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Metabolic and urinary profiles in normal-weight, overweight and obese dogs: clinical response to an individualised weight loss programme

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ABSTRACT

Canine overweight and obesity mirrors human trends, emphasizing a One Health perspective. This prospective study evaluated haematological, biochemical and urinary parameters, alongside systolic blood pressure (SBP), in client-owned dogs: normal-weight (NW, $n = 10$), overweight (OW, n = 10) and obese (OB, n = 10). Overweight and obese dogs followed a 150-day caloric restriction programme (high-protein, high-fibre, low-energy) with biweekly veterinary follow-up.

At baseline, triglycerides were significantly higher in OB (126.6 mg/dL) compared to controls (57.6 mg/dL; $p < 0.05$). While serum total protein, globulin, C-reactive protein and calcium were significantly higher in OB ($p < 0.05$), mean values remained within clinical reference intervals. Baseline SBP in OB (191.3 mmHg) was significantly higher ($p < 0.05$) than in NW. Post-intervention, mean weight loss reached 9.43% (OW) and 9.83% (OB). Success in achieving ideal body condition was higher in OW (87.5%) than OB (30%) ($p < 0.05$). In obese group, hypertriglyceridaemia normalised (126.6 to 78.4 mg/dl; $p < 0.01$) and SBP significantly decreased (191.3 to 174 mmHg; $p < 0.05$). Programme success strongly correlated with improved physical activity ($p < 0.05$) and quality of life ($p < 0.01$).

These findings confirm that canine obesity induces metabolic and inflammatory dysregulation, even in apparently healthy dogs. Results demonstrate that structured nutritional programmes can significantly mitigate these alterations, although certain inflammatory markers, like C-reactive protein, may not fully reach control levels within the study timeframe. Notably, clinically relevant improvements occurred before achieving ideal body condition, highlighting the efficacy of early intervention and sustained veterinary-owner engagement in weight management strategies.

1. Introduction

Overweight and obesity constitute a major public health problem (Bartges et al., 2017). According to the World Health Organization (WHO, 2025), worldwide prevalence more than doubled between 1990 and 2022. By 2022, nearly 60% of adults were affected, while the combined prevalence among children and adolescents reached 20%. These figures underscore an alarming and progressive upward trend in global adiposity rates.

Parallel to this human trend, obesity represents the most frequent nutritional disorder and a critical chronic disease in companion animals. It is now widely considered a pandemic health problem (Bartges et al., 2017; de Godoy, 2018; Larsen and Villaverde, 2016; Montoya-Alonso et al., 2017). According to numerous studies conducted in different countries, 34–59.3% of companion dogs are overweight (Courcier et al., 2010; Loste et al., 2012; Lund et al., 2006; Mao et al., 2013; McGreevy et al., 2005; Montoya-Alonso et al., 2017; Porsani et al., 2020b; Usui et al., 2016). Furthermore, the incidence within this species continues to rise (Bartges et al., 2017; Montoya-Alonso et al., 2017; Phungviwatnikul et al., 2022). Consequently, a One Health approach to obesity has been

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proposed (Bartges et al., 2017).

Obesity is defined as an excess of body fat that compromises health status (Laflamme, 2006; Ramos-Plá, 2017). In dogs, a body weight 10–19% above ideal is classified as overweight, whereas an increase exceeding 20% constituted obesity (Kintzer, 2017; Ramos-Plá, 2017; Ratsch et al., 2022). The aetiology is multifactorial, comprising intrinsic factors such us breed, reproductive status and concomitant diseases, and extrinsic determinants. The latter include related to owner lifestyle (e.g., being overweight or obese, sedentary lifestyle, advanced age) and perception (Nijland et al., 2010), diet, and medical or surgical treatments (Ramos-Plá, 2017).

Notably, pet owners often project their personal nutritional habits onto their pets; this anthropomorphic approach frequently leads to hyperalimentation to strengthen emotional bonds (Linder et al., 2021; Suarez et al., 2012; Muñoz-Prieto et al., 2018). Additionally, suboptimal husbandry, including inadequate feed storage, poor bowl hygiene and high dietary variability, may negatively impact systemic health (Luisana et al., 2022; Raspa et al., 2023). Despite these known risks, a significant knowledge gap persists regarding whether metabolic perturbations manifest progressively or are exclusive to clinical obesity.

Clinically, the diagnosis of overweight and obesity in small animals is performed during the physical examination using the body weight and body condition score. The nine-point scale is currently the most widely accepted system (Laflamme, 1997; WSAVA, World Small Animal Veterinary Association, 2013). Treatment usually requires a controlled weight loss programme focused on increased physical activity and energy restriction via therapeutic diets (Blanchard et al., 2004; German et al., 2007, German et al., 2009, German et al., 2015b; Yaissle et al., 2004). In this regard, high-fibre, high-protein and low-energy commercial diets provide adequate satiety, avoiding malnutrition and muscle-mass loss (Linder et al., 2012; Porsani et al., 2020a). Crucially, owner commitment and disease recognition are pivotal to therapeutic success (Larsen and Villaverde, 2016; Porsani et al., 2020a).

Beyond simple energy storage, adipose tissue is now recognised as an active endocrine organ. It secretes numerous hormones and proteins, termed adipokines (such as adiponectin and leptin), chemokines, and cytokines, facilitating communication between peripheral tissues and the brain (German et al., 2009). Obesity induces a state of chronic, low-grade systemic inflammation and, along with overweight, predisposes dogs to several comorbidities, thereby reducing quality of life (QoL) and life expectancy (German et al., 2009, 2012b; Kealy et al., 2002; Montoya et al., 2023; Salt et al., 2019). Consequently, excess adiposity is specifically linked to insulin resistance and diabetes mellitus, hyperlipidaemia, respiratory and cardiovascular diseases, osteoarthritis, neoplasia, and renal dysfunction (German, 2006; German et al., 2009, 2012b; Ratsch et al., 2022). For these reasons, and considering the magnitude of the at-risk canine population, obesity is a major welfare concern (German et al., 2015b).

Despite extensive research into the metabolic dysregulation and inflammation associated with canine obesity, findings remain inconsistent (Adolphe et al., 2014; Barić Rafaj et al., 2017; Carzoli et al., 2025; Cavalcante et al., 2023; German et al., 2009; Pang et al., 2023; Phungviwatnikul et al., 2022; Tvarijonaviciute et al., 2011, 2012a; Vecchiato et al., 2023; Veiga et al., 2008). A significant gap remains in the literature: current studies often fail to distinguish between different degrees of adiposity. Consequently, it is unclear leaving a knowledge gap regarding whether inflammatory and metabolic perturbations manifest progressively or are exclusive to clinical obesity. Furthermore, it is yet to be determined whether the restitution of biochemical parameters requires reaching an ideal body weight, and how owner compliance modulates the trajectory of success.

To address these uncertainties, the present study aimed to evaluate the impact of overweight and obesity on blood and urinary parameters, and to monitor their evolution in response to a weight loss programme in client-owned dogs.

2. Materials and methods

2.1. Animals and study design

This prospective clinical study was conducted at the Endocrinology Service of the veterinary teaching hospital (Hospital Veterinario de la Universidad de Zaragoza, Spain) from February to December 2023. Patient recruitment was conducted through a multi-channel outreach strategy, including the distribution of informational flyers, targeted electronic mailings, and professional social media platforms to publicise the research initiative. A power analysis was not performed a priori; the sample size was determined by convenience, based on the availability of subjects. The final sample size was determined by the number of overweight and obese subjects successfully recruited according to the predefined inclusion criteria. Subsequently, an equivalent number of normal-weight dogs were enrolled to ensure balanced cohorts ($n = 10$ per group), thereby maintaining statistical robustness for comparative analysis.

A total of 66 candidates were initially screened for eligibility. However, 36 dogs were subsequently excluded for failing to meet the stringent inclusion criteria, resulting in a final study population of 30 client-owned adult dogs. To ensure that all dogs were healthy before enrolment (except for being overweight or obese), a complete patient history was obtained during the initial visit, and a physical examination was conducted. Furthermore, body weight (BW), body condition score (BCS), muscle condition and systemic blood pressure (SBP) were recorded. In addition, baseline laboratory analyses were performed, including complete blood count (CBC), a serum biochemistry panel, a urinalysis (including specific gravity, urine strip and urine sediment) and a faecal parasitological analysis. Information about nutritional management (diet: type, commercial brand, number of feedings per day, calculation of the amount of feed; snacks: type, frequency), food-seeking behaviour, physical activity level and QoL was assessed by the veterinarian after a discussion with the owner, using the questionnaire developed by Flanagan et al., 2017 and detailed in supplementary material (S1).

Dog owners were determined to be eligible for the study if they consented to their dog participating (S2), they agreed to bring their dogs back for the assessment and if they were readily contactable by telephone or email. Dogs were suitable for the enrolment if they were of adult age, healthy (except for being overweight or obese), no prior history of an adverse reaction to food, not requiring a therapeutic diet (other than a weight loss diet) and not having a significant concurrent disease.

After enrolment, all reasons for subsequent exclusion were systematically documented. Reasons for suspending participation included failure to attend scheduled appointments, dietary non-compliance due to refusal or aversion to the prescribed food, or failure of the owner to comply with the protocol. Additionally, the onset of unrelated illnesses during the course of the study was also considered a reason for potential trial suspension. In such cases, the veterinarian assessed the dog's condition to determine the appropriateness of continuing the study, with decisions being based on the nature and severity of the illness, the required treatment and the overall welfare of the patient.

Based on the BCS, the 30 dogs were categorised into three distinct groups, according to a 9-point body condition scale chart (Freeman et al., 2011): control or normal-weight (NW) ($n = 10$) (BCS: 4–5/9), overweight (OW) (n = 10) (BCS: 6–7/9), and obese (OB) (n = 10) (BCS: 8–9/9). Signalment data (breed, age and sex) for each dog was systematically collected from each dog's respective owner.

During the six-week period prior to the commencement of the present study, all dogs were fed a maintenance dry diet, characterised by the following properties: PURINA® PROPLAN® Medium Adult Everyday Nutrition (Protein 26%, Fat 16%, Carbohydrate 40.5%, Crude fibre 2%, Moisture 8%, Metabolisable energy 3895 kcal/kg). The daily food ration was calculated on the basis of the equations proposed by the

FEDIAF (2025) with regard to activity (MER (kcal/day) = 110 x BW $(kg)^{0,75}$).

An individualised weight loss programme was formulated for overweight and obese dogs encompassing daily rations, meal frequency, and recommended exercise, based on their BCS. These dogs were exclusively fed a specific commercial weight loss dry diet high in protein and fibre, and low in calories (PURINA® PROPLAN® Canine OM Obesity Management [Protein 29%, Fat 6%, Carbohydrate 41%, Crude fibre 10%, Moisture 7.5%, Metabolisable energy 2961 kcal/kg]). Any additional foodstuffs were strictly avoided. The initial daily food ration was calculated based on the equations proposed by the FEDIAF (2025) for obese prone adults (MER (kcal/day) $\leq$ 90 x BW $(kg)^{0,75}$). The diet was progressively introduced throughout a week. For most dogs, the daily portion was divided into two meals per day: one in the morning and one in the evening. Additionally, the owners also weighed each daily portion precisely using an electronic gram scale.

A total of 10 follow-up visits were conducted every two weeks to assess and modify the weight loss nutritional programme as necessary. During these visits, a complete physical examination, including measurement of SBP (oscillometric petMAP graphic II, Ramsey Medical, Inc.), was performed, BW, BCS and muscle mass were recorded. The weekly weight loss rate was calculated as a percentage according to the previous assessments of BW and number of weeks between reassessments. A review of the weight loss programme was also performed to evaluate the owner's level of adherence to it. The expected rate of weight loss was between 0.5% and 2.0% of the starting BW per week (German et al., 2007) and the caloric intake was re-adjusted, if necessary, based on the level of weight loss. If the weekly weight loss was less than 0.5%, a 10% reduction in energy allocation was implemented. Conversely, if the weight loss exceeded 2% per week, the portion was increased by 10%.

2.2. Sampling collection

Blood samples were collected by venipuncture after 12 h of fasting, at day 0 (T0) in the three groups of dogs, and then at day 60 (T60) and day 150 (T150) in overweight and obese dogs. Blood cell counts were conducted using an automated blood cell counter (ProCyte Dx, IDEXX Laboratories, Westbrook, ME, USA) on whole blood collected in ethylenediaminetetraacetic acid tubes (EDTA K3E 1.3 mL, SARSTEDT, Sarstedtstraβe, Germany). In addition, biochemistry panels (including glucose, creatinine, blood urea nitrogen, phosphorus, calcium, total protein, albumin, globulin, alanine aminotransferase, gamma-glutamyl transferase, alkaline phosphatase, total bilirubin, cholesterol, triglycerides, sodium, potassium, chloride and C-reactive protein) were analysed in a Catalyst One analyser (IDEXX Laboratories, Westbrook, ME, USA) with plasma collected in heparin tubes (Lithium heparin LH 1.3 mL, SARSTEDT, Sarstedtstraβe, Germany), and obtained after centrifugation for 10 min at 3000 *g*. The blood samples were analysed and processed individually on the day of sampling in all dogs. A rigorous internal quality control protocols were maintained within the hospital's laboratory. Analytical consistency was ensured through daily equipment calibration and the execution of all procedures by the same technical personnel.

The owners collected a sample of the first morning urine through free-catch in a sterile container at T0 (NW, OW and OB groups), and at T60 and T150 (OW and OB groups), to perform a complete urinalysis. The urine specific gravity was measured by a refractometer and the chemical properties were analysed using urinary dipsticks (Uranotest® 11C, Urano Vet SL, Spain). The sediment, obtained after centrifugation (5 min at 2500 *g*), was examined by microscopy. Furthermore, faecal parasitological analyses were performed by flotation and sedimentation at T0 from stool samples taken by the owners over the previous three consecutive days.

2.3. Ethics statement

Before the study, owners of all participating animals gave informed consent in writing. Owners were allowed the opportunity to ask any questions and to confirm or decline participation. The selected diet for this study was commercially available, and its administration was always indicated by clinical criteria as part of the treatment. As compensation for participating, owners were not charged for the study visits and received both diets free of charge. All procedures were carried out under Project License PI41/22 approved on 25 July 2022 by the Ethics Committee for Animal Experiments from the University of Zaragoza.

2.4. Statistical analysis

Statistical analysis of the results was performed using a computer programme (Statview SAS Institute 5.0.1). Due to the sample size ($n \leq 50$), the Shapiro-Wilk test was used to test for normality.

A comparative study of the means was carried out among the three groups (NW, OW, OB) for the continuous variables (BCS, weight, % of weight loss, kcal of ME per kg, SBP, and serum and urinary analyte data) at the beginning of the study (T0); between the OW and OB groups at the different follow-up times (T60, T150); as well as between NW (T0) and OW and OB at the end of the study (T150). Parametric tests (unpaired *t*-test and ANOVA) were used when the distribution of variables was normal (data represented in the text as mean ± standard deviation (SD)). If any of the data followed a non-normal distribution (data represented in the text as median [range]), non-parametric tests (Mann-Whitney and Kruskal-Wallis) were selected.

The evolution of parameters in the same individual at different follow-up times was analysed by a paired t-test when all data followed a normal distribution or by the Wilcoxon test if data did not follow a normal distribution at any of the times.

The Chi-square test (Fisher's exact test) and Spearman's test were used to examine the association between qualitative and quantitative variables, respectively. The relationship between weight loss and success in achieving ideal BC was assessed with different aspects of nutritional management, including calories consumed, food-seeking behaviour, physical activity and QoL. Differences were considered statistically significant at a 95% confidence level ($p < 0.05$).

3. Results

3.1. Characteristics of canine population

The information gathered from the three groups of dogs is summarised in Table 1. A total of 14 females (46.67%) and 16 males (53.33%) were included in the study. Most dogs were neutered, with only 23% of the animals being intact. The specific range of breeds represented is detailed in Table 1. Statistical analysis showed no significant variations in the distribution of breeds, sex, reproductive status, or age between the groups.

At the beginning of the study, the median BW was 12.75 kg (2.65 to 31.7 kg) for NW dogs, 22.56 kg (2.75 to 41.6 kg) for OW dogs and 31.3 kg (7.5 to 78 kg) for OB dogs.

The information collected on the feeding habits of dogs showed that the majority of the dogs (80%) ($n = 24$; NW = 9, OW = 9, OB = 6) were fed dry commercial food, while a smaller proportion (6.7%) (n = 2, NW = 1, OB = 1) were fed a combination of wet and dry food, and the remaining 13.3% ($n = 4$; OW = 1, OB = 3) were fed a mixture of dry commercial and homecooked food. Many owners (33.3%) ($n = 10$; NW = 3, OW = 3, OB = 4) did not measure the daily amount of food consumed, while 40.0% ($n = 12$; NW = 5, OW = 5, OB = 2) used a measuring cup, and only 26.7% ($n = 8$; NW = 2, OW = 2, OB = 4) weighed it on an electronic gram scale. Most dogs (66.7%; $n = 20$; NW = 8, OW = 4, OB = 8) received two daily rations of food, while a small

Table 1
Baseline characteristics of the study dog population.

Group	Sex (%)		Reproductive status (%)		Age (years) Mean ± SD	BW (kg) Mean ± SD	BCS Mean ± SD	Breeds
	Female	Male	Intact	Neutered				
Normal-weight (n = 10)	60	40	30	70	5.30 ± 2.83	12.75 ± 10.09	4.65 ± 0.58	5 MB, 2 DA, 1 BR, 1 PO, 1 YT
Overweight (n = 10)	50	50	10	90	7.60 ± 3.24	22.56 ± 13.96	6.70 ± 0.42	2 LR, 1 BR, 1 GR, 1 GS, 1 MA, 1 PM, 2 MB, 1 YT
Obese (n = 10)	30	70	30	70	6.40 ± 1.84	31.30 ± 24.36	8.60 ± 0.52	3 LR, 2 MB, 1 BE, 1 GR, 1 SM, 1 PM, 1 YT

BW: body weight. BCS: Body Condition Score (based on a 9-point scale).
Breed acronyms: BE, Beagle; BR, Braque; DA, Dachshund; GR, Golden Retriever; GS, German Shepherd; LR, Labrador Retriever; MA, Maltese; MB, Mixed Breed; PM, Pomeranian; PO, Pointer; SM, Spanish Mastiff; YT, Yorkshire Terrier.

Table 2
Comparison of serum and urine parameter concentrations (mean ± SD or median [range]) among the normal-weight, overweight, and obese dogs at baseline (T0).

Parameters	Normal-weight	Overweight	Obese	*p-value NW-OW*	*p-value NW-OB*	*p-value OW-OB*
Glucose (mg/dL) [74–143]	94.60 ± 13.02	93.80 ± 8.78	93.56 ± 11.76	0.7213	0.7251	0.9594
Creatinine (mg/dL) [0.5–1.8]	0.96 ± 0.22	1.01 ± 0.25	0.97 ± 0.24	0.6364	0.95	0.7035
BUN (mg/dL) [7–27]	17.60 ± 4.30	16.5 ± 4.33	15.33 ± 5.31	0.5756	0.3189	0.6049
Phosphorus (mg/dL) [2.5–6.8]	3.77 ± 0.85	3.82 ± 0.89	3.85 ± 0.69	0.8991	0.8140	0.9240
Calcium (mg/dL) [7.9–12]	9.45 ± 0.44	9.76 ± 0.45	10.13 ± 0.52	0.1359	**0.0061**	0.1099
Total protein (g/dL) [5.2–8.2]	6.29 ± 0.45	6.61 ± 0.43	6.87 ± 0.53	0.1209	**0.0195**	0.2590
Albumin (g/dL) [2.3–4.0]	3.03 ± 0.24	3.08 ± 0.27	3.00 ± 0.30	0.6723	0.8104	0.5457
Globulin (g/dL) [2.5–4.5]	3.24 ± 0.27	3.53 ± 0.33	3.74 ± 0.54	**0.0482**	**0.0186**	0.3073
ALT (U/L) [10–125]	67.80 ± 24.72	44.50 [37–85]	56 [42–125]	0.0547	0.7749	0.0863
ALP (U/L) [23−212]	51.40 ± 15.86	49.80 ± 13.38	78 [40–219]	0.7435	0.1117	0.0584
GGT (U/L) [0−11]	0 [0−21]	0	0 [0–9]	0.3458	0.9999	0.2888
Total bilirubin (mg/dL) [0–0.9]	0.1 [0.1–0.3]	0.1	0.1	0.1693	0.1456	0.9999
Cholesterol (mg/dL) [110−320]	174.30 ± 47.13	207.10 ± 48.39	217.89 ± 56.29	0.1421	0.0838	0.6589
Triglycerides (mg/dL) [10−100]	57.60 ± 20.48	69 [51–232]	126.62 ± 51.07	0.0603	**0.0059**	0.4489
Sodium (mmol/L) [144–160]	152.00 ± 2.60	152.60 ± 2.22	149.87 ± 2.03	0.5943	0.0825	**0.0163**
Potassium (mmol/L) [3.5–5.8]	3.92 ± 0.33	4.09 ± 0.27	3.93 ± 0.29	0.2364	0.9403	0.2393
Chloride (mmol/L) [109–122]	112.33 ± 1.80	110.7 ± 2.31	110.67 ± 2.74	0.1068	0.1468	0.9774
C reactive protein (mg/dL) [0.0–1.0]	0.32 ± 0.21	0.2 [0.1–1.2]	0.7 [0.2–4.6]	0.4022	**0.0217**	**0.0211**
Urine specific gravity	1055 [1020–1060]	1035.25 ± 11.80	1035.2 ± 18.86	0.0893	0.0820	0.8931
Urine pH	6 [5–7]	6.5 ± 0.93	6 [5–7]	0.1044	0.7022	0.0838

ALP: alkaline phosphatase. ALT: alanine aminotransferase. BUN: blood urea nitrogen. GGT: gamma-glutamyl transferase. NW: normal-weight. OB: obese. OW: overweight.
Statistical analysis was performed using unpaired *t*-tests and Mann-Whitney *U* tests. A p-value <0.05, indicated in bold, was considered statistically significant.

number were fed three times a day (16.7%; $n = 5$; OW = 3, OB = 2) or ad libitum (16.7%; n = 5; NW = 2, OW = 3). The majority of dogs (86.7%; $n = 26$; NW = 8, OW = 9, OB = 9) received treats or snacks (dental sticks, biscuits, bread, table scraps, fruit) on a near-daily basis.

At the preliminary visit (T0), food-seeking behaviour was quantified using a scale from 0 to −3, where 0 represented the physiological baseline (behaviours restricted to pre-meal times) and negative integers denoted progressive pathological deviations. A score of −1 (mild anxiety) indicated occasional inter-meal food-seeking; −2 (moderate) reflected frequent inter-meal and postprandial behaviours; and − 3 (severe) represented near-continuous food-related distress.

NW dogs showed no food-seeking anxiety (score 0). In contrast, this behaviour was markedly present in the OW group (60% score − 1; 30% score − 2; 10% score − 3) and particularly severe in the OB cohort, where 70% of individuals reached the maximum score of −3. A statistically significant difference ($p < 0.001$) was observed in mean scores between groups (NW = 0, OW = −1.5 ± 0.7, OB = −2.7 ± 0.48).

A comparative analysis of the dogs' lifestyle scores was conducted, revealing that 11 dogs (36.7%; $n = 11$; OW = 2, OB = 9), 13 dogs (43.3%; $n = 13$; NW = 5, OW = 7, OB = 1), and 6 dogs (20%; $n = 6$; NW = 5, OW = 1) had an owner-reported activity score of −1 (low activity), 0 (normal activity) and 1 (high activity), respectively. There was a statistically significant difference ($p < 0.001$) in the mean activity score among NW (0.5 ± 0.53), OW (−0.1 ± 0.57) and OB dogs (−0.9 ± 0.32). The owner-reported QoL score showed that 7 OB dogs (23.3%) had a QoL score of −1 (low), only 1 OB dog (3.3%) had a score of 0 (normal) and the remaining NW and OW dogs (73.3%) had a QoL score of 1 (good). A significant difference ($p < 0.001$) was observed in the mean QoL score of the OB group (−0.5 ± 0.85) compared to the NW and OW groups (1.0).

3.2. Baseline characteristics

3.2.1. Serum and urine analyses

The haematological and urinary parameters evaluated between the three groups of dogs at the beginning of the study are shown in Table 2. The most frequently observed out-of-range serum parameter was triglyceride (TG) concentration, which was elevated in 10 out of 28 dogs (OW = 3, OB = 7). This was followed by C-reactive protein (CRP) concentration, which was elevated in 4 out of 28 dogs (OW = 1, OB = 3). Additionally, elevated concentrations of alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT) were observed in one obese dog and one normal-weight dog, respectively. Statistically significant differences ($p < 0.05$) were observed, particularly between the NW and OB groups. The mean concentrations of calcium (Ca) ($p = 0.0061$), total protein ($p = 0.0195$), globulin ($p = 0.0186$), TG ($p = 0.0059$), and CRP ($p =$

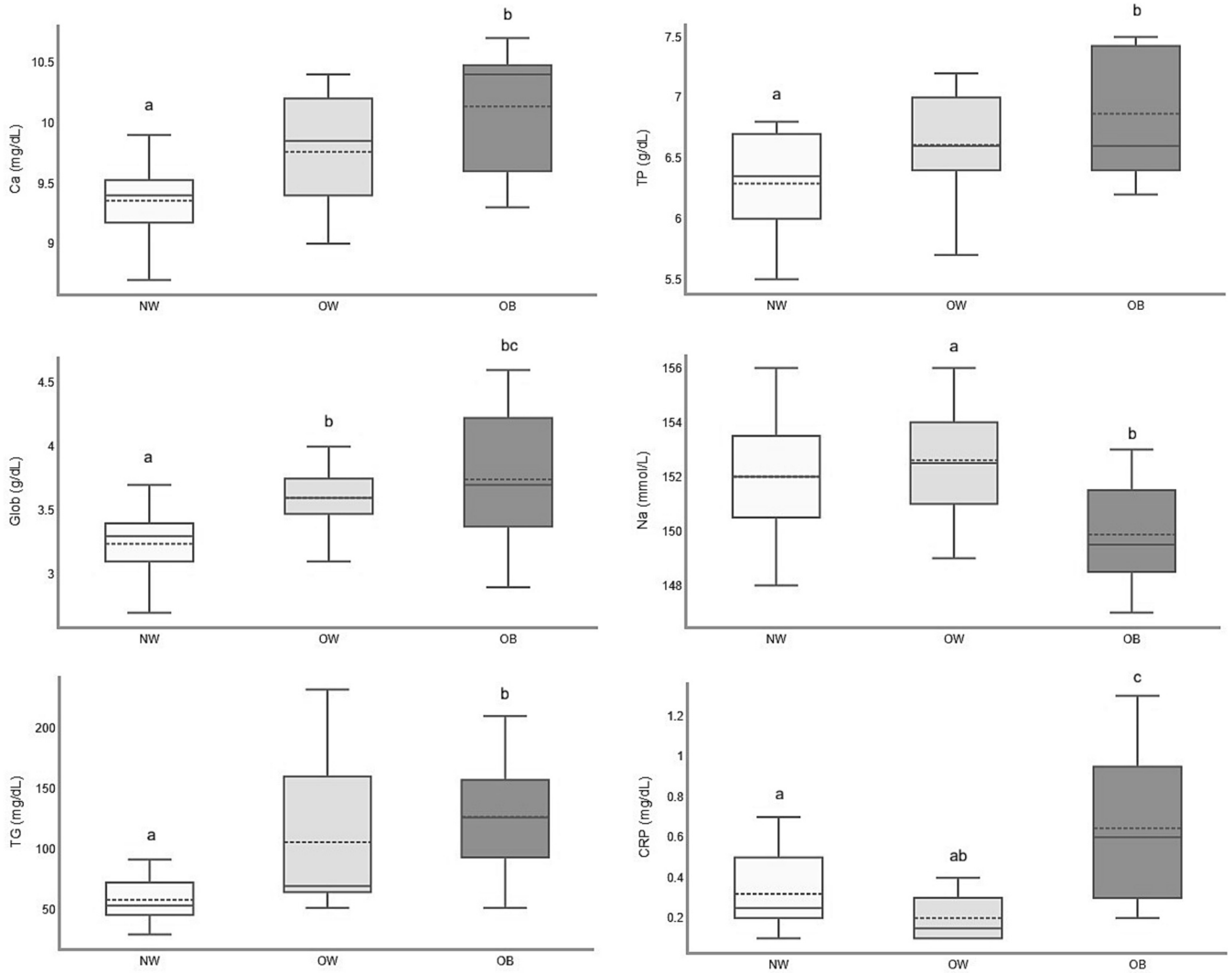

Fig. 1. Box-and-whisker plot showing the mean serum concentrations of calcium (Ca), total protein (TP), globulin (Glob), triglycerides (TG), C reactive protein (CRP) and sodium (Na) among the study groups (NW: normal-weight; OW: overweight; OB: obese) at baseline (T0). Different letters indicate statistically significant differences between groups ($p < 0.05$).

0.0217) were significantly higher in the OB group compared to the NW group. Moreover, CRP levels were also significantly higher in the OB group compared to the OW group ($p = 0.0211$). Additionally, mean globulin concentrations were slightly elevated in OW dogs compared to NW dogs ($p = 0.0482$). In contrast, sodium (Na) levels were lower in the OB group compared to the OW group ($p = 0.0163$) (Fig. 1). Although differences were observed between groups for the parameters mentioned above, all remained within the established reference interval, except for TG and CRP, whose concentrations exceeded the upper limit.

3.2.2. Blood pressure

At T0, the mean systemic blood pressure values for NW dogs (159.2 mmHg; range: 127–194 mmHg) were significantly lower ($p = 0.0432$) than those for OB dogs (191.33 mmHg; range: 148–270 mmHg). In accordance with ACVIM criteria, hypertension (>160 mmHg) was identified in 10% of NW, 67% of OW, and 80% of OB dogs. Moreover, 44% of overweight and obese individuals displayed SBP values exceeding 180 mmHg, a threshold associated with high risk of target organ damage (Acierno et al., 2018). However, no significant differences were observed between the OW group (168.78 mmHg; range: 120–200 mmHg) and the OB group (Table 3).

3.3. Effects of the weight loss programme

During the course of the study, two animals from the OW group were excluded: one due to the owner's failure to comply with follow-up requirements, and the other due to the development of a severe illness.

3.3.1. Weight loss outcomes and body score condition

Table 4 summarises all data related to weight changes in OW and OB dogs undergoing an individualised weight loss programme. Fig. 2 shows the progression of BCS at the two-weekly controls. All OB and OW dogs lost weight, with 10 out of 18 (55.6%) achieving an optimal body condition by the end of the study (T150) without any concomitant loss of muscle mass. The success rate of OW dogs in achieving their ideal weight (87.5%) was significantly higher than that of the OB group (30%). The statistical analysis confirmed a significant overall reduction in BCS ($p < 0.001$), which was significantly associated with the group variable ($X^2 = 5.951$; $p = 0.0109$).

The evolution of individual weight loss rates (0.5% to 2% according to German et al., 2007) varied between groups and temporal points. At T60, while 44.4% of OW and 70% of OB dogs achieved or exceeded the expected rate, 30% of OW and 20% of OB dogs demonstrated weight gain. This trend persisted at T150, where 40% of OW and 30% of OB dogs failed to reduce body mass. These results could be attributable to inconsistent owner compliance and the persistent provision of extra treats. However, as shown below, many dogs improved their BCS despite modest weight loss. This discrepancy likely reflects a shift in body composition, specifically a reduction in adiposity coupled with an increase in lean muscle mass, which has a higher density than fat.

Table 3
Mean systolic blood pressure (SBP) values in the three groups of dogs at baseline (T0) and in the overweight and obese groups at the control visits (T60, T150) during the weight loss phase.

GROUP	T0	T60	T150	*p-value*
Normal-weight	159.20 ± 21.30 [a]			
Overweight	168.78 ± 26.27 [ab]	169.12 ± 38.72	180.12 ± 28.54	
Obese	191.33 ± 40.82 [b ‘]	170.70 ± 45.39 [‘]	174 ± 34.78 [‘]	0.0498 (T0-T150)
p-value	0.0432 (NW-OB)			

NW: normal-weight. OB: obese.
Different superscripts indicate statistically significant differences ($p < 0.05$): letters indicate comparisons between groups, and symbols indicate comparisons between time points within the same group.

Table 4
Weight loss outcomes in overweight and obese dogs during the weight loss programme. Data are expressed as mean ± SD.

Group	Parameter	T0	T60	T150	Overall
Overweight	Body weight (kg)	22.56 ± 13.96	23.71 ± 13.33	22.15 ± 12.93	
	BCS	6.70 ± 0.42	5.62 ± 0.74	4.87 ± 0.64	
	Weight loss (%)[a]		2.79 ± 5.40	6.73 ± 6.28	9.43 ± 6.80
	Rate of weight loss[b]		0.35 ± 0.68	0.56 ± 0.52	0.39 ± 0.35
	Target body weight reached (number [%])			7 (87.5%)	
	Daily energy intake[c]	76.31 ± 7.68	72.10 ± 9.98	76.51 ± 13.13	74.97 ± 9.75
Obese	Body weight (kg)	31.30 ± 24.36	29.61 ± 23.76	28.27 ± 22.49	
	BCS	8.60 ± 0.52	7.60 ± 0.84	6.65 ± 1.25	
	Weight loss (%)[a]		6.21 ± 4.13	3.86 ± 4.65	9.83 ± 5.82
	Rate of weight loss[b]		0.78 ± 0.51	0.32 ± 0.39	0.36 ± 0.27
	Target body weight reached (number [%])			3 (30%)	
	Daily energy intake[c]	74.83 ± 6.37	65.66 ± 11.66	59.29 ± 11.35	64.62 ± 8.04

BCS: Body condition score (based on a 9-point scale).
[a] Expressed as the percentage of body weight relative to the previous time point. Positive values indicate a net loss; negative values indicate a net gain.
[b] Expressed as the percentage of starting body weight loss per week.
[c] Expressed as kcal of ME per $kg^{0.75}$ of target body weight.

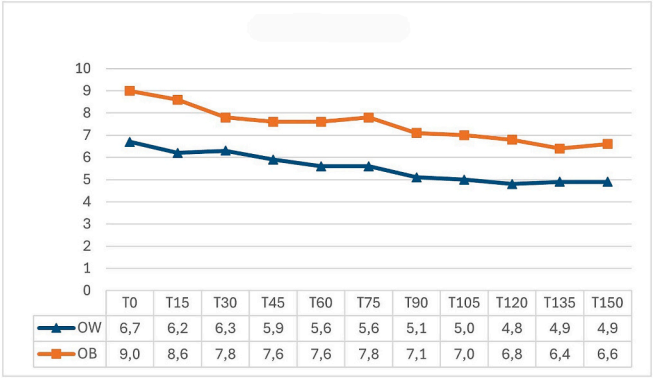

Fig. 2. Changes in the mean Body Condition Score (BCS) in overweight (OW) and obese (OB) dogs recorded at two-weekly follow-up visits over the 150-day study period.

Table 4 summarises the mean weight change data for both groups. The mean overall percentage of weight loss relative to starting BW was 9.43% (−3.64% to 16.22%) for OW dogs and 9.83% (−0.95% to 16.76%) for OB dogs. The analysis of weight loss progression across stages showed that OW dogs lost an average of 2.79% (−5.45% to 9.13%) of their BW from the initial (T0) to T60, and 6.73% (0.00% to 20.51%) from T60 to T150. For the OB group, the mean percentage of weight loss during the initial period (T0 to T60) was 6.21% (1.33% to 12.58%), and 3.86% (−3.03% to 10.77%) between T60 and T150.

In OW dogs, a significant decrease in mean BCS was observed across the study time points ($p < 0.05$). Moreover, a significant reduction in mean BW was observed by the end of the study ($p < 0.05$), despite a

minor increase during the initial two months. The mean percentage of weight loss was significantly higher ($p = 0.0254$) at T150 than at T60, although no significant differences were found in the weekly weight loss rate throughout the study.

For OB dogs, the mean BCS also significantly decreased ($p < 0.05$) during the follow-up visits. Significant differences were observed in mean BW between the commencement of the restricted calorie phase and the subsequent visits (T60, $p = 0.008$; T150, $p = 0.012$). However, no significant differences were observed in mean BW between T60 and T150. Regarding weekly weight loss, the mean percentage was significantly higher ($p = 0.016$) in the initial two months in comparison to the mean percentage loss experienced over the course of the entire study.

The mean overall rate of weekly weight loss, expressed as a percentage of starting BW lost per week, was similar in OW and OB dogs: 0.39% (−0.09 to 0.90%) and 0.36% (−0.17% to 0.68%), respectively. However, differences were noted in the evolution of weekly weight loss between groups. In the OB group, the weekly loss was significantly higher during the first two months in comparison to the overall weekly loss throughout the study ($p = 0.016$). Conversely, in the OW group, the percentage of weight loss was significantly more pronounced in the final weeks ($p = 0.025$).

Differences were also detected concerning daily energy intake (expressed as kcal of ME per $kg^{0,75}$ BW). In the OW group, energy intake was significantly lower ($p < 0.05$) at T60 than at other study time points during the study. Additionally, a significant increase in caloric intake ($p < 0.05$) was observed in the OB group at the beginning of the study compared to the levels recorded during the weight loss intervention. Moreover, the mean daily energy intake overall during the caloric restriction phase was 74.97 (65.87–94.53) in OW dogs and 64.62 (54.18–82.05) in OB dogs, a difference that was statistically significant ($p = 0.025$). This difference was particularly evident at T150, where there was a significant decrease in energy intake ($p = 0.011$) in the OB group compared to the OW group.

3.3.2. Serum and urine analyses

During the caloric restriction phase, no significant changes were observed in haematological parameters in either the OW or OB dogs. The biochemical and urinary parameters of both groups at T0, T60, and T150, along with their statistical analysis, are presented in Tables 5 and 6.

Within the OW group, statistically significant differences were observed in mean serum levels of albumin, cholesterol, CRP, and urine specific gravity. Despite these changes, all parameters remained within their normal reference ranges (Table 5, Fig. 3). Specifically, a significant decrease in cholesterol concentration was observed in association with weight loss at T60 ($p = 0.0050$) and T150 ($p = 0.0079$). Conversely, CRP concentration increased significantly by the study's conclusion ($p = 0.0167$). Nevertheless, one OW dog exhibited persistently elevated CRP levels throughout the study. Furthermore, urine specific gravity consistently increased throughout the observation period: T0-T150 ($p =$

Table 5
Comparison of serum and urine parameters (mean ± SD or median [range]) in overweight dogs during the weight loss phase at baseline (T0), T60, and T150.

Parameters	OW T0	OW T60	OW T150	*p*-value *T0-T60*	*p-value T0-T150*	*p-value T60-T150*
Glucose (mg/dL) [74–143]	93.80 ± 8.78	91.87 ± 8.32	86.25 ± 8.97	0.5842	0.1395	0.2691
Creatinine (mg/dL) [0.5–1.8]	1.01 ± 0.25	1.06 ± 0.19	1.01 ± 0.22	0.1705	0.05791	0.1036
BUN (mg/dL) [7–27]	16.50 ± 4.33	18.62 ± 4.03	19.62 ± 5.10	0.4622	0.3661	0.6900
Phosphorus (mg/dL) [2.5–6.8]	3.82 ± 0.89	3.84 ± 0.66	3.70 ± 0.83	0.7452	0.3576	0.6666
Calcium (mg/dL) [7.9–12]	9.76 ± 0.45	9.72 ± 0.34	9.66 ± 0.25	0.5983	0.3174	0.4721
Total protein (g/dL) [5.2–8.2]	6.61 ± 0.43	6.52 ± 0.29	6.57 ± 0.30	0.9298	0.7503	0.6767
Albumin (g/dL) [2.3–4.0]	3.08 ± 0.27	3.05 ± 0.23	3.21 ± 0.24	0.8850	**0.0025**	0.614
Globulins (g/dL) [2.5–4.5]	3.53 ± 0.33	3.49 ± 0.18	3.36 ± 0.28	0.9134	0.3164	0.3607
ALT (U/L) [10–125]	44.50 [37–85]	42.50 [30–129]	50.75 ± 18.06	0.4452	0.6740	0.6744
ALP (U/L) [23–212]	49.80 ± 13.38	52.12 ± 11.63	59.50 ± 18.13	0.4669	0.2218	0.1745
GGT (U/L) [0–11]	0	0	0 [0–1]	–	–	–
Total bilirubin (mg/dL) [0–0.9]	0.1	0.1 [0.1–0.3]	0.1 [0.1–0.3]	0.1797	0.1025	0.5809
Cholesterol (mg/dL) [110–320]	207.10 ± 48.39	175.00 ± 37.00	173.50 ± 30.82	**0.0050**	**0.0079**	0.7306
Triglycerides (mg/dL) [10–100]	69.00 [51–232]	75.50 [51–180]	86.37 ± 20.74	0.7792	0.7794	0.5754
Sodium (mmol/L) [144–160]	152.60 ± 2.22	153.75 ± 1.83	153.75 ± 1.39	0.0956	0.2096	–
Potassium (mmol/L) [3.5–5.8]	4.09 ± 0.27	4.16 ± 0.44	4.32 ± 0.44	0.9346	0.3285	0.3792
Chloride (mmol/L) [109–122]	110.70 ± 2.31	112.37 ± 2.13	112.00 [112–116]	0.1682	0.1118	0.6845
C reactive protein (mg/dL) [0.0–1.0]	0.2 [0.1–1.2]	0.2 [1.0–1.7]	0.5 [0.4–1.3]	0.1573	**0.0167**	0.1193
Urine specific gravity	1035.25 ± 11.80	1036.00 [1032–1060]	1055.00 [1034–1060]	0.2072	**0.0277**	**0.0422**
Urine pH	6.50 ± 0.93	6.71 ± 0.76	6.75 [6.00–7.00]	0.5775	0.8608	0.3961

ALP: alkaline phosphatase. ALT: alanine aminotransferase. BUN: blood urea nitrogen. GGT: gamma-glutamyl transferase. OW: overweight.
Statistical analysis was performed using unpaired *t*-tests and Mann-Whitney *U* tests. A *p*-value <0.05, indicated in bold, was considered statistically significant.

Table 6
Comparison of serum and urine parameters (mean ± SD or median [range]) in obese dogs during the weight loss phase at baseline (T0), T60, and T150.

Parameters	OB T0	OB T60	OB T150	*p*-value *T0-T60*	*p-value T0-T150*	*p-value T60-T150*
Glucose (mg/dL) [74–143]	93.56 ± 11.76	97.50 ± 8.92	99.70 ± 18.62	0.3828	0.5624	0.6531
Creatinine (mg/dL) [0.5–1.8]	0.97 ± 0.24	0.90 ± 0.21	0.83 ± 0.16	0.0509	**0.0302**	0.1323
BUN (mg/dL) [7–27]	15.33 ± 5.31	15.10 ± 5.38	14.94 ± 5.79	0.3284	0.8833	0.6079
Phosphorus (mg/dL) [2.5–6.8]	3.85 ± 0.69	3.80 [2.70–4.10]	3.91 ± 3.90	0.1219	0.7627	0.1719
Calcium (mg/dL) [7.9–12]	10.13 ± 0.52	9.97 ± 0.40	10.02 ± 0.33	0.0943	0.5230	0.3708
Total protein (g/dL) [5.2–8.2]	6.87 ± 0.53	6.83 ± 0.27	6.83 ± 0.27	0.6106	0.5052	>0.9999
Albumin (g/dL) [2.3–4.0]	3.00 ± 0.30	3.01 ± 0.35	3.19 ± 0.39	0.8358	0.4024	0.3042
Globulin (g/dL) [2.5–4.5]	3.74 ± 0.54	3.78 ± 0.34	3.64 ± 0.33	0.8508	0.4865	0.3837
ALT (U/L) [10–125]	56.00 [42.00–125.00]	51.00 [29.00–238.00]	56.20 ± 15.65	0.5533	0.3621	0.7989
ALP (U/L) [23–212]	78.00 [44.00–219.00]	79.20 ± 43.09	79.11 ± 47.74	0.1685	0.7671	0.2973
GGT (U/L) [0–11]	0 [0–9]	0 [0–9]	0 [0–14]	0.7855	0.6547	>0.9999
Total bilirubin (mg/dL) [0–0.9]	0.10	0.10 [0.10–0.30]	0.23 ± 0.16	–	**0.0158**	**0.0084**
Cholesterol (mg/dL) [110−330]	217.89 ± 56.29	171.40 ± 32.61	177.56 ± 48.90	**0.0062**	**0.0375**	0.5347
Triglycerides (mg/dL) [10–100]	126.62 ± 51.07	112.70 ± 35.66	78.43 ± 27.49	0.5628	0.1852	**0.0075**
Sodium (mmol/L) [144–160]	149.87 ± 2.03	152.5 ± 2.01	154.22 ± 2.05	**0.0260**	**0.0034**	0.2256
Potassium (mmol/L) [3.5–5.8]	3.83 ± 0.29	3.50 [3.40–4.20]	4.14 ± 0.40	0.7209	0.1930	0.1072
Chloride (mmol/L) [109–122]	110.67 ± 2.74	112.00 [104.00–114.00]	112.67 ± 2.00	0.2558	0.0679	**0.0464**
C reactive protein (mg/dL) [0.0–1.0]	0.70 [0.20–4.60]	0.65 [0.10–2.50]	0.64	0.7983	0.3122	0.6741
Urine specific gravity	1035.2 ± 18.86	1035.55 ± 10.48	1034.1 ± 20.34	0.5299	0.8461	0.3284
Urine pH	6.00 [5.00–7.00]	6.05 ± 0.95	6.00 [5.00–6.50]	0.3363	0.5164	0.3805

ALP: alkaline phosphatase. ALT: alanine aminotransferase. BUN: blood urea nitrogen. GGT: gamma-glutamyl transferase. OB: obese.
Statistical analysis was performed using unpaired *t*-tests and Mann-Whitney U tests. A *p*-value <0.05, indicated in bold, was considered statistically significant.

0.0277), T60-T150 ($p = 0.0422$).

With regard to the OB group, statistically significant differences were detected in serum levels of creatinine, total bilirubin, cholesterol, triglycerides, sodium and chloride (Table 6, Fig. 4). However, the mean values remained within their respective reference ranges. Conversely, the initial mean serum TG concentration, which was above the reference range, progressively decreased at T60 and was normalised by T150 ($p = 0.0075$).

A comparison of the evolution of analytes between the OW and OB groups during the weight loss phase revealed significant differences in mean serum levels of calcium, total proteins and globulin (Table 7). At T60 control, mean serum total protein and globulin concentrations were significantly higher ($p = 0.0369$, $p = 0.0420$, respectively) in the OB group compared to the OW dogs. By the end of the study, mean serum Ca concentration was slightly higher ($p = 0.0235$) in OB dogs compared to OW dogs and mean urine pH was significantly lower ($p = 0.0123$) in OB dogs than in OW dogs. At T60, the mean TG concentration was higher in OB dogs compared to OW dogs, showing a strong tendency towards statistical significance ($p = 0.058$). However, by T150, the mean TG concentration in the OB group had decreased, approaching values observed in the OW group.

The results obtained at the end of the weight loss programme for the parameters of the OB and OW dogs (T150) were then compared with those of the NW group included at the beginning of the study (T0) (Table 8, Fig. 5). The statistical analysis showed that in the OB group, mean serum levels of Ca ($p = 0.005$), total protein ($p = 0.005$), globulins ($p = 0.009$) and CRP ($p = 0.007$) were significantly higher than in the NW group at both baseline and the conclusion of the study (Table 2, Table 8). The mean bilirubin concentration, while showing no baseline difference, was also significantly higher ($p = 0.025$) in the OB group by the end of the study. Conversely, TG serum concentrations decreased during the weight loss programme, resulting in no statistically significant differences between the OB and NW groups at the end of the study.

In the OW group, mean CRP ($p = 0.029$), TG ($p = 0.010$), and urinary pH ($p = 0.001$) values were significantly higher at the study's conclusion than in the NW group, a difference not observed at baseline. However, globulin concentration, which was significantly higher in OW at baseline, showed no difference after the weight loss programme.

3.3.3. *Systemic blood pressure*

Table 3 summarises the mean SBP measurements for OW and OB dogs during the weight loss phase. In the OW group, SBP showed a progressive, non-significant increase. Conversely, SBP for the OB group significantly decreased by the end of the study ($p = 0.0498$). Despite these intra-group trends, no significant differences in mean SBP were observed between the OW and OB groups during the weight loss phase. Notably, both study groups exhibited higher SBP compared to the NW dogs (Table 8).

3.3.4. *Quality and lifestyle*

Owner questionnaires administered during the follow-up visits revealed an increase in physical activity and an improvement in QoL for

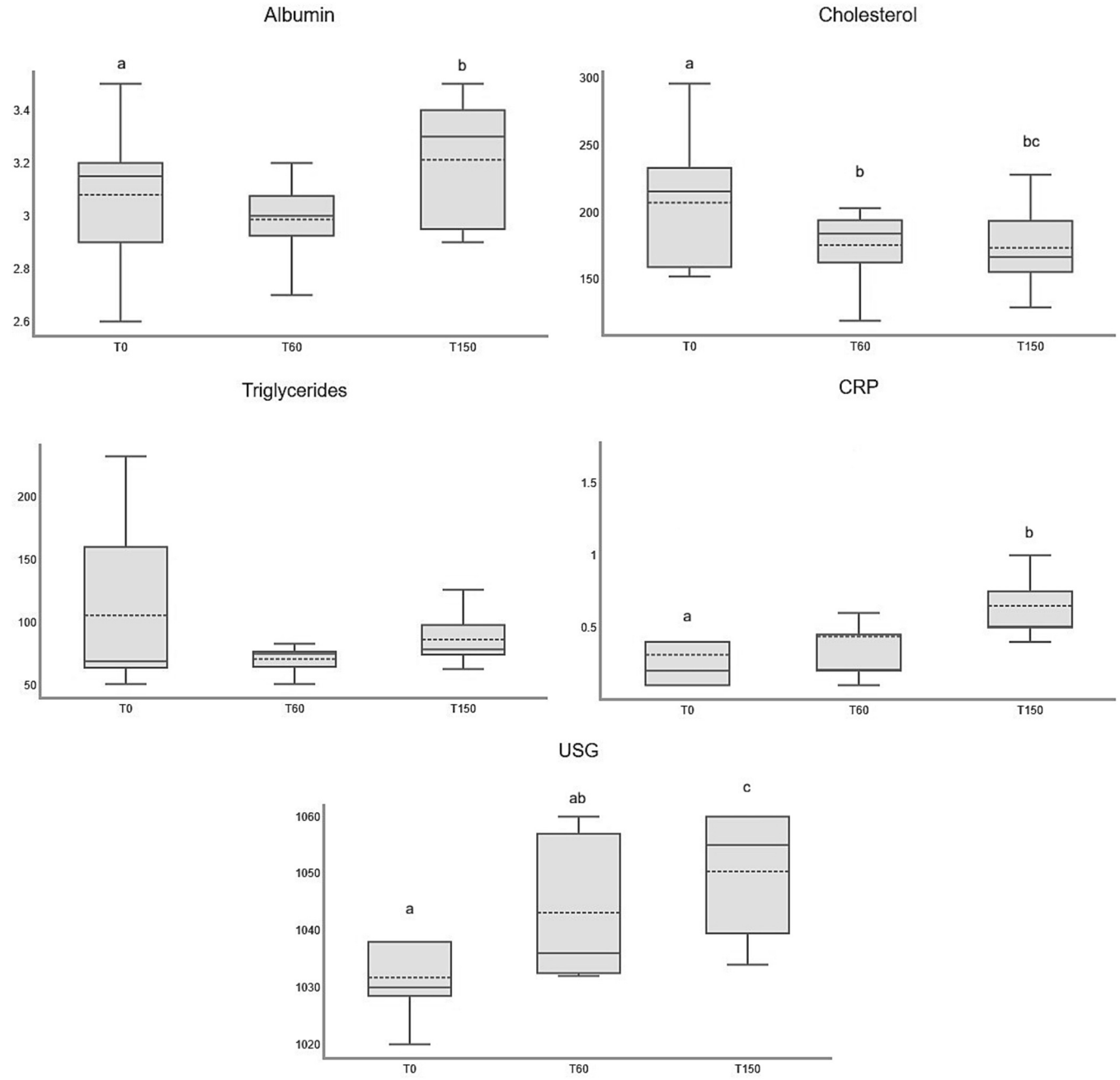

Fig. 3. Box-and-whisker plot representing the serum and urine parameters that showed significant changes during the weight loss phase (T0, T60, T150) in overweight dogs. CRP: C reactive protein. USG: urine specific gravity. Different letters indicate statistically significant differences ($p < 0.05$) between time points.

most dogs throughout the study. Furthermore, a reduction in food-seeking behaviour was observed in both OW and OB dogs. All owners whose dogs achieved a satisfactory weight loss rate adhered to the recommendations provided by the veterinary team.

Interestingly, at the study's outset, all owners assessed their obese pets as being of normal-weight or merely overweight. However, by the end of the study, their assessment of their pet's body condition more closely aligned with that assigned by the researchers.

Statistical analysis of variables related to nutritional management, dietary habits, and lifestyle revealed a significant correlation between achieving optimal body condition at the end of the study and activity levels ($X^2 = 6.024$; $p = 0.0492$), QoL ($X^2 = 10.884$; $p = 0.0043$) and food-seeking behaviour ($X^2 = 6.525$; $p = 0.0383$). Almost all dogs with normal activity levels (6 out of 7) and high activity levels (1 out of 1) achieved their ideal weight. In contrast, most dogs with low activity levels (7 out of 10) did not reach their optimal weight. Regarding QoL, all owners in the OW group reported a good QoL for their pets at the commencement of the study. In contrast, almost all owners in the OB group (7 out of 10) reported a poor QoL for their pets. Significantly, almost all dogs with good QoL (9 out of 10) reached their ideal weight. Quality of life was also significantly associated with activity levels ($X^2 = 10.909$; $p = 0.0276$). Furthermore, food-seeking behaviour was found to be significantly associated with achieving optimal body condition. Specifically, the majority of dogs exhibiting anxious food-seeking behaviour (8 out of 14) did not achieve optimal body condition by the conclusion of the study, whereas all dogs with low levels of food-seeking behaviour did so (4 out of 4).

Furthermore, the Mann-Whitney U test revealed a significant relationship between daily energy intake and the achievement of optimal body condition ($U = 10$; $p = 0.008$). The mean daily kcal consumed per

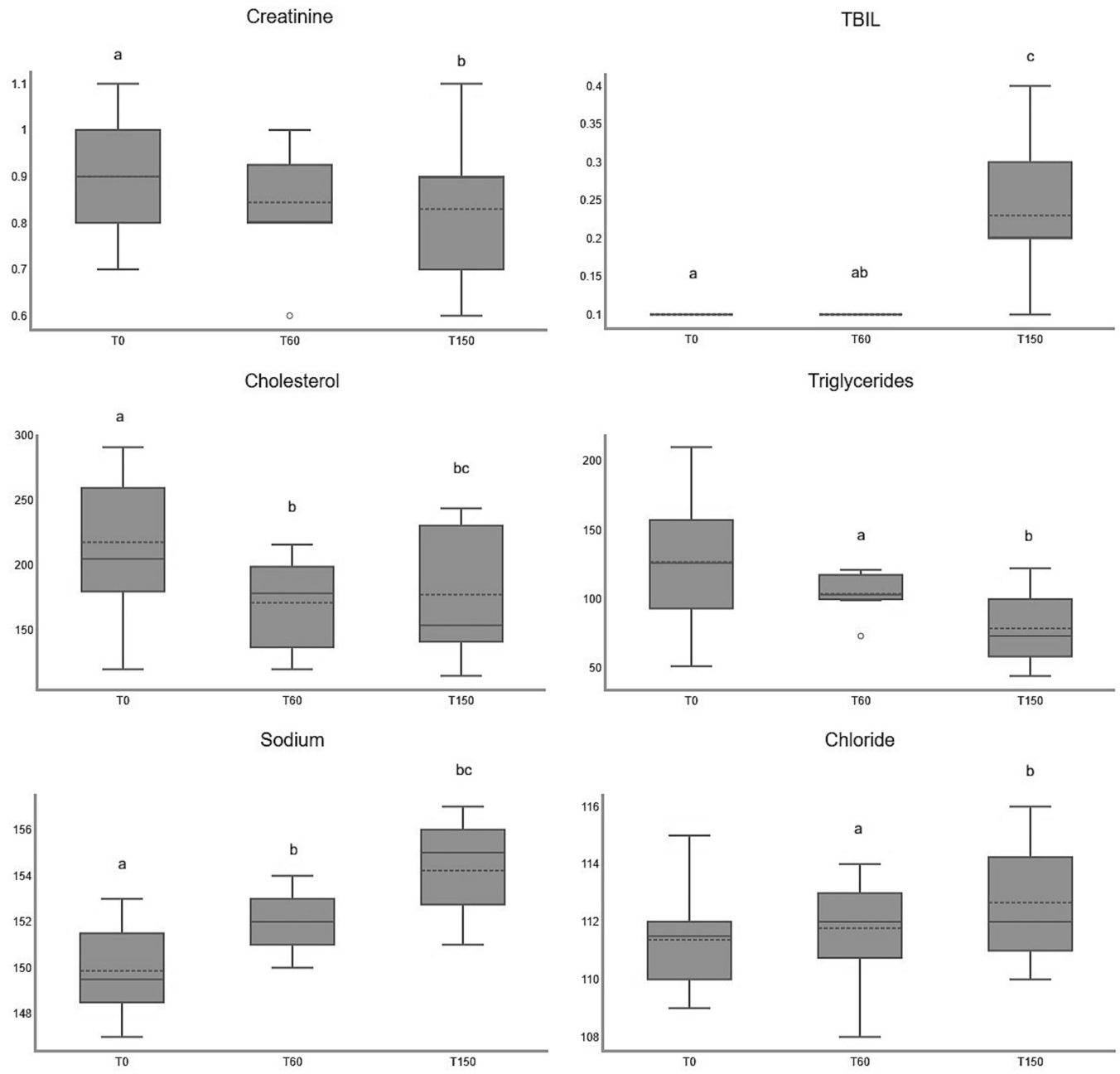

Fig. 4. Box-and-whisker plot representing the serum and urine parameters that showed significant changes during the weight loss phase (T0, T60, T150) in obese dogs. TBIL: total bilirubin. Different letters indicate statistically significant differences ($p < 0.05$) between time points.

kg of BW by dogs during the weight loss plan showed a good positive correlation with the BCS achieved at T150 ($r = 0.65$).

4. Discussion

All enrolled dogs were considered healthy based on clinical examination performed at the beginning of the weight loss period (T0), with the sole exception of being overweight or obese. The cohort was categorised using the 9-point body condition scale, which is currently the most widely accepted and validated clinical tool for standardising adiposity assessment in small animal practice (Freeman et al., 2011). This system ensures high inter-observer reliability compared to other subjective methods. There is a lack of consensus in the literature regarding the categorisation of dogs as NW, OW, or OB. While numerous studies have categorised OW dogs within the OB category (Jeusette et al., 2005; Peña et al., 2008; Radakovich et al., 2017; Tropf et al., 2017; Tvarijonaviciute et al., 2012b; Vecchiato et al., 2023), others have considered a BCS of 6/9 to be NW (Parker and Freeman, 2011; Radakovich et al., 2017). This variability complicates the comparison of our findings with previously published data, as most existing literature assumes similar pathophysiological and biochemical alterations in both OW and OB dogs.

Overweight and obesity are progressive diseases with a high prevalence in the canine species. These conditions result in poor welfare due to an increased risk of developing several concurrent diseases (Chandler, 2016; Clark and Hoenig, 2016; German et al., 2017; Mosing et al., 2013; Tropf et al., 2017 Tvarijonaviciute et al., 2012a; Wynn et al., 2016), and have a negative impact on QoL and longevity (Courcier et al., 2010; German et al., 2012a; Kealy et al., 2002; Montoya et al., 2023; Yam et al., 2016). As in human medicine, excess body fat in dogs is most often

Table 7
Comparison of serum and urine parameters (mean ± SD or median [range]) among overweight (OW) and obese (OB) dogs at T60 and T150 time points.

Parameters	OW T60	OB T60	*p-value OW-OB T60*	OW T150	OB T150	*p-value OW-OB T150*
Glucose (mg/dL) [74–143]	91.87 ± 8.32	97.5 ± 8.92	0.1901	86.25 ± 8.97	99.7 ± 18.62	0.0801
Creatinine (mg/dL) [0.5–1.8]	1.06 ± 0.19	0.9 ± 0.21	0.1107	1.01 ± 0.22	0.83 ± 0.16	0.0544
BUN (mg/dL) [7–27]	18.62 ± 4.03	15.1 ± 5.38	0.1099	19.62 ± 5.10	14.94 ± 5.79	0.0913
Phosphorus (mg/dL) [2.5–6.8]	3.84 ± 0.66	3.80 [2.70–4.10]	0.1946	3.70 ± 0.83	3.91 ± 3.90	0.5369
Calcium (mg/dL) [7.9–12]	9.72 ± 0.34	9.97 ± 0.40	0.1879	9.66 ± 0.25	10.02 ± 0.33	**0.0235**
Total protein (g/dL) [5.2–8.2]	6.52 ± 0.29	6.83 ± 0.27	**0.0369**	6.57 ± 0.30	6.83 ± 0.27	0.0772
Albumin (g/dL) [2.3–4.0]	3.05 ± 0.23	3.01 ± 0.35	0.7871	3.21 ± 0.24	3.19 ± 0.39	0.8886
Globulin (g/dL) [2.5–4.5]	3.49 ± 0.18	3.78 ± 0.34	**0.0420**	3.36 ± 0.28	3.64 ± 0.33	0.0796
ALT (U/L) [10–125]	42.50 [30–129]	51.00 [29–238]	0.5338	50.75 ± 18.06	56.2 ± 15.65	0.5026
ALP (U/L) [23–212]	52.12 ± 11.63	79.20 ± 43.09	0.1051	59.50 ± 18.13	79.11 ± 47.74	0.2926
GGT (U/L) [0–11]	0	0 [0–9]	0.2226	0 [0–1]	0 [0–14]	0.8617
Total bilirubin (mg/dL) [0–0.9]	0.10 [0.10–0.30]	0.10 [0.10–0.30]	0.3780	0.10 [0.10–0.30]	0.23 ± 0.16	0.2389
Cholesterol (mg/dL) [110–320]	175.00 ± 37.00	171.40 ± 32.61	0.7861	173.50 ± 30.82	177.56 ± 48.90	0.8431
Triglycerides (mg/dL) [10–100]	75.50 [51–180]	112.70 ± 35.66	0.0558	86.37 ± 20.74	78.43 ± 27.49	0.5349
Sodium (mmol/L) [144–160]	153.75 ± 1.83	152.50 ± 2.010	0.1924	153.75 ± 1.39	154.22 ± 2.05	0.5913
Potassium (mmol/L) [3.5–5.8]	4.16 ± 0.44	3.50 [3.4–4.2]	0.5308	4.32 ± 0.44	4.14 ± 0.40	0.3860
Chloride (mmol/L) [109–122]	112.37 ± 2.13	112.00 [104–114]	0.6838	112.00 [112–116]	112.67 ± 2.00	0.5130
C reactive protein (mg/dL) [0.0–1.0]	0.20 [1.0–1.7]	0.65 [0.1–2.5]	0.0869	0.50 [0.4–1.3]	0.64	0.4621
Urine specific gravity	1036.00 [1032–1060]	1035.55 ± 10.48	0.1835	1055.00 [1034–1060]	1034.10 ± 20.34	0.0776
Urine pH	6.71 ± 0.76	6.05 ± 0.95	0.1562	6.75 [6–7]	6.00 [5–6.5]	**0.0123**

ALP: alkaline phosphatase. ALT: alanine aminotransferase. BUN: blood urea nitrogen. GGT: gamma-glutamyl transferase. OB: obese. OW: overweight.
Statistical analysis was performed using unpaired *t*-tests and Mann-Whitney U tests. A p-value <0.05, indicated in bold, was considered statistically significant.

due to a positive imbalance between food intake and energy expenditure.

Causes of overweight and obesity in dogs have been broadly categorised as either animal-related or human-related. Middle-aged adult dogs (Courcier et al., 2010; Pegram et al., 2021; Suarez et al., 2022) and certain breeds, such as Labrador Retrievers and Golden Retrievers (Haddad, 2024; Raffan et al., 2016; Wallis and Raffan, 2020), are more prone to developing these pathologies. In the present study, the mean age of dogs was over five years in the three groups, and five Labrador Retriever and two Golden Retriever were included in the OW and OB groups.

The majority of the dogs included in the OW and OB groups were neutered. A significant correlation has been identified between neutering and the development of obesity in companion animals. A study by Robertson (2003) revealed that neutered dogs have a 2.8-fold increased probability of developing obesity. Furthermore, behavioural changes such as decreased physical activity and increased appetite have been observed in neutered dogs. Conversely, studies have demonstrated a decline in energy requirements following castration (Bermingham et al., 2014; Colliard et al., 2006; Jeusette et al., 2004; Mao et al., 2013; Phungviwatnikul et al., 2020). These findings confirm that neutering is a significant risk factor for the development of canine obesity and veterinary guidance at this stage is essential to prevent the subsequent onset of overweight or obesity in dogs (Haddad, 2024).

Owner behaviour, lifestyle and human-animal interaction are considered key factors influencing a dog's weight. A high quantity of food, often of poor nutritional quality, in conjunction with a lack of regular exercise, are among of the main contributors to canine overweight and obesity. Before the intervention, most owners relied on measuring cups, an unreliable method linked to widespread overfeeding (German et al., 2011). Additionally, the majority of dogs in the study were fed table scraps and treats on a near-daily basis and the majority of OW and OB dogs exhibited signs of food anxiety. Numerous authors have demonstrated that the consumption of homemade food, table scraps and treats has a detrimental effect on the development of obesity in dogs (Bland et al., 2010; Courcier et al., 2010; Gartner et al., 2025; Haddad, 2024; Heuberger and Wakshlag, 2011; Lund et al., 2006; Mao et al., 2013; Robertson, 2003; Sallander et al., 2010). The activity levels of all the OB dogs and the majority of the OW group were found to be significantly lower than those of the NW dogs. These findings are consistent with those reported by other authors, who have established a correlation between low physical activity and an increased risk of developing overweight and obesity (Bjørnvad et al., 2019; Bland et al., 2010; Courcier et al., 2010; Kluess et al., 2021; Mao et al., 2013; Orsolya Julianna et al., 2020; Robertson, 2003). Furthermore, owners considered that OB dogs had a poorer QoL than OW or NW dogs. A notable limitation of this study is that the questionnaire employed to assess QoL has not been formally validated. While this may affect the sensitivity of the behavioural metrics, we believe the potential for bias is mitigated by the fact that our findings align closely with research utilising

Table 8
Comparison of serum and urine parameters and systolic blood pressure (SBP) (mmHg) (mean ± SD or median [range]) between normal-weight (NW) dogs at baseline (T0) and overweight/obese dogs at the 150-day time point (T150) following weight loss intervention.

Parameters	NW T0	OW T150	OB T150	*p*-value *NW T0-OW T150*	*p-value NW T0-OB T150*
Glucose (mg/dL) [74–143]	94.60 ± 13.02	86.25 ± 8.97	99.7 ± 18.62	0.091	0.576
Creatinine (mg/dL) [0.5–1.8]	0.96 ± 0.22	1.01 ± 0.22	0.83 ± 0.16	0.617	0.144
BUN (mg/dL) [7–27]	17.60 ± 4.3	19.62 ± 5.10	14.94 ± 5.79	0.385	0.26
Phosphorus (mg/dL) [2.5–6.8]	3.77 ± 0.85	3.70 ± 0.83	3.91 ± 3.90	0.899	0.812
Calcium (mg/dL) [7.9–12]	9.45 ± 0.44	9.66 ± 0.25	10.02 ± 0.33	0.216	**0.005**
Total protein (g/dL) [5.2–8.2]	6.29 ± 0.45	6.57 ± 0.30	6.83 ± 0.27	0.128	**0.005**
Albumin (g/dL) [2.3–4.0]	3.03 ± 0.24	3.21 ± 0.24	3.19 ± 0.39	0.134	0.289
Globulins (g/dL) [2.5–4.5]	3.24 ± 0.27	3.36 ± 0.28	3.64 ± 0.33	0.37	**0.009**
ALT (U/L) [10–125]	67.80 ± 24.72	50.75 ± 18.06	56.20 ± 15.65	0.111	0.229
ALP (U/L) [23–212]	51.40 ± 15.86	59.50 ± 18.13	79.11 ± 47.74	0.337	0.129
GGT (U/L) [0–11]	0 [0–21]	0 [0–1]	0 [0–14]	0.999	0.939
Total bilirubin (mg/dL) [0–0.9]	0.10 [0.1–0.3]	0.10 [0.1–0.3]	0.23 ± 0.16	0.4	**0.025**
Cholesterol (mg/dL) [110–320]	174.30 ± 47.13	173.5 ± 30.82	177.56 ± 48.90	0.966	0.885
Triglycerides (mg/dL) [10–100]	57.6 ± 20.48	86.37 ± 20.74	78.43 ± 27.49	**0.010**	0.118
Sodium (mmol/L) [144–160]	152.00 ± 2.60	153.75 ± 1.39	154.22 ± 2.05	0.103	0.062
Potassium (mmol/L) [3.5–5.8]	3.92 ± 0.33	4.32 ± 0.44	4.14 ± 0.40	0.053	0.214
Chloride (mmol/L) [109–122]	112.33 ± 1.8	112 [112–116]	112.67 ± 2.00	0.319	0.715
C reactive protein (mg/dL) [0.0–1.0]	0.32 ± 0.21	0.50 [0.40–1.30]	0.64	**0.029**	**0.007**
Urine specific gravity	1055 [1020–1060]	1055 [1034–1060]	1034.10 ± 20.34	0.924	0.09
Urine pH	6.00 [5–7]	6.75 [6–7]	6.00 [5–6.5]	**0.001**	0.902
SBP (mmHg)	159.20 ± 21.30	180.12 ± 28.54	174.00 ± 34.78	0.109	0.314

ALP: alkaline phosphatase. ALT: alanine aminotransferase. BUN: blood urea nitrogen. GGT: gamma-glutamyl transferase. NW: normal-weight. OB: obese. OW: overweight.
Statistical analysis was performed using unpaired *t*-tests and Mann-Whitney U tests. A *p*-value <0.05, indicated in bold, was considered statistically significant.

established, validated QoL instruments (Flanagan et al., 2017; Gartner et al., 2025; German et al., 2012b; Yam et al., 2016). Nevertheless, the use of a non-validated tool necessitates a cautious interpretation of the absolute scores, and future studies should prioritise the use of psychometrically validated scales to enhance comparative robustness.

Prior to inclusion in the study, dogs were fed the same maintenance diet for a period of six weeks to minimize the potential effect of the diet on the analysed parameters. The daily food rations were calculated using the FEDIAF (2025) equations, considering activity levels (MER [kcal/day] = 110 x BW [kg]$^{0.75}$). Minor variations in the concentrations of certain serum parameters were observed based on the animals' BCS, with the majority of values remaining within the reference interval. Hypertriglyceridaemia was the most prevalent biochemical abnormality observed, particularly in OB dogs and to a lesser degree in the OW group; conversely, all dogs in the NW group showed normal values. The present findings are consistent with those of earlier research (Carzoli et al., 2025; Gomez-Fernandez-Blanco et al., 2024; Kim et al., 2025; Oba et al., 2023; Piantedosi et al., 2016; Piantedosi et al., 2020; Sarikaya and Gökçe, 2025; Tvarijonaviciute et al., 2012a). As demonstrated in previous studies (Forster et al., 2018; Gomez-Fernandez-Blanco et al., 2024; Kim et al., 2025; Oba et al., 2023;), marginally elevated cholesterol concentrations were also identified in the OB and OW groups. However, these differences did not reach statistical significance. The presence of dyslipidaemia in OW and OB dogs has been demonstrated to increase the risk of developing comorbidities such as pancreatitis, liver disease, ocular disease or gastrointestinal disorders and may be related to the severity of the obesity (McKenzie et al., 2025; Porsani et al., 2020a, 2020b; Xenoulis and Steiner, 2015). Furthermore, the increased concentration of CRP, an acute phase protein, in the OB group suggests a state of low-grade chronic inflammation related to the condition of obesity, as has been previously described in dogs (German et al., 2009; Radakovich et al., 2017; Vecchiato et al., 2023) and in humans (Cohen et al., 2021; Das, 2001; Stępień et al., 2014). In contrast, no significant difference was observed between the NW and OW groups, a result that aligns with the findings of Gomez-Fernandez-Blanco et al. (2024). The effect of obesity on canine cardiac function remains to be elucidated; however, the present results are consistent with those of recent studies describing hypertension in obese dogs (Carzoli et al., 2025Piantedosi et al., 2016; Piantedosi et al., 2020; Tvarijonaviciute et al., 2012b).

The characteristics of the prescribed weight loss diet are consistent with the recommendations of previous studies, as well as the European Pet Food Industry (FEDIAF, 2025) which recommends caloric restriction (metabolic energy content below 3.275 kcal/g) and a diet formulated to be high in protein (above 25%), and a dry matter fibre content below 12% (German et al., 2015a, 2015b; Vanelli et al., 2025). It has been demonstrated that this formulation is efficacious in promoting weight

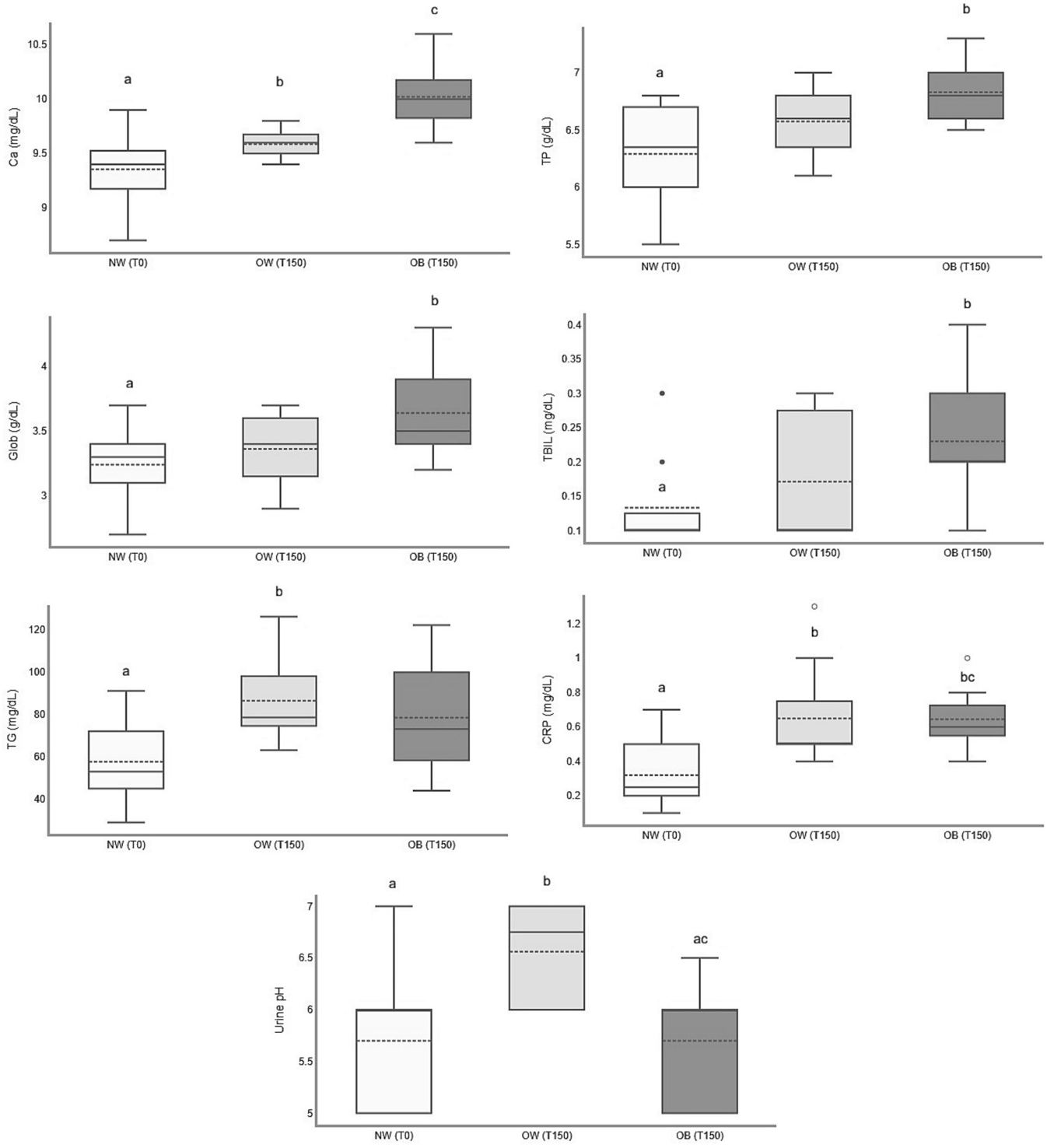

Fig. 5. Box-and-whisker plot representing serum and urine parameters that showed differences between normal-weight (NW) dogs at baseline and overweight (OW) and obese (OB) dogs at the end of study (T150). Ca: calcium. TP: total protein. Glob: globulins. TBIL: total bilirubin. TG: triglycerides. CRP: C reactive protein. Different letters indicate statistically significant differences between groups ($p < 0.05$).

and fat loss, whilst preserving muscle mass and optimising body composition. The equation developed by FEDIAF for obesity-prone dogs was used to estimate the daily energy requirements for weight loss, and thereby ascertain the initial ration (MER [kcal/day] $\leq$ 90 x BW $[kg]^{0.75}$) (FEDIAF, 2025). During the study, energy requirements were individually adjusted for each patient according to their weight loss evolution and body condition score. In the OW group, daily energy intake remained consistent throughout the entire weight loss period. For the OB group, a slightly lower daily caloric intake than in OW dogs was initiated, which necessitated a further reduction in the final months to achieve the proposed goals. The amount of caloric restriction required to achieve weight loss varies significantly among dogs, and a comparison of results with those of previous studies is complicated. Furthermore, the success of a weight loss programme for spayed females and mixed breed dogs is contingent upon a lower calorie requirement per metabolic BW (Vendramini et al., 2022). The daily energy intake of OB dogs at the

conclusion of the weight loss programme was higher than that described by Oba et al. (2023) (53.77 ± 8.25 kcal/kg$^{0.75}$) and a bit lower (64 [41–95] kcal/kg$^{0.75}$) than the study of Vecchiato et al. (2023). Dogs in the OB group demonstrated a higher degree of body fat, indicating that their mean daily energy intake must be lower than that of OW dogs to achieve adequate weight reduction. This necessity for stricter restriction in more severely obese dogs is primarily due to a significantly slower average rate of weight loss (0.48% vs 0.71% per week) and a greater loss of lean tissue mass compared to those with lower adiposity. Notably, this accelerated loss of lean tissue persists even after accounting for the overall percentage of weight lost, which directly contributes to a more pronounced decrease in metabolic rate. While the starting caloric intake might be similar, overweight dogs often preserve more lean mass, allowing them to maintain a higher metabolic rate and higher caloric requirement compared to more obese dogs, whose metabolic rate drops more sharply due to lean tissue catabolism and adaptive thermogenesis (Broome et al., 2023). These findings emphasise the necessity of precise body condition assessment and distinction between overweight and obese dogs, as their rates of weight loss, energy requirements, and the time required to achieve target goals vary.

The current study revealed significant metabolic changes in both the OW and OB dog groups during the weight loss phase. These findings emphasise the metabolic and physiological impacts of weight management and are consistent with recent evidence highlighting the association between BW and various clinical markers. Specifically, the observed decrease in serum cholesterol and triglycerides concurrent with weight loss is a consistent finding in both human and canine medicine (Nussbaumerova and Rosolova, 2023; Oba et al., 2023; Pan et al., 2023; Phungviwatnikul et al., 2022; Tvarijonaviciute et al., 2012a; Vekic et al., 2023). It is widely acknowledged that canine obesity is frequently associated with hyperlipidaemia, and a reduction in adipose tissue mass through dietary intervention has been demonstrated to enhance lipid profiles and insulin sensitivity (Gayet et al., 2004). The efficacy of the weight loss protocol is evidenced by the normalisation of initial hypertriglyceridaemia in the OB group and the significant drop in cholesterol levels in the OW and OB groups. The results obtained provide substantial support for the hypothesis that weight reduction constitutes an efficacious therapeutic strategy for the mitigation of metabolic dysregulation associated with excess adiposity (Carzoli et al., 2025; Cavalcante et al., 2023; Veiga et al., 2008).

Serum C-reactive protein concentration was found to be significantly higher in the OB dogs than in the other two groups. While the OB dogs showed a slight downward trend in CRP as their body condition improved and weight was lost, their values remained elevated in comparison to the NW cohort. Furthermore, the unexpected increase in CRP observed in the OW group during the weight loss phase, suggests that obesity-related inflammation is complex and not invariably responsive to short-term caloric restriction. These findings are consistent with the work of other authors (Vecchiato et al., 2023), who suggest that inflammatory conditions related to obesity are not necessarily responsive to caloric restriction, regardless of the type of obesity, and that a longer study duration may be necessary, as not all animals may reach their optimal weight within the given timeframe.

A comparison of the OB and OW groups revealed significant differences in serum levels of total proteins, globulin, and calcium during the weight loss phase. The higher concentrations of total protein and globulin in the OB group at T60 may suggest a state of chronic low-grade inflammation. As demonstrated in previous studies, obese dogs frequently have elevated serum protein and globulin levels, particularly within the alpha-2 globulin fraction (Carzoli et al., 2025; Piantedosi et al., 2016, 2020; Safadi et al., 2021; Vieira et al., 2022). This finding, when considered in conjunction with the existing body of evidence from human medicine linking obesity to the activation of the complement system (Al Haj Ahmad and Al-Domi, 2017; Oberbach et al., 2011; Shim et al., 2020), suggests a comparable inflammatory response in canines. As complement proteins in dogs are located within the beta globulin fraction (Kaneko et al., 2008), this could be responsible for the increase in this fraction observed in the obese dogs in our study. While a specific link between obesity and altered serum calcium has been less studied, the present findings are consistent with a previous report (Radakovich et al., 2017). The authors hypothesised that higher calcium concentrations in overweight/obese dogs may be attributed to a decreased serum water fraction in these animals.

Although the majority of these biochemical fluctuations remained within established reference intervals and were classified as mild, such findings must be interpreted with caution. While some evidence suggests that these changes may lack immediate clinical significance (German et al., 2009; Oba et al., 2023), their persistence at the upper limit of the physiological range may serve as an early biomarker of incipient metabolic dysregulation. These subtle but consistent alterations likely represent a pre-clinical inflammatory state that precedes overt systemic disease, suggesting that relying solely on whether a value falls within a reference interval may overlook critical metabolic shifts occurring during the progression of adiposity (Linder et al., 2012). Furthermore, the evolution of these parameters warrants continued investigation; as noted by Tvarijonaviciute et al. (2012b) and Piantedosi et al. (2016), a lack of resolution in these markers, even in the absence of clinical signs, indicates a protracted impairment of metabolic homeostasis. Consequently, these fluctuations should be closely monitored as they may signify the early stages of obesity-related comorbidities that persist despite weight reduction.

Published data regarding the impact of a weight loss programme on SBP in dogs reveals considerable heterogeneity (Carzoli et al., 2025; Piantedosi et al., 2016, 2020; Tvarijonaviciute et al., 2012b). In the present study, a statistically significant decrease was observed in the OB group by the conclusion of the study, although the final value remained higher than that of the NW dogs. In contrast, the OW group exhibited consistent SBP levels during the initial phase of the study, with an increase observed at the final measurement. Given that a proportion of dogs did not achieve their target body condition, these results suggest that a longer duration of follow-up may be essential to accurately assess the long-term impact of weight reduction on SBP.

The study corroborates the notion that owner-perception bias constitutes a substantial initial impediment to treatment, as the majority of owners underestimated their pets' obesity status at the outset (Kluess and Jones, 2023; Teixeira et al., 2020; Webb et al., 2020). However, the subsequent alignment of owner assessment suggests that the veterinary follow-up effectively improved owner literacy. It is evident that the process of actively monitoring weight and receiving veterinary guidance not only helps the dog lose weight but also educates the owner, leading to a more precise assessment of their pet's health status. This change in owner perception is a critical element for the long-term maintenance of weight and the prevention of future weight gain.

Successful weight loss has been found to be highly correlated with improvements in physical activity and QoL, thereby reinforcing the existence of a positive feedback loop in which enhanced mobility facilitates sustained weight management (German et al., 2012a). Furthermore, the QoL of most OB dogs was reported as poor, indicating that severe obesity significantly impairs daily function, a decline that was largely reversed upon successful weight loss.

Behavioural factors and caloric control presented specific challenges: dogs exhibiting anxious food-seeking behaviour were more likely to fail to achieve optimal BCS (Gartner et al., 2025). This finding suggests that the implementation of adjunctive behavioural modification strategies may be beneficial for these patients (Marliani et al., 2024). The studies indicated that the strongest predictor of success was adherence to the prescribed dietary regimen, with a significant correlation observed between the daily energy intake and the achievement of the target ($U = 10$; $p = 0.008$). Furthermore, a robust positive correlation was observed between mean daily kilocalorie consumption and final BCS ($r = 0.65$). This finding serves to reinforce the notion that precise and sustained caloric restriction constitutes the primary factor in achieving

therapeutic weight loss.

4.1. The present study was subject to several limitations

Methodological Limitations: the use of BCS to categorise each dog as NW, OW or OB is an imperfect measure of body fat mass, but a good correlation between BCS (nine-point scale) and dual-energy X-ray absorptiometry was reported (Bjørnvad et al., 2017). Despite its extensive utilisation, the BCS is inherently subjective and influenced by factors such as breed, morphology, and individual fat distribution patterns (Jeusette et al., 2010). However, to mitigate this potential challenge, all cases were assessed by highly-trained veterinarians, where higher inter-observer agreement is anticipated (German et al., 2006).

Population-related limitations: the reliance on a convenience sample of 30 client-owned dogs, as opposed to research subjects, introduces variability in signalment and environment, which may limit the generalisability of the findings. To mitigate the impact of nutritional variability, however, both the pre-study maintenance diet and the weight loss diet were provided to all dogs. This heterogeneity is conducive to the external validity and clinical relevance of our findings in relation to the general canine population.

Duration-related Limitations: the 150-day timeframe was insufficient for all patients to attain ideal condition. According to previous studies, the median duration of a complete weight loss cycle is nine months, with some dogs requiring more than twelve months to reach target (Flanagan et al., 2017; German et al., 2015b). However, the short-term design continues to emphasise that clinical benefits precede complete weight loss. Owners have reported a consistent enhancement in physical activity and an improvement in QoL during the intervention. Concurrently with these clinical benefits, biochemical parameters, specifically TG and cholesterol, showed a favourable trend. These findings provide robust evidence that the therapeutic benefits associated with weight reduction become manifest well in advance of attaining an ideal BCS.

In conclusion, the results of this investigation demonstrate that canine overweight and obesity are associated with evident metabolic alterations and health risks compared to normal-weight dogs. The study confirmed that dietary restriction, based on a high-protein, high-fibre, and low-calorie diet, produces significant physiological benefits in client-owned dogs, effectively mitigating the severe metabolic consequences of these conditions. The reduction in body weight and fat mass induced by caloric restriction resulted in improved lipid profiles; however, serum CRP concentrations did not exhibit a consistent or complete resolution. Notably, significant clinical improvements were manifest prior to patients attaining their ideal BCS, suggesting that metabolic recovery initiates early in the weight loss process.

Furthermore, owner commitment, the establishment of healthy feeding and exercise habits, and continuous veterinary team support were identified as crucial determinants for achieving optimal body condition and maintaining it over time. The present study highlights the importance of assessing body condition in all dogs and identifying the onset of overweight as early as possible to implementing individual changes for each patient. These modifications are intended to impede the progression of the disease and enhance the QoL. Importantly, these findings open a promising future research line focused on identifying early biomarkers of metabolic dysregulation associated with overweight and obesity. Detecting these shifts before overt clinical signs emerge would allow for the implementation of proactive dietary strategies and personalised management plans to arrest disease progression. Future research should also involve longitudinal assessments to determine the permanence of these metabolic improvements and ensure the long-term enhancement of quality of life in these patients.

Declaration of generative AI in scientific writing

During the preparation of this manuscript, the authors used Gemini (Google) for text editing (grammar, structure, spelling, punctuation, and formatting). The authors have reviewed and edited the output and take full responsibility for the content of this publication.

CRediT authorship contribution statement

Araceli Loste: Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Marta Borobia:** Writing – review & editing, Writing – original draft, Resources, Investigation, Funding acquisition, Conceptualization. **Alberto García:** Writing – review & editing, Resources, Investigation, Data curation, Conceptualization. **Lucía Escobar:** Writing – review & editing, Visualization, Resources. **Laura Navarro:** Writing – review & editing, Visualization, Resources, Investigation, Formal analysis, Data curation, Conceptualization.

Informed consent statement

Informed consent (Supplementary Materials: S2) was obtained from all the owners of the animals involved in this study.

Institutional review board statement

This study was approved by the Ethics Committee for Animal Experiments of the University of Zaragoza and authorized by the competent authority (Project License PI41/22).

Declaration of competing interest

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Appendix A. Supplementary data

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