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Effects of overweight on serum 25-hydroxyvitamin D macro and microelements and hematological parameters in healthy adult client-owned cats

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Minerals and trace elements are crucial for maintaining normal body metabolism, but limited knowledge exists about their connection to being overweight in cats. This study was conducted to evaluate potential changes in hematological factors and the levels of minerals or trace elements in the serum of overweight cats. A cross-sectional study was carried out, and seventy-two client-owned, healthy adult cats were randomly selected from those visiting veterinary clinics in Mashhad. The cats were divided into two groups on the basis of their Body Condition Score (BCS): the ideal weight group (BCS = 5) and the overweight group (BCS ≥ 6). Serum concentrations of calcium, phosphorus, iron, magnesium, zinc, copper, and 25(OH) vitamin D and hematological parameters, including red blood cell (RBC) count, white blood cell (WBC) count, hemoglobin, mean corpuscular hemoglobin (MCH), corpuscular hemoglobin concentration (MCHC), hematocrit, mean corpuscular volume (MCV), and red cell distribution width (RDW), were measured. Correlations between the variables were also determined. Overweight cats had significantly higher levels of iron, hematocrit, hemoglobin, RBC, and MCHC. Conversely, the levels of phosphorus, RDW, WBC, neutrophils, and monocytes were significantly lower in overweight cats than in those with ideal body weights. There were no significant differences in the other parameters measured between overweight and ideal-weight cats. Additionally, in the entire population and the normal-weight group, 25(OH) vitamin D showed a positive correlation with calcium and a negative correlation with magnesium. In conclusion, being overweight in cats causes changes in their hematological parameters and the concentrations of certain elements in blood serum, specifically iron and phosphorus. These changes were in reference interval thus, the clinical significance of these alterations requires further investigation especially in obese cats.

Keywords Body condition score (BCS), Cats, Hematology, Overweight, Trace elements, 25(OH)vitamin D

Excess weight and body fat amount result from an imbalance between food intake and energy expenditure[1]. The prevalence of overweight and obesity has also increased in companion animal populations, similar to trends observed in humans. Earlier studies reported that 6–12% of cats were overweight. However, recent research has indicated that 19–29% of cats are overweight and that 6–8% are obese, raising serious concerns about weight-related diseases in veterinary medicine. Overweight and obesity in cats can lead to serious health problems, such as diabetes mellitus, urinary tract diseases, hepatic lipidosis, lameness[2], respiratory problems, dermatopathy, and neoplasms[3].

Vitamin D is a fat-soluble compound. Unlike humans, dogs and cats cannot synthesize vitamin D through their skin, so they must obtain it from their diet. In humans, obesity has been associated with lower amounts of vitamin D. This is because fat tissue stores vitamin D, causing it to be absorbed and retained more in fat. As a result, less vitamin D is available in the bloodstream[4]. However, this effect has not been confirmed in dogs[5]. Additionally, people with more body fat tend to have more vitamin D stored in their fat tissues. If vitamin D intake is reduced or there is a severe deficiency of cholecalciferol, the amount of vitamin D in the blood does not

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decrease quickly, as vitamin D is gradually released from fat tissue[6]. Overall, these observations emphasize the complex relationship between vitamin D and body fat.

A variety of physiological and biochemical processes depend on minerals and trace elements, which are necessary micronutrients[7]. For example, copper acts as a cofactor in several enzymatic reactions that are necessary for cell growth and development. Moreover, zinc-containing metalloenzymes and metalloproteins are involved in a wide range of cellular processes, including metabolism, redox signaling, gene expression, and maintaining membrane structure[8].

There is evidence suggesting that an imbalance in minerals may be linked to the development and worsening of various health conditions[7,9]. However, research on the effects of trace elements and minerals related to overweight and obesity in veterinary medicine is relatively limited compared with studies conducted in humans. Previous studies have indicated a correlation between overweight/obesity and trace element contents in humans[6,10,11] and dogs[12]. Cihan et al.. (2023) investigated the relationship between obesity in dogs and the concentrations of certain minerals in their bodies. Compared with the control group, the obese group presented significantly higher serum iron concentrations. However, no significant relationships were observed with other elements, such as copper, zinc, calcium, phosphorus, and magnesium[12]. However, findings on the relationship between obesity and trace element status are contradictory, underscoring the need for further research.

In general, there are many more overweight cats than obese cats. Given that being overweight can be a warning of obesity, it seems essential to investigate potential disorders in the population of overweight cats. Furthermore, it is necessary to determine whether being overweight may predispose them to alterations in hematological parameters and the levels of minerals and trace elements. While there has been extensive research on how being overweight affects health markers (such as blood parameters and mineral concentrations) in humans, similar studies in cats are very limited. We hypothesized that that feline overweight does not influence hematological parameters, the 25(OH) vitamin D concentration, or the amounts of minerals and trace elements. In this study, we investigated whether being overweight leads to changes in blood components, 25(OH) vitamin D, minerals, and trace elements in cats.

Materials and methods

This manuscript reports on a study involving domestic cats and adheres to the ethical standards set forth in the 1964 Declaration of Helsinki and its later amendments. All animal studies were approved by the appropriate ethics committee of Ferdowsi University of Mashhad (IR.UM.REC.1401.028, 30.10.2021), ensuring compliance with ethical guidelines for the treatment of animals in research. In addition, the authors claim that the study is reported in accordance with the ARRIVE guidelines. 72 healthy adult cats were randomly selected from those visiting veterinary clinics in Mashhad, located in northeastern Iran, for routine check-ups (36.2972°N, 59.6067°E). The inclusion criteria for this research were general health, non-pregnant or non-lactating status, and an age exceeding 7 months. To assess the cats' health, clinical examinations, including rectal temperature, capillary refill time (CRT), oral examination, lymph node and abdominal palpation, and respiratory and heart examinations (including heart rate and heart sounds), and orthopedic evaluations, were performed by an experienced veterinarian. In addition, hematological and biochemical tests, as well as histories of vaccination, surgery, anti-parasitic therapy within the past six months, and medication use in the past month, were evaluated. Only cats considered healthy on the basis of these criteria were included in the study. Cats that were non-fasting, pregnant, lactating, less than 7 months of age, restless or had received medications or dietary supplements in the past month were excluded from the study.

All the cat owners involved in the study completed a form to provide their informed consent and filled out a questionnaire with details about their cats. The questionnaire collected information on the cats' age, sex, reproductive status, breed, living conditions, diet, and overall health.

The cats had a variety of diets, including commercial cat food, homemade food, or a combination of both. Therefore, the amounts of vital minerals and vitamin D in their meals could not be accurately measured.

To evaluate the physical condition of the cats, we employed a 9-point Body Condition Scoring (BCS) method[13]. This system is widely recognized and has been used in recent studies[3,14,15]. The cats were divided into two groups on the basis of their BCS:

Ideal Group: This group included 36 healthy adult cats with a BCS of 5, indicating an ideal body condition (mean age: 28.75 months, median age: 18.5 months). The mean weight of these cats was 3.78 kg (median: 4 kg). The cats in this group were of various breeds, including domestic shorthair (DSH, $n=22$), Persian ($n=10$), Siamese ($n=1$), Scottish ($n=2$), and mixed breeds ($n=1$). There were 13 females and 23 males, with an equal split of neutered ($n=18$) and intact ($n=18$) cats. Additionally, eight cats were kept outdoors, and 28 cats were kept indoors. The cats were fed either a commercial diet ($n=12$), homemade food ($n=4$), or both ($n=20$). The cats in this group ranged in age from 7 months to 10 years. These cats ranged in age from 7 months to 2 years ($n=23$), > 2–7 years ($n=11$), and more than 7 years ($n=2$).

Overweight Group: To simplify the analysis, both overweight and obese cats were categorized together as "overweight." This group consisted of 36 adult cats (mean age: 40.86 months, median age: 33 months), with a BCS of 6 or higher. Among them, 16 had a BCS of 6, 14 had a BCS of 7, 4 had a BCS of 8, and 2 had a BCS of 9. The mean weight of these cats was 5.6 kg (median: 5.5 kg). This group included breeds such as domestic shorthair (DSH, $n=21$), Persian ($n=9$), British ($n=1$), Scottish ($n=3$), and mixed breeds ($n=2$). Among these, 12 were females, and 24 were males. Additionally, 26 of the cats were neutered, and 10 were intact. Four cats were kept outdoors, while 32 were kept indoors. The cats were fed either a commercial diet ($n=17$), homemade food ($n=2$), or both ($n=17$). Their ages ranged from 9 months to 11 years. The cats ranged in age from 7 months to 2 years ($n=15$), >2–7 years ($n=19$), and more than 7 years ($n=2$).

The cats were fasted for 12 h before blood was collected. Prior to sampling, the cats were allowed adequate time to acclimate to the room and reach a calm, relaxed state while becoming familiar with their surroundings. A total of 7 mL of blood was collected from the jugular vein when the cat was calm and rested. Two mL of blood was placed into a tube containing the anticoagulant EDTA (Farzaneh Arman CO, Isfahan, Iran) for hematological analysis. The remaining blood was transferred into clot activator tubes (Hebei Xinle Sci & Tech Co., Shijiazhuang, China) to separate the serum. The samples were immediately placed on ice and transported to the laboratory. In the laboratory, the blood samples were centrifuged for ten minutes at 1800 × g. After being frozen at -20 °C for one week, the serum samples were moved to -80 °C for long-term storage until they were ready for analysis. The hematological tests were conducted within one hour of sample collection.

Various parameters, including calcium, phosphorus, magnesium, zinc, copper, and iron, were measured in the blood of the cats. Commercial reagents from Biorex Fars (Shiraz, Iran) and Pars Azmoon (Tehran, Iran), as well as an autoanalyzer (Biotecnica, BT 1500, Rome, Italy), were used for these measurements. Control serum (Pars Azmoon, Tehran, Iran) was used to assess the accuracy of the measurements.

Hematological parameters, including red blood cells (RBCs), white blood cells (WBCs), hemoglobin, mean corpuscular hemoglobin (MCH), corpuscular hemoglobin concentration (MCHC), hematocrit, mean corpuscular volume (MCV), and red cell distribution width (RDW), were measured via the Nihon Kohden Veterinary Cell Counter (Celltac α, MEK-6450, Tokyo, Japan).

Blood smears were prepared and stained with a commercial hematological stain (Wright-Giemsa solution from Roozazmoon, Tehran, Iran) to perform the differential white blood cell count and also examine for hemoparasites. Smears were examined microscopically for hemoparasites previously reported in cat in Iran, including hemotropic Mycoplasma spp., Cytauxzoon spp., Babesia spp., and Hepatozoon spp. At least 20 fields within the monolayer region of each smear were carefully evaluated, and all samples were negative for hemoparasites.

Serum 25(OH) vitamin D concentrations were measured via ELISA with a commercial diagnostic kit for 25(OH) vitamin D (Pishgaman Sanjesh, Tehran, Iran) that had previously been validated in our laboratory (Ferdowsi University Veterinary Hospital Laboratory, Mashhad, Iran) for animal serum. The interassay and intra-assay CVs of the kit were determined via fully nested ANOVA and were 9.78% and 5.58%, respectively. The lower and higher limits of detection are 4 ng/ml and 120 ng/ml, respectively. The test sensitivity was 1.8 ng/ml. Additionally, the specificity for detecting various forms of vitamin D, including vitamin D2, vitamin D3, 25(OH) vitamin D2, and 25(OH)vitamin D3, is < 0.1%, < 0.1%, 83%, and 100%, respectively.

The data analysis was conducted via SPSS software (IBM, SPSS version 23, USA). The Shapiro-Wilk test was applied to evaluate the normality of the variable distributions. The calcium, magnesium, RBC, Hgb, MCV, MCH, and RDW had a normal distribution, whereas the other measured data, including phosphorus, zinc, copper, iron, HCT, and MCHC, had a non-normal distribution. For normally distributed data, the t-test was employed; for non-normally distributed data, the Mann-Whitney U test was utilized. The results of the t-test are shown as the means ± SEs, whereas the Mann-Whitney U test results are presented as the medians and percentiles (25th and 75th). $p \leq 0.05$ was considered significant. Furthermore, correlations among the variables were examined for the entire sample, as well as separately for the normal and overweight groups. Pearson's correlation was used for normally distributed data, and Spearman's correlation was used for non-normally distributed data. $p \leq 0.05$ was considered as significant correlation.

Results

Overweight cats had significantly higher amounts of iron, hematocrit, hemoglobin, RBC, and MCHC compared to ideal weight cats ($p \leq 0.05$, Table 1). Conversely, overweight cats had significantly lower concentrations of phosphorus, RDW, WBC, neutrophils, and monocytes than ideal cats did ($p \leq 0.05$, Table 1). There were no significant differences in the other measured parameters between the two trial groups ($p > 0.05$, Table 1).

Additionally, we found that 25(OH) vitamin D was positively correlated with calcium and negatively correlated with magnesium in the overall population and the ideal group (Table 2).

Discussion

Overweight/obesity are recognized as important health problems in companion animal medicine[2]. These conditions may lead to changes in hematological factors and abnormal metabolism of essential nutrients. Minerals and trace elements are important for maintaining normal metabolism in the body because they support numerous enzymatic processes by stabilizing proteins and enzymes and serving as cofactors[16]. Given that overweight and obesity are common in the feline population, this study aimed to determine the differences in 25(OH)vitamin D, minerals and trace elements amounts, and hematological factors between ideal and overweight/obese cats. We showed that overweight cats had significantly greater amounts of PCV, HG, RBC, and MCHC. However, RDW was significantly ($p \leq 0.05$) lower in overweight cats. Martins et al.. studied 45 apparently healthy cats and divided them into three groups on the basis of their body condition score: control, overweight, and obese. Minimal changes in hematological parameters were found, except for increases in RDW and MCV[3]. Similarly, in a large cross-sectional retrospective study involving 100,854 men and 105,853 women from the UK Biobank cohort, overweight and obese individuals presented increases in hematocrit, hemoglobin, and red blood cell count[17]. There may be a link between hematological variations and being overweight or obese via the action of hormones such as estrogens, glucocorticoids, and leptin. Erythropoietin (EPO), a hormone critical for red blood cell production, may not be the only factor in erythropoiesis among overweight/obese individuals[17]. In chronic kidney failure, where EPO generation is reduced due to kidney impairment, hemoglobin levels are higher with a higher Body Mass Index (BMI)[18]. Furthermore, compared with patients with a normal weight, obese people need lower doses of EPO to maintain hemoglobin levels, suggesting that

Variable	Ideal	Overweight	*P*-value	Reference interval
25(OH)D (ng/ml)	74.78 ± 6.04	82.04 ± 5.7	0.385	-
Magnesium (mg/dl)	2.85 ± 0.04	2.84 ± 0.06	0.941	1.5–2.5[2]
Calcium (mg/dl)	9.63 ± 0.15	9.45 ± 0.09	0.297	6.2-10.2[1]
Phosphorous (mg/dl)	6.45 (4.92–7.15)	5.05 (4.41–6.1)	***0.003***	4.5–8.1[1]
Zinc (μg/dl)	111 (98.22–134.75)	117 (87.7–141)	0.836	-
Copper (μg/dl)	77.5 (64.72–97.07)	70.85 (53.52–88.5)	0.153	-
Iron (μg/dl)	140.5 (129.25–153.75)	167 (139.25–176)	***0.004***	68-215[1]
HCT (%)	33.05 (31.17–36.4)	37 (32.57–40.37)	***0.008***	24–45[3]
Hemoglobin (g/dl)	10.83 ± 0.26	12.37 ± 0.23	***0.000***	8.0-15[3]
RBC ($\times 10^{12}$/L)	7.93 ± 0.22	8.71 ± 0.20	***0.011***	5.0-10.0[3]
MCV (fL)	42.18 ± 0.46	42.27 ± 0.56	0.906	39–55[3]
MCH (pg)	13.74 ± 0.18	14.34 ± 0.27	0.073	13–17[4]
MCHC (%)	32.35 (31.50–33.55)	33.20 (32.5–33.8)	***0.023***	31–35[3]
RDW (%)	14.7 ± 0.16	14.23 ± 0.12	***0.026***	17–21[3]
WBC ($\times 10^9$/L)	6.700 (5.150–9.400)	5.250 (4.600–8.575)	***0.035***	5.5-19.5[3]
Neutrophil ($\times 10^9$/L)	3.981 (3.117–5.356)	2.755 (2.256–4.992)	***0.018***	2.5-12.5[3]
Lymphocyte ($\times 10^9$/L)	2.353 (1.668–3.168)	1.942 (1.504–2.787)	0.102	1.5–7[3]
Eosinophil ($\times 10^9$/L)	0.349 (0.203–0.567)	0.364 (0.196–0.525)	0.665	0-1.5[3]
Monocyte ($\times 10^9$/L)	0.129 (0.62–0.342)	0.052 (0–0.127)	***0.005***	0-0.85[3]

Table 1. Minerals, trace elements, and hematological parameters in cats with ideal and overweight BCS. For normally distributed data, the table shows the mean ± SE values. For non-normally distributed data, the median with the 25th and 75th percentiles are presented. A p-value of ≤ 0.05 was considered statistically significant. Reference intervals for the cat:[1]Kaneko et al. (2008) and[2]Willard et al. (2012);[3]Rizzi et al. (2010) and[4] Krimer et al. (2011). Abbreviations: 25(OH)vitamin D, 25(OH)D; HCT, hematocrit; RBC, red blood cell count; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW, red blood cell distribution width; WBC, white blood cell count.

Variable	Group	25(OH)D	Mg	Ca	*p*	Zn	Cu	Fe	HCT	Hg	RBC	MCV	MCH	MCHC	RDW	WBC
25(OH)D	Ideal	1	-0.408*	0.346*	-0.020	-0.024	-0.223	-0.029	0.114	0.124	0.165	-0.194	-0.166	-0.020	0.153	-0.051
	Overweight	1	-0.136	0.114	-0.135	-0.104	-0.125	0.090	-0.314	-0.120	-0.192	-0.066	0.145	0.154	-0.052	0.156
	Total	1	-0.241*	0.240*	-0.110	-0.076	-0.123	0.017	-0.063	0.060	0.028	-0.122	0.039	0.086	0.038	0.009
Mg	Ideal		1	-0.032	-0.108	0.211	-0.037	0.066	-0.192	-0.116	-0.137	-0.061	0.139	0.309	0.022	-0.093
	Overweight		1	-0.009	0.032	0.502**	0.132	0.256	0.278	0.289	0.319	-0.128	-0.131	0.083	0.212	-0.027
	Total		1	-0.018	-0.047	0.344**	0.076	0.176	0.085	0.096	0.119	-0.104	-0.051	0.125	0.114	-0.032
Ca	Ideal			1	0.093	0.143	-0.099	0.007	0.215	0.296	0.307	-0.307	-0.156	0.156	0.037	0.111
	Overweight			1	0.193	0.019	0.145	-0.001	-0.037	0.049	0.083	-0.239	-0.060	-0.127	-0.096	0.058
	Total			1	0.162	0.085	0.035	-0.051	0.081	0.122	0.173	-0.261*	-0.122	0.046	0.029	0.125
P	Ideal				1	-0.315	0.064	-0.167	0.044	-0.010	0.171	-0.279	-0.333*	-0.171	0.339*	0.596**
	Overweight				1	-0.082	-0.010	0.242	-0.105	-0.242	-0.095	0.058	-0.033	-0.108	0.119	0.156
	Total				1	-0.205	0.066	-0.141	-0.123	-0.245*	-0.037	-0.145	-0.279*	-0.162	0.344**	0.514**
Zn	Ideal					1	0.433**	0.469**	-0.252	-0.117	-0.202	-0.131	0.133	0.389*	-0.248	-0.280
	Overweight					1	0.681**	0.169	0.193	0.197	0.114	0.164	0.191	0.277	-0.104	0.211
	Total					1	0.633**	0.374**	0.009	0.041	-0.009	0.005	0.053	0.209	-0.156	-0.064
Cu	Ideal						1	0.190	-0.050	-0.057	-0.057	-0.035	-0.028	0.007	-0.125	0.115
	Overweight						1	-0.086	0.122	0.220	0.220	-0.043	0.212	0.245	0.037	0.429**
	Total						1	0.029	-0.068	-0.002	-0.058	-0.026	0.084	0.217	-0.047	0.254*
Fe	Ideal							1	-0.052	0.078	-0.130	0.077	0.264	0.346*	-0.349*	-0.302
	Overweight							1	-0.009	-0.055	-0.094	0.188	0.049	-0.099	0.064	0.028
	Total							1	0.057	0.122	-0.005	0.107	0.177	0.232	-0.198	-0.215

Table 2. The correlation values between certain measured indices in the whole population, ideal and overweight cats. * and ** denote significant correlation at $p < 0.05$ and $p < 0.01$, respectively. 25(OH)vitamin D, 25(OH)D; Mg, magnesium; Ca, calcium; P, phosphorous; Zn, zinc; Cu, copper; Fe, iron; HCT, hematocrit; RBC, red blood cell count; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW, red blood cell distribution width; WBC, white blood cell count.

other factors can compensate for EPO deficits[17,19,20], with one of the most prominent candidates being leptin. Leptin, a hormone produced by fat tissue[17], is elevated in overweight individuals and may stimulate red blood cell production[20]. Moreover, glucocorticoids contribute to the production of red blood cells and are linked to obesity[17]. Furthermore, estrogens produced in fat tissue increase iron availability and suppress hepcidin secretion, facilitating red blood cell production and supporting the renewal of blood stem cells[17]. In this study, overweight cats showed a significant decrease in RDW. This finding contrasts with the results of Martins et al., who reported an increase in RDW in overweight and obese cats. Martins indicated that further studies are needed to clarify the cause[3]. In human studies, the association between obesity and RDW alterations has been investigated more extensively[21–23]. Fujita et al. reported higher RDW in overweight adolescents compared to individuals with normal weight; however, they did not observe an increase in RDW in overweight mice[22]. In the present study, we also observed a decrease in RDW in overweight cats. This finding is most likely attributed to a reduced number of immature red blood cells in circulation and does not appear to be associated with chronic inflammation. The lower WBC counts in overweight cats further support the absence of inflammatory processes in these cats. Given the scarcity of veterinary research on this topic, additional studies are warranted to further investigate the relationship between overweight and RDW in cats and to clarify the possible underlying mechanisms.

WBC counts in overweight cats in our study were significantly ($p \le 0.05$) lower than those in cats with ideal body weights. However, they remain within the reference interval. Martins et al.. (2023) reported no significant changes in the WBC count, their study revealed a downward trend in the WBC count from the ideal weight group to the overweight group in cats[3]. In contrast, other studies have shown that WBC counts tend to increase with overweight and obesity in humans[24–26], dogs[27], and cats[28]. Based on the studies cited, it has been suggested that overweight/obesity may induce low-grade systemic inflammation, which could result in elevated WBC counts. Nevertheless, some studies, particularly in humans[29] and dogs[1], did not observe an increase in WBC counts with overweight or obesity. The reduced WBC counts in overweight cats suggest that the hypothesis of low-grade inflammation in these cats is invalid, indicating that other factors are responsible for this decline. Despite the statistical significance of the difference between overweight and ideal cats, its clinical significance is unclear, as the WBC levels remain within the normal range for both groups.

It has long been known that vitamin D plays an important role in maintaining the balance of calcium and phosphorus in the body. For many years, it was thought that these are the only functions of vitamin D in metabolic processes. However, recent investigations have increasingly concentrated on exploring other effects of vitamin D[4]. This study revealed a significant ($p \le 0.05$) positive relationship between total calcium concentrations in the body and 25(OH) vitamin D concentrations. This result is consistent with vitamin D's well-established roles in maintaining normal serum calcium concentrations, which include boosting calcium reabsorption in the kidneys, improving calcium absorption in the intestines, and facilitating calcium release from bones[30]. Additionally, the present study revealed a significant negative correlation between magnesium concentrations and 25(OH) vitamin D contents. Previous studies have indicated a positive interaction between magnesium and 25(OH) vitamin D, as magnesium serves as a cofactor in the enzymatic processes responsible for activating or deactivating vitamin D. Furthermore, magnesium absorption in the intestines might be increased by active vitamin D[31,32]. It has been demonstrated that vitamin D supplementation increases magnesium concentrations in some cases. On the other hand, taking too many supplements could have the reverse effect and lower serum magnesium amounts[32].

Calcium and magnesium are crucial for maintaining the ion balance, the structure of mitochondrial membranes, oxidative phosphorylation, and the production of ATP. Phosphorus is involved in many metabolic processes, particularly energy metabolism and enzymatic reactions. In addition, iron has several vital functions, including being part of hemoglobin and myoglobin, immune regulation, energy metabolism, and other enzymatic reactions[8]. Few studies have investigated phosphorus concentrations in overweight dogs and cats[3,12,27]. In the studies available, no significant changes in phosphorus concentrations were observed[3,12,27], but in the present study, phosphorus concentrations were found to be significantly lower in overweight animals than in those with an ideal body weight. Similar results[33–35] were observed in investigations involving humans, whose serum phosphorus concentrations were controlled within the normal range. Food intake triggers the release of insulin and the process of phosphorylating proteins and carbohydrates. This, in turn, increases the absorption of phosphorus from the blood into the skeletal muscles and liver, leading to a decrease in the level of phosphate in the bloodstream. Insufficient phosphorus intake limits ATP production, which signals to the brain to increase food intake, leading to overeating. Reduced phosphorus concentrations and diminished ATP synthesis lead to decreased thermogenesis and may increase weight gain efficiency. Low phosphorus concentrations are also associated with lower energy expenditure and physical activity, as well as impaired oxygen transport to tissues[35]. Insulin can also promote phosphorylation, which can potentially reduce the availability of phosphorus, affecting its use in processes such as ATP synthesis[36]. However, it is unclear whether different phosphate concentrations affect weight increase or whether weight gain causes phosphate disruption. Hence, long-term research is necessary to better examine this association.

Iron is essential for many metabolic processes, such as oxygen transport, cell growth, and gene expression regulation. However, in excess, it can be detrimental and increase oxidative stress[37]. The results of present study revealed that iron amounts were significantly ($p \le 0.05$) higher in overweight cats than in those with an ideal weight. A study on dogs revealed a similar increase in iron concentrations as the body condition score (BCS) increased, and the authors attributed this to overeating[12]. Overweight and obese people frequently have reduced iron concentrations in humans, which is believed to be due to chronic inflammation caused by excess weight[38]. However, the reason for the differences in the reported results between dogs and humans is not clear and requires further investigation.

Our study has some limitations that need to be addressed in subsequent investigations. Although there were significant differences in a few parameters between the two groups, these differences remained within the normal range, raising questions about their potential clinical importance. This study recognizes the typical drawbacks of observational research, especially cross-sectional research, in that the associations described may not be causal and require further investigation via alternative methodologies.

Furthermore, the cats were owned by clients and fed a variety of diets (homemade, commercial, or a combination), which made it difficult to determine the precise nutritional value of their food. We attempted to balance the two groups in terms of age, sex, breed, nutrition, housing conditions, etc., to minimize their impact. However, since this is not entirely achievable, there remains a risk that these factors may still influence the results. More than half of the overweight cats had a BCS of 6–7, whereas fewer had a BCS of 8–9, suggesting varying degrees of overweight. Furthermore, the study did not account for breed or neuter status when the groups were selected, which could have influenced the results.

In conclusion, the results of this study indicate that overweight in cats is significantly associated with changes in hematological parameters and the concentrations of certain minerals and trace elements, including serum iron and phosphorus. While these changes may contribute to clinical disorders in overweight cats, the values observed in this study remained within the reference range. Therefore, further research with a larger sample size and especially in obese cats with stricter control of factors such as diet, age, and breed, along with the measurement of additional trace elements, is necessary to determine the precise clinical significance and potential consequences of these changes.

Data availability

The data that support the findings of this study are not openly available due to reasons of sensitivity and are available from the corresponding author upon reasonable request. Data are located in controlled access data storage at Ferdowsi University of Mashhad.

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E.V: writing-original draft; M.M: methodology establishment; E.V: sample collection and measurements; M.M.: data analysis; M.M.: funding acquisition; M.M and E.V.: review and editing final manuscript. All the authors have read and agreed to the published version of the manuscript.

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Declarations

Competing interests
The authors declare no competing interests.

Ethics approval and consent to participate
This manuscript reports on a study involving domestic cats and adheres to the ethical standards set forth in the 1964 Declaration of Helsinki and its later amendments. All animal studies were approved by the appropriate ethics committee of Ferdowsi University of Mashhad (IR.UM.REC.1401.028, 30.10.2021), ensuring compliance with ethical guidelines for the treatment of animals in research. The involvement of each cat was approved by its owner. Cat owners provided informed consent for participation and filled out a questionnaire about their cats.

Additional information
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