disorders, which encompass a group of risk factors linked to hyperinsulinemia and hypoadiponectinemia (Tvarijonaviciute *et al.*, 2012). The relationship between obesity and obesity-related diseases, particularly metabolic disorders, appears to hinge on adipokines (Piantedosi *et al.*, 2016). The most studied adipokine in both human and veterinary medicine is leptin, whose plasma levels rise in proportion to the mass of adipose tissue (Radin *et al.*, 2009).

In contrast, while the dynamics of certain serum adipokines, such as adiponectin and resistin, are well described in human and murine models during obesity (de Oliveira Leal and Mafra, 2013), the understanding of these dynamics in veterinary medicine remains incomplete. Some studies have indicated that serum adiponectin concentrations are lower in OB dogs compared to lean ones (Piantedosi *et al.*, 2016; Tropf *et al.*, 2017; Muñoz-Prieto *et al.*, 2020), while others suggest that obesity does not significantly affect serum adiponectin levels (Verkest *et al.*, 2011; Mori *et al.*, 2013).

Although elevated serum resistin levels in OB humans have been linked to atherosclerosis, pancreatitis, insulin resistance, and type II diabetes mellitus, there are limited reports on the relationship between serum resistin and obesity in dogs, with some studies

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showing no significant impact of obesity on resistin concentrations in canines (Eirmann *et al.*, 2009; Kleine *et al.*, 2020).

This dysregulation in adipokine production associated with obesity is accompanied by insulin resistance (de Marchi *et al.*, 2020; Ramos and Castillo, 2020) and is linked to various hematological (Safadi *et al.*, 2021; Vieira *et al.*, 2022), biochemical (Barić Rafaj *et al.*, 2016; Piantedosi *et al.*, 2016; Ramos and Castillo, 2020), hormonal (Lee *et al.*, 2014; Cihan and Tural, 2019; Ramos and Castillo, 2020), and cardiovascular alterations (Piantedosi *et al.*, 2016; de Marchi *et al.*, 2020). To our knowledge, there have not been observational reports associating obesity with urinalysis in veterinary medicine concerning dogs. Furthermore, most studies do not take an integrative approach, particularly regarding urinalysis and hematology, which are essential for understanding the impact of obesity on tissues and organs. Therefore, this study aims to clarify the serum adipokine profile in OB and OW dogs, along with its associations with hematological, biochemical, hormonal, and cardiovascular parameters.

Materials and Methods

Inclusion and exclusion criteria

Dogs aged between 1.5 and 10 years, regardless of gender or breed, were recruited for this study. Only healthy dogs (besides obesity) were included, and all dogs underwent a clinical examination, along with hematological tests, blood chemistry analysis, urinalysis, and a hormone panel assessing thyroid and adrenal function. Dogs that showed poor clinical examination results or blood values indicative of other health issues, aside from obesity, were excluded from the study.

Groups conformation

Ninety dogs were initially recruited for the study; however, six were excluded due to comorbidities. This included five dogs that had thyroid test results indicating hypothyroidism and one dog that showed clinical signs consistent with hypercortisolism.

Among the 84 dogs that remained in the study, they were classified using the nine-point body condition score (BCS) chart developed by Laflamme (1997). The classifications were as follows: normal weight (NW; BCS 4–5; $n = 33$), (OW; BCS 6–7; $n = 28$), and (OB; BCS 8–9; $n = 23$). The dog's weights were measured using a Kretz Vet Scale model 150K4AOBC7RAR from Santa Fe, Argentina.

Cardiovascular evaluation

Systolic blood pressure (SBP), diastolic blood pressure (DBP), mean blood pressure (MBP), and cardiac frequency (CF) were assessed using an oscillometric monitor (Suntech Vet 30), following the methodology outlined by Acierno *et al*. (2018). Patients with a SBP greater than 160 mm Hg were classified as hypertensive.

Sampling

Blood samples were collected after a 12-hour fasting period, always in the morning hours. The samples were divided into three tubes: the first portion was placed in an Ethylenediaminetetraacetic acid (EDTA)-Sodium fluoride tube for glycemia analysis, the second portion was placed in a K3-EDTA tube for hematological studies, and the last portion was stored in a tube without anticoagulant for serum collection after clotting and centrifugation at 3,500 rpm for 10 minutes. The serum was then divided into two fractions; the first was used immediately for biochemical analysis, while the second fraction was frozen at −80°C for hormone processing. First morning urine samples were collected via free catch and stored in designated containers, refrigerated until processing.

Adipokines and cytokines

Leptin and resistin concentrations were measured using canine-specific ELISA kits (EZCL-31K and RAB1017, respectively; Millipore, Saint Louis, USA). Adiponectin levels were assessed using a high-sensitivity human adiponectin ELISA kit (RD191023100; BioVendor R&D, Brno, Czech Republic), which has been previously validated for use in dogs (Tvarijonaviciute *et al.*, 2010). Serum concentrations of TNF-α and IL-6 were determined using canine-specific Quantikine ELISA kits (CA6000 and CATA00, respectively; R and D Systems Inc., Minneapolis, MN). All kits were utilized according to the manufacturer's guidelines, ensuring that the intra-assay coefficient of variation (CV) was less than 10% in all cases.

Hormones

Insulin concentrations were measured by radiometric assays (IRMA), using the INS-IRMA kit (DIA Source Immune Assays S.A., Belgium). Serum total T4 (TT4), free T4 (FT4), TSH, and cortisol concentrations were determined through solid-phase competitive chemiluminescent enzyme immunoassay (IMMULITE 1000), using commercial kits (Siemens, Diagnostic Product Corporation, Los Angeles, CA). The intra-assay and inter-assay CV was less than 10% for all cases.

Hematology

Blood counts were conducted using a Mythic 18 Vet automated system (Orphée, Geneva, Switzerland), with reagents and controls sourced from the same supplier. Subsequently, May-Grunwald Giemsa staining was performed to evaluate the blood smear, utilizing a Nikon Eclipse E100 optical microscope.

Biochemistry and protein electrophoresis

Serum biochemistry Serum biochemistry analysis was conducted using the CB350i automated equipment (Wiener Lab Group, Rosario, Argentina). This analysis utilized commercial reagents and controls obtained from the same supplier. The following parameters were measured: glycemia, triglycerides, alanine aminotransferase, aspartate aminotransferase, serum alkaline phosphatase, total protein, albumin, globulins,

total cholesterol, High density lipoprotein (HDL) cholesterol, Low density lipoprotein (LDL) cholesterol, total bilirubin, urea, and creatinine. C-reactive protein (CRP) levels were determined using a canine-specific dry-chemistry assay (FUJI DRI CHEM SLIDE vc-CRP, Fujifilm, Japan). Protein electrophoresis was performed with an automated capillary electrophoresis instrument (Minicap, Sebia, Barcelona, Spain). For all metabolites measured, the CV for the controls used were less than 10%.

Urinalysis

The chemical properties of urine were assessed using Human Combina s11 test strips, following the manufacturer's instructions. For the evaluation of physical properties, urine specific gravity (USG) was measured using a refractometer. Additionally, turbidity and color were assessed. To obtain the urinary sediment, the sample was centrifuged at 2,000 rpm for 5 minutes, and the sediment was examined using a Nikon Eclipse optical microscope.

Proxies

The Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) and Homeostasis Model Assessment of β-cell Function (HOMA-β) indices were calculated using the HOMA calculator software version 2.2.3 (Oxford University).

Statistical analysis

Normality was assessed using the Kolmogorov-Smirnov test through the PROC UNIVARIATE procedure in the Statistical Analysis System (SAS). Non-normally distributed data were log-transformed for further analysis. The mixed procedure (SAS Studio, Version 3.8, Enterprise Edition) was used for data analysis, incorporating the body condition group as a fixed effect. Age category, sex, and gonadal status were examined as covariates and retained in the model if they were statistically significant. The weight of the study population was adjusted according to the distance from the ground to the scapular-humeral joint. Sex proportions were analyzed using a chi-square test. Associations between variables were evaluated using Pearson's correlation coefficient, employing the CORR procedure (PROC CORR, SAS). A p-value of less than 0.05 was considered statistically significant, while p-values ranging from 0.05 to 0.10 were regarded as indicative of a trend.

Ethical approval

The experimental methodology was approved by the ethics committee (CHEA file number 953/19).

Results

Study population

Regarding age, the means of the NW, OW, and OB groups were 6.3 ± 0.5, 6.9 ± 0.5, and 6.6 ± 0.6 years, respectively, with no differences between groups. The proportion of male/female in the NW group was 12/21, while that of the OW group was 10/18, and that of the OB group was 11/12, with no differences between the proportions of each group ($p = 0.6$). Most of the dogs (53.5%, $n = 45$) were mixed breed, followed by Golden Retriever (10.6%, $n = 9$), Labrador Retriever (9.5%, $n = 8$), German Shepherd and French Bull Dog (4.8%, $n = 4$ each), Yorkshire Terrier, Rottweiler, Poodle and Beagle (2.4%, $n = 2$ each), Pitbull, Pug, Border Collie, Basset Hound, Saint Bernard and Boxer (1.2%, $n = 1$ each). Regarding weight, which was adjusted according to the size (distance from the ground to the scapular-humeral joint), the NW group presented an average of 17.7 ± 1.6 kg, while that of the OW group was 27.6 ± 1.8 and that of the OB group was 36.9 ± 1.8 kg, all groups being different with each other ($p < 0.0001$).

Blood pressure and CF

The OB group had higher SBP (159.9 ± 5.2 mmHg) compared to OW (144.1 ± 4.8 mmHg, $p = 0.045$) and NW (145.3 ± 4.8 mmHg, $p = 0.030$). MBP was also higher in the OB group (114.9 ± 3.5 mmHg) than OW (103.8 ± 3.3 mmHg, $p = 0.03$) and tended to be higher in NW (106.2 ± 3.3 mmHg, $p = 0.09$). DBP and CF were not affected by BCS.

Adipokines, cytokines, thyroid hormones, and basal cortisol

Leptin serum concentrations increased with BCS ($p < 0.0001$; Fig. 1A). Adiponectin was also affected by BCS ($p = 0.01$), being lower in the OB group compared to OW and NW ($p = 0.04$ and $p = 0.005$, respectively) (Fig. 1B). Serum resistin was higher in the OB group compared to the NW ($p = 0.02$). The OW group tended to have higher levels of serum resistin than the NW ($p = 0.08$), while no differences were observed between the OW and OB groups ($p = 0.60$) (Fig. 1C).

The concentrations of TNF-α and IL-6 in most samples were below the sensitivity of the tests used in this study (7.8 and 31.3 pg/ml, respectively). Total T4, Free T4, TSH, and basal cortisol were not affected by BCS (data not shown).

Glucose and lipid metabolism

Blood glucose was higher ($p = 0.02$) and tended to be higher ($p = 0.10$) in the OB group compared to NW and OW, respectively (Fig. 2A). Insulinemia was affected by body condition ($p < 0.0001$), being almost two times higher in OB patients compared to NW patients ($p < 0.0001$, Fig. 2B). OW patients had higher insulin concentrations than NW patients ($p = 0.02$) and lower insulin concentrations than OB animals ($p = 0.02$). The HOMA-IR index increased according to BCS ($p < 0.0001$, Fig. 2C), being higher in OB and OW than in NW canines ($p < 0.0001$ and $p = 0.02$, respectively). OW dogs had lower HOMA-IR index values than OB dogs ($p = 0.02$). The HOMA-β index was higher in OB patients compared to NW patients ($p = 0.03$, Fig. 2D). The concentration of serum triglycerides was affected by BCS ($p = 0.02$), being almost two-fold greater in OB animals than in NW animals ($p = 0.005$, Fig. 2E). OW dogs tended to have higher concentrations than NW ($p < 0.10$) but did not differ from OB canines. Serum total

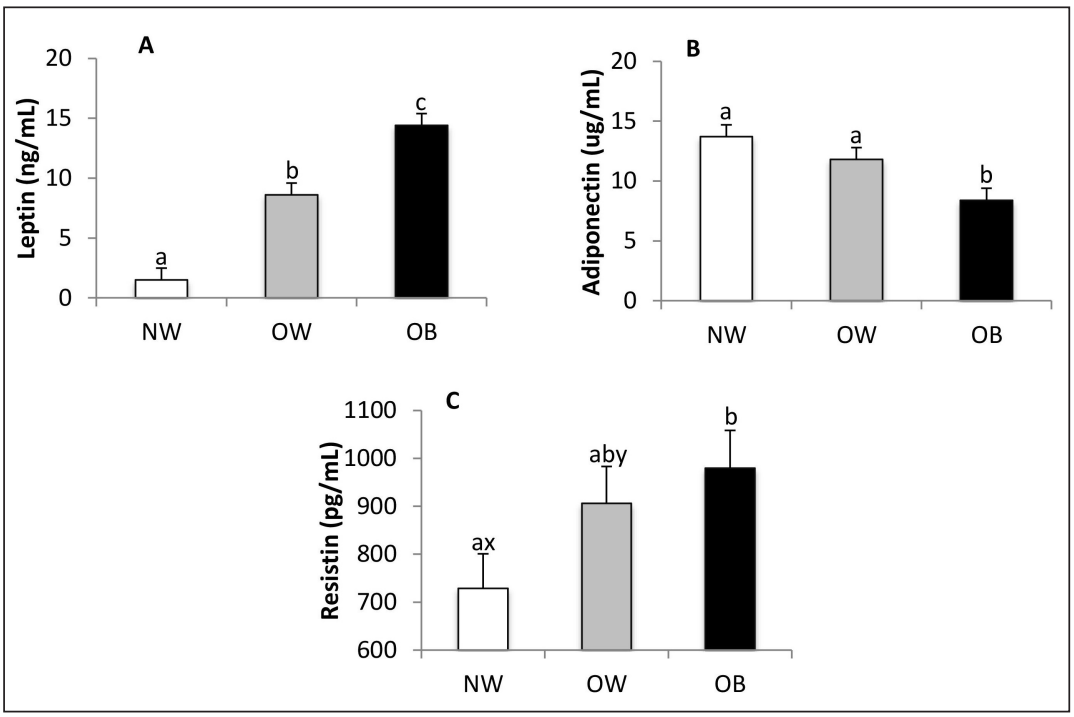

Fig. 1. Serum leptin (A), adiponectin (B), and resistin (C) concentrations in NW, BCS 4–5, OW, BCS 6–7, and OB, BCS 8–9 dogs. *a* versus *b* $p < 0.05$. *x* versus *y* $p \leq 0.1$.

cholesterol, HDL cholesterol, LDL cholesterol, and HDL/LDL ratio were not affected by BCS.

Biochemistry and urinalysis

Serum total protein concentration was affected by BCS ($p = 0.01$) (Fig. 3A), being higher in the OB group when compared to the NW group ($p = 0.0027$) and tending to be higher in OW canines than in NW canines ($p = 0.09$). Albumin tended to be affected by BCS ($p = 0.07$) (Fig. 3B), since its concentration was higher in OW canines in relation to the NW group ($p = 0.03$). Serum globulin concentration was affected by BCS ($p = 0.02$) (Fig. 3C), being higher in OB canines compared to the group of NW animals ($p = 0.006$) and tending to be higher in the group of OB canines compared to the OW group ($p = 0.09$). According to serum electrophoresis, beta globulins tended to be affected by BCS ($p = 0.055$), being higher in the OB group compared to the NW ($p = 0.02$) (Fig. 3D). No other protein fraction was affected ($p > 0.1$).

The serum concentration of hepatic enzymes, bilirubin, and CRP were not affected by BCS.

While serum urea concentration was not affected by BCS, creatinine concentration was ($p = 0.001$). Creatinine concentrations were higher in the NW group compared to the OB ($p = 0.0003$) and OW groups ($p = 0.03$) and tended to be lower in OB canines than in OW canines ($p = 0.09$) (Fig. 4A). USG was affected by BCS ($p = 0.006$), being higher in the NW group than in the OW and OB groups ($p = 0.003$ and $p = 0.009$, respectively) (Fig. 4B). Urinary pH and proteinuria were not influenced by BCS.

Hematology

BCS did not affect erythrocyte count, hemoglobin, hematocrit, mean corpuscular volume, or mean corpuscular hemoglobin. The mean corpuscular hemoglobin concentration (MCHC) was lower in the OB group when compared to NW ($p = 0.03$; Fig. 5A). Total leukocyte count was affected by BCS ($p = 0.003$) (Fig. 5B), being higher in OW and OB animals compared to NW ($p = 0.02$ and $p = 0.006$, respectively), with no differences between them. Neutrophils were affected by BCS (Fig. 5C), being higher in OW and OB patients when compared with NW canines ($p = 0.0003$ and $p = 0.002$, respectively). Band neutrophils, lymphocytes, monocytes, and eosinophils were not affected by BCS.

Correlations

Serum insulin was positively correlated with serum triglycerides, cholesterol, and CRP ($r^2 = 0.73$, $r^2 = 0.56$, and $r^2 = 0.51$, respectively, $p < 0.0003$ for all), and serum triglycerides were correlated with serum cholesterol ($r^2 = 0.81$, $p < 0.0001$).

Discussion

In the cardiovascular evaluation, OB dogs exhibited higher SBP compared to NW and OW dogs. This finding aligns with previous studies conducted on dogs (Tebaldi *et al.*, 2012; José Lahm *et al.*, 2016;

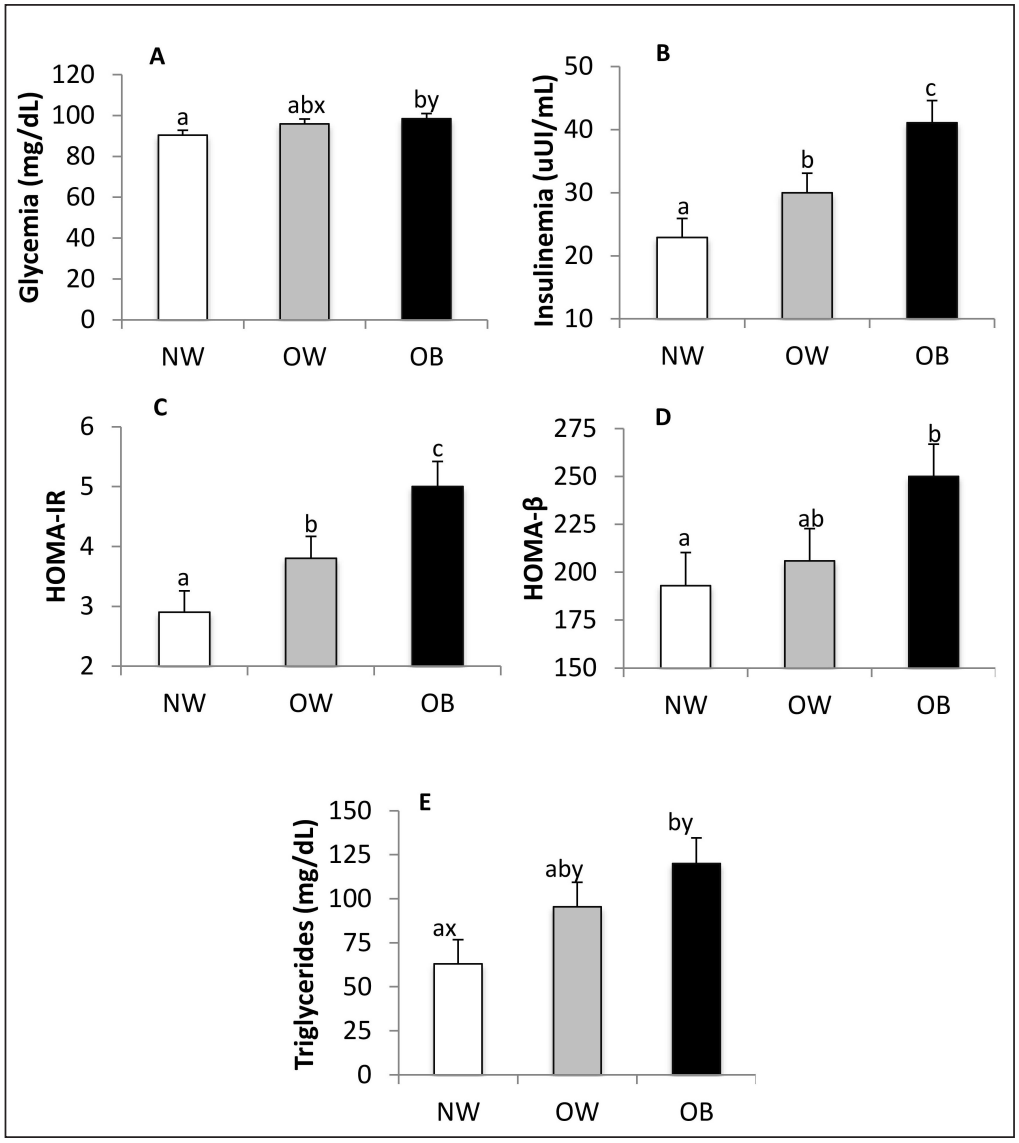

Fig. 2. Glucose (A) and insulin (B) concentrations, HOMA-IR (C) and HOMA-β (D), and triglycerides (E) concentrations in NW, BCS 4–5, OW, BCS 6–7, and OB, BCS 8–9 dogs. *a* versus *b* $p < 0.05$. *x* versus *y* $p \leq 0.1$.

Piantedosi *et al.*, 2016). However, other researchers have reported no significant difference in SBP based on BCS (Tropf *et al.*, 2017; de Marchi *et al.*, 2020; Piantedosi *et al.*, 2020). In human and murine models, activation of the renin-angiotensin-aldosterone system has been observed in OB individuals, contributing to arterial hypertension (Boustany *et al.*, 2004; Engeli *et al.*, 2005). Additionally, leptin has been shown to stimulate the ventromedial hypothalamus in mice, which increases sympathetic activity and levels of catecholamines, potentially leading to an increase in SBP in OB animals (Satoh *et al.*, 1999). Furthermore, insulin resistance and hyperinsulinemia associated with obesity have also been linked to elevated SBP (López *et al.*, 2014).

Leptin serum concentration increases with BCS, which aligns with several studies conducted on dogs (Jeusette *et al.*, 2005; Ishioka *et al.*, 2007; Lee *et al.*, 2014; Park *et al.*, 2014, 2015; Piantedosi et al., 2016; Palatucci *et al.*, 2018; Piantedosi *et al.*, 2020). In contrast, adiponectin is inversely associated with BCS, consistent with findings from several researchers (Ishioka *et al.*, 2006; Eirmann *et al.*, 2009; Lee *et al.*, 2014; Park *et al.*, 2015; Piantedosi *et al.*, 2016; Tropf *et al.*, 2017). Regarding resistin, there are only two published reports about this adipokine in dogs, which contradict our findings by indicating that BCS does not affect serum

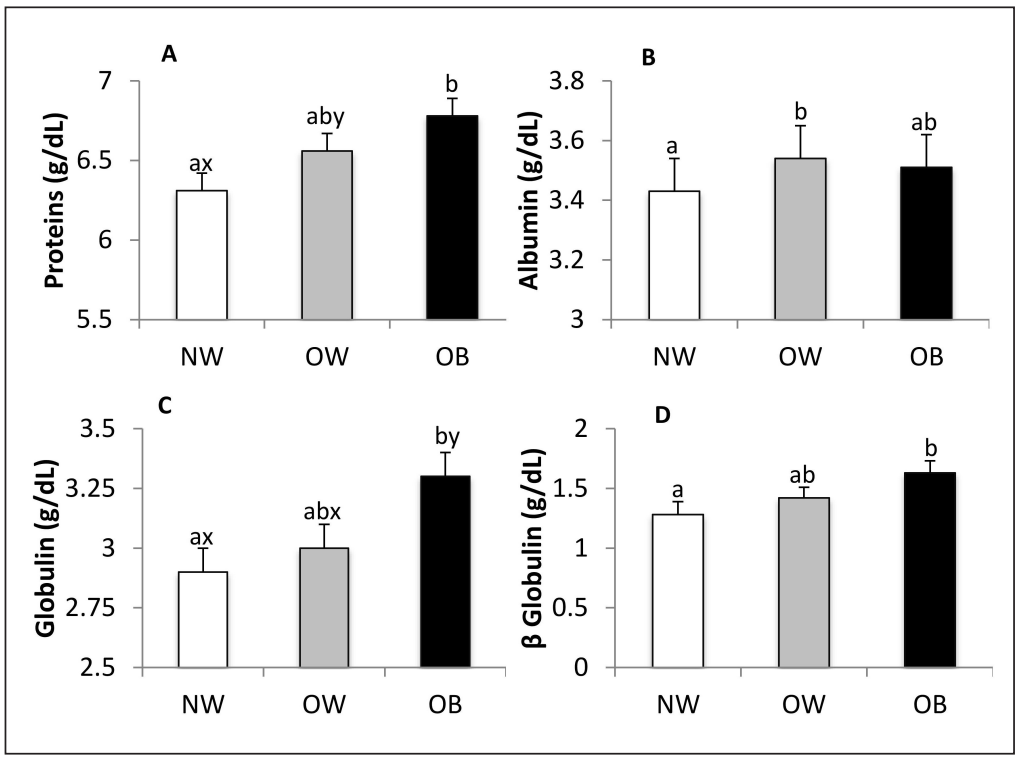

Fig. 3. Total protein (A), albumin (B), globulin (C), and beta-globulin (D) concentrations in NW, BCS 4–5, OW, BCS 6–7, and OB, BCS 8–9 dogs. *a* versus *b* $p < 0.05$. *x* versus *y* $p \leq 0.1$.

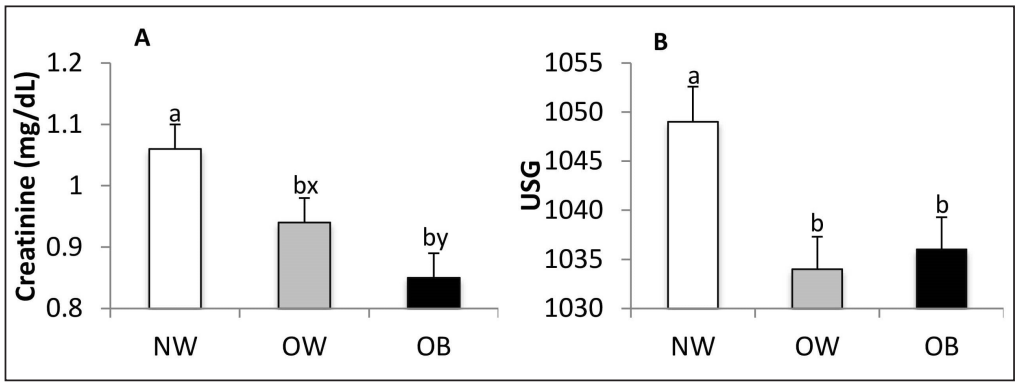

Fig. 4. Serum creatinine concentration (A) and USG (B) NW, BCS 4–5, OW, BCS 6–7, and OB, BCS 8–9 dogs. *a* versus *b* $p < 0.05$. *x* versus *y* $p \leq 0.1$.

resistin concentration (Eirmann *et al.*, 2009; Kleine *et al.*, 2020). However, studies in mice and humans support our findings, showing that serum resistin levels are higher in OB individuals (Patel *et al.*, 2003; Adeghate, 2004). Resistin influences carbohydrate metabolism and may promote diabetes by inducing insulin resistance through type 4 toll-like receptor activation (Kim *et al.*, 2007; Tarkowski *et al.*, 2010). In our study, the observed higher concentrations of leptin and resistin, along with lower levels of adiponectin in OB dogs, could contribute to insulin resistance. Indeed, the data on adipokines correlates with the higher serum insulin concentrations and HOMA indexes found in OB dogs, reflecting the metabolic effort to maintain glucose levels within reference ranges. Additionally, insulin resistance may explain the increased serum triglyceride concentrations observed in OB dogs, which has been previously documented by other researchers (José Lahm *et al.*, 2016; Piantedosi *et al.*, 2016; Tropf *et al.*, 2017; Cihan and Tural, 2019; de Marchi *et al.*, 2020; Safadi *et al.*, 2021). The underlying mechanism may involve the downregulation of endothelial

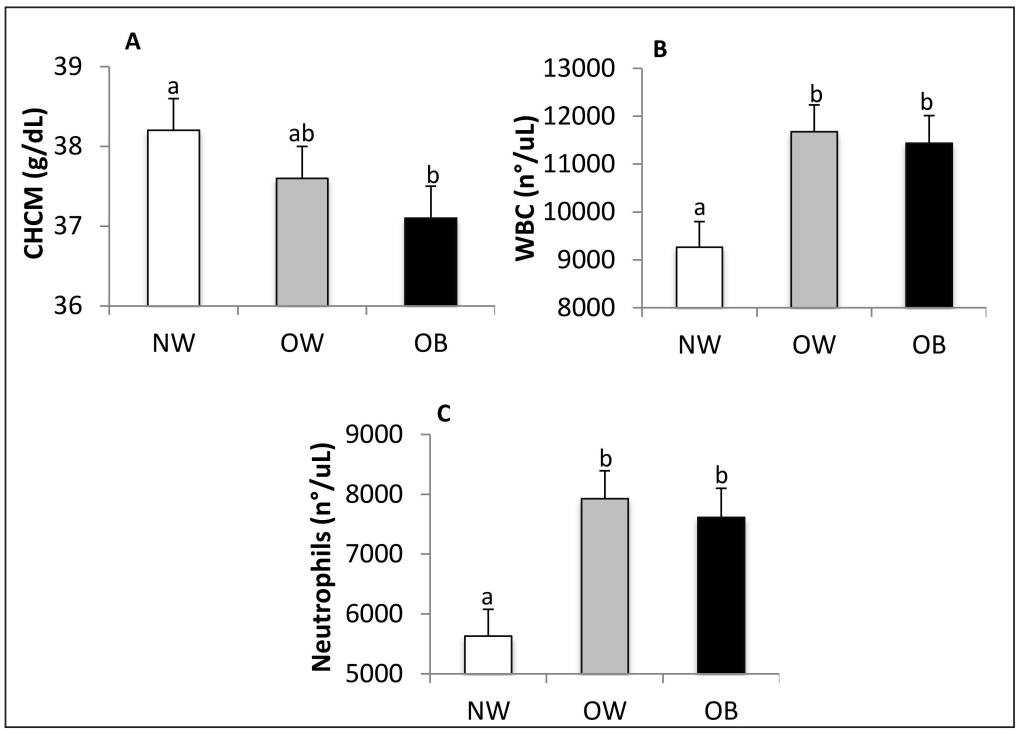

Fig. 5. MCHC (A), total leukocyte count (B), and neutrophil count (C) in NW, BCS 4–5, OW, BCS 6–7, and OB, BCS 8–9 dogs. *a* versus *b* $p < 0.05$. *x* versus *y* $p \leq 0.1$. CHCM,MCHC. WBC, white blood cells.

lipoprotein lipase, leading to decreased triglyceride clearance (Brown and Goldstein, 2008), and/or a form of selective liver insulin resistance where insulin fails to downregulate gluconeogenesis while continuing to stimulate fatty acid synthesis (Shimomura *et al.*, 2000). A higher BCS was associated with an increased concentration of serum total proteins, primarily due to a rise in serum globulins, particularly β globulins. Previous studies have reported increases in total serum proteins and globulins in OB dogs (Piantedosi *et al.*, 2016; Safadi *et al.*, 2021; Vieira *et al.*, 2022). Two research reports examined the electrophoretic protein profile of globulins in OB dogs and found that the observed differences were mainly due to the α fraction, specifically the α2 globulins (Piantedosi *et al.*, 2016; Piantedosi *et al.*, 2020). In contrast, studies in human medicine have noted activation of the complement system in OB individuals, which is evident from higher concentrations of C3 and C3b proteins (Oberbach *et al.*, 2011; Al Haj Ahmad and Al-Domi, 2017; Shim *et al.*, 2020). In dogs, these complement proteins are categorized within the β globulin fraction (Kaneko *et al.*, 2008), which may explain the increase in this fraction observed in OB dogs in our study.

Regarding renal function, serum creatinine levels decrease as BCS increases. Our findings align with those of Barić Rafaj *et al*. (2017), while other studies, such as those by Piantedosi *et al.* (2016), Bosco *et al.* (2018), Forster *et al.* (2018), Pongkan *et al.* (2020), Safadi *et al.* (2021), and Vieira *et al.* (2022), reported no significant effects of BCS on serum creatinine concentration. Since serum creatinine levels are associated with lean body mass, we suggest that the lower serum creatinine levels observed in the OB group may be explained by a loss of lean body mass, particularly muscle mass. In fact, muscle mass loss observed in humans with insulin resistance is attributed to ectopic fat deposits in skeletal muscle and the secretion of associated cytokines, which adversely affect muscle mass and function (Meex *et al.*, 2019). Sarcopenic obesity in humans has been linked to insulin resistance in both men and women (Kim *et al.*, 2013). Another possible explanation for our findings is that OB individuals tend to have higher creatinine clearance, both calculated and estimated, as reported in human studies (Tobar *et al.*, 2013). Although still within normal ranges, lower urinary density (USG) was observed in OB and OW dogs compared to those of NW. We found no observational reports regarding the relationship between BCS and urinary density in dogs. In canines with induced obesity, an increase in glomerular filtration rate has been described (Henegar *et al.*, 2001), which supports our findings.

Overall, the data regarding USG and serum creatinine, along with serum globulin levels, suggest that even within reference values, there are functional alterations in organs and tissues due to obesity. If these alterations persist over time, they may lead to a loss of homeostasis.

Research on CRP levels in canines is limited, and the findings are inconsistent. Veiga *et al*. (2008) reported that OB dogs have lower blood CRP levels compared to lean dogs while Barić Rafaj *et al.* (2017) identified significantly higher plasma CRP levels in OW and OB dogs compared to those with an ideal BCS. Besides, Tvarijonaviciute *et al.* (2012) found no change in CRP levels in canines following a weight loss treatment while Wakshlag *et al.* (2011) observed a decrease in CRP levels after a weight reduction program.

In terms of hematology, MCHC was found to be lower in the OB group compared to the NW group. This observation aligns with findings in humans, where an increase in pelvic circumference was linked to a decrease in MCHC (Vuong *et al.*, 2014). Additionally, total leukocyte counts and absolute neutrophil counts were higher in the OW and OB groups compared to the NW group. However, scientific literature on these variables presents mixed results. While several authors did not find significant differences in leukocyte counts (Barić Rafaj *et al.*, 2017; Forster *et al.*, 2018; Safadi *et al.*, 2021; Vieira *et al.*, 2022), Barbosa *et al.* (2019) reported a lower total leukocyte count in OB patients, with a notable decrease in lymphocytes. Furthermore, although Forster *et al.* (2018) and Vieira *et al.* (2022) concluded that there were no significant differences in total leukocyte counts, they did note a tendency for higher leukocyte counts in OB canines. Studies in humans and mice have shown that bone marrow stem cells possess leptin receptors, which directly stimulate the formation of granulocyte-monocyte colonies, the precursor cells for neutrophils (Purdy and Shatzel, 2021). Therefore, the increased leptin levels observed in OB dogs may promote granulopoiesis, resulting in elevated neutrophil and total leukocyte counts.

Conclusion

OB and OW canines present marked changes in carbohydrate and lipid metabolites that reflect a deterioration in insulin sensitivity as body condition increases and a compensatory hyperinsulinemia. Moreover, novel results associated with obesity are the alterations of serum resistin, the β globulin fraction, creatinine, urinary density, and neutrophils that, even within normal ranges, suggest alterations of homeostasis.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Authors' contributions

Adrián Carzoli: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Visualization, Writing—original draft, Ana Meikle: Conceptualization, Methodology, Validation, Writing-review and editing, Supervision, Funding acquisition. Paula Pessina: Conceptualization, Methodology, Validation, Resources, Writing-review and editing, Supervision, Project administration, Funding acquisition.

Data availability

The data supporting this study's findings are sensitive and not openly available. They can be obtained from the corresponding author upon reasonable request.

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