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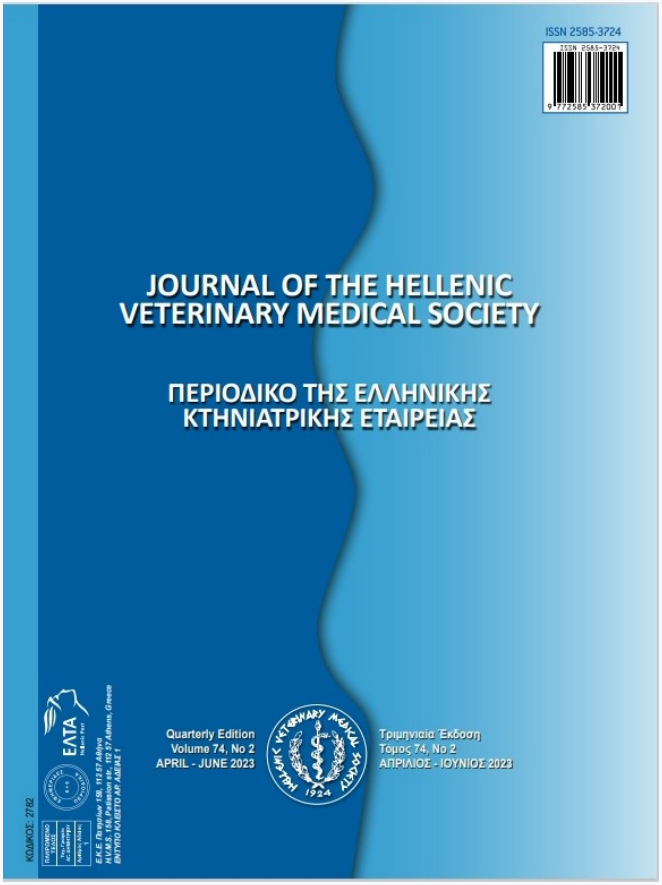

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Research article
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Comparison of selected levels of serum elements / minerals in obese dogs

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ABSTRACT: Obesity is the most common metabolic disorder defined as excessive adipose tissue accumulation in the body. Elements and minerals are crucial for biological metabolism. There have been limited studies based on the relationship between elements/minerals and obesity in dogs. The study was aimed to evaluate possible alterations of selected serum elements/minerals -copper (Cu), iron (Fe), zinc (Zn), selenium (Se), chromium (Cr), nickel (Ni), calcium (Ca), phosphorus (P), magnesium (Mg), boron (B) and cobalt (Co)- in obese dogs. The study groups were categorized as obese group (n:20) and controls (n:10) with different breeds and genders due to body condition scoring system cared under similar conditions fed with dry commercial food once daily. Serum elements/minerals in serum were analyzed by using inductively coupled plasma-optical emission spectrophotometer (ICP-OES). Obese dogs had statistically higher serum Fe levels compared with healthy controls. A positive correlation was found between serum levels of Cr-Ni; Cu-Mg; Cu-Se; Mg-Se; Mg-Ca; Mg-P; B-Co; B-Ca; Ni-Co; Co-Ca and Ca-P in obese dogs. It has been accepted that obesity is a pro-inflammatory process priorly initiating in adipose tissue. Higher serum Fe levels in obese dogs might be related with inflammation during the obesity process. Moreover, the strong correlations among other discussed elements/minerals even with no statistical alterations of serum levels in obese dogs should be elucidated with further studies. Elements and minerals might be evaluated as biomarkers for the determination of effects of obesity in dogs.

***Keywords*:** Obesity; Dog; Serum elements; Serum minerals

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INTRODUCTION

Obesity is the most common inflammatory metabolic disorder defined as excessive adipose tissue accumulation in the body (Cortese et al., 2019). Studies from different parts of the world have reported that the incidence of obesity is more than 50% in the dog population (Courcier et al., 2010; Mao et al., 2013). Although some diseases (i.e., hypothyroidism and hyperadrenocorticism), pharmaceuticals (i.e.; glucocorticoids and anti-convulsive drug-induced polyphagia) can cause obesity, there is a negative correlation between energy intake and energy expenditure (Cortese et al., 2019). Therefore, excessive food intake or inadequate energy use can lead to positive energy balance. Various factors may be involved in this balance, including genetics, amount of physical activity, and energy content of the diet (Burkholder et al., 2000; Cayir et al., 2014). The effect of genetics has been reported in dogs such as Labrador Retriever, Cairn Terrier, Cavalier King Charles Spaniel, Scottish Terrier, Cocker Spaniel and some giant breeds (Gosselin et al., 2007). Effects of gender has also been reported as a predisposing factor in studies with dogs; females are considered to be overly prone to obesity. Another cause identified in dogs is restricted lifestyle and middle age. In addition, the diet can lead to the development of obesity. Many studies have reported that obesity can have detrimental effects on dogs' health and lifespan. It is known that obesity is mostly accompanied by high body mass index (BMI), hyperlipidemia, diabetes mellitus, cardiovascular diseases, neoplastic diseases, fertility problems, urogenital problems, orthopedic disorders and dermatological diseases in domestic animals (Edney and Smith, 1986; Burkholder et al., 2000).

About thirty of the known elements are currently thought to be necessary or important for the normal health and growth of animals. Some of them are called essential trace elements since they are sensitive enough to be measured in tissues (Rucker et al., 2008). Trace elements and minerals are known to play a vital role in different metabolic processes in the body. They are reported to be essential nutrients with regulatory, immunological and antioxidant functions as essential components or cofactors of enzymes throughout metabolism (Ates Alkan et al., 2019).

In recent years, studies on humans and animals have investigated the relationship between obesity and elements/minerals (Azab et al., 2014; Demerdash, 2015; Karatela and Ward, 2016). Although there have been many studies demonstrating the relationship between obesity and trace elements in humans, there have been recently no study in animals, especially in dogs. As we could reach the literature, this was the first study to analyze the relation of selected elements/minerals with obese and dogs with normal body condition. We aimed to investigate alterations of selected elements/minerals in obesity pathogenesis in dogs.

MATERIALS AND METHODS

Dogs and study design

All the dogs included in the study were admitted to Hospital Animal Teaching, Faculty of Veterinary Medicine, University of Bursa Uludag for routine control without clinical signs of any disease. According to the 5-grade clinical definition of body condition scoring (Brooks et al., 2014), which was accepted by the American Animal Hospital Association (AAHA), dogs with 3 (normal body condition) or 5 (obese body condition) scores were included in the study groups. The study population consisted of totally 30 dogs with different breeds aged between 3-7 years, 20 obese (obese group; 9 female/11 male) and 10 healthy dogs (control group; 5 female/5 male) with normal body condition. All dogs were fed with quality dry commercial food once daily without any treatment and/or supplementation consuming tap water. Information related with of their feeding interval as starting from one year age from their owners. This study was approved by the Ethical Committee of Bursa Uludag University Animal Experiments obtaining the consent of the owners.

Blood collection and preparation of blood samples

Blood samples were collected from dogs in dry vacuum tubes from *vena cephalica antebrachii* between 10:00 and 11:00 in the morning, in April and May. Blood samples were centrifuged at 1200 rpm for 10 minutes using a centrifuge (Laboratory Centrifuge M4812P-Elektromag Lab. Equipments Inc. Co. B7/153 Ikitelli, Istanbul, Turkey) following clotting. The obtained serum samples were transferred to cryo tubes and frozen at -80 °C (WISD Ultra Low Temperature Freezer SWUF-300, DAIHAN Scientific Co. Korea) until the time of analysis.

Measurement of serum elements and minerals

In order to eliminate adsorbed metals on the glassware being used, all the glassware was kept in 10 % (v/v) nitric acid solution before use. These were then cleaned with deionized water and dried in an oven overnight at 100 ºC. The analyses of serum elements/minerals in serum were determined by using inductive-

ly coupled plasma-optical emission spectrophotometer (ICP-OES 6000- Thermo Fisher Scientific Inc., Istanbul, Turkey) at the Laboratory of Trace Element Analysis at Department of Biophysics, Faculty of Cerrahpasa Medicine at University of Istanbul-Cerrahpasa.

ICP-OES was operated under the suitable conditions including selecting the suitable wavelength for Cu (324.754 nm), Fe (259.940 nm), Zn (206.200 nm), Se (196.090 nm), Cr (267.716 nm), Ni (221.647 nm), Ca (317.933 nm). P (177.495 nm), Mg (285.213 nm), B (249.773 nm) and Co (228.616 nm), respectively. The plasma operating conditions for ICP-OES system in this study were 15 l/min plasma gas flow rate, 0.5 argon carrier flow rate, 1.51/min sample flow rate, and elution flow rate. The speed of the peristaltic pump was 100 rpm. Transport lines were made using a 1.25-mm-i.d. polytetrafluoroethylene tubing. Each measurement was performed three times and averages were used for the analysis. Results were expressed in microgram per milliliter (ppm = µg/mL) and mg/L of serum (Tarhan et al., 2016; Aslan et al., 2018; Ates Alkan et al, 2019).

Reagents

Test standards for ICP-OES analysis were prepared from proper standard solutions containing 1000 µg/mL for each tested element/mineral obtained from Chem Lab NV (Belgium). All reagents were of analytical reagent grade, and deionized water was used. Stock solutions of elements/minerals were prepared by taking appropriate amounts of standards in deionized water. Test solutions were prepared immediately before use. Doubly distilled deionized water was used in the current study. To reduce the risk of contamination from ambient air and dust, all work was performed on a clean bench. All the glassware used was cleaned by being soaked in with 10 % (v/v) nitric acid solution for 1 day before use. These were rinsed thoroughly with deionized water and dried in an oven overnight at 100 °C (Tarhan et al., 2016).

Statistical Analysis

Statistical analysis of numerical data of the variables examined in dogs was performed using the Sigma Plot 14.0 statistical package program (Systat Software Inc. CA, USA). For this purpose, the arithmetic mean (X) and standard deviation (Sx) of the parameters were calculated. Differences in $p \leq 0.05$ values were considered statistically significant. The student T-test and Mann-Whitney U test were used to determine the differences between the obese and the control group. Spearman's correlation test was performed to analyze correlation.

RESULTS

There was no statistical significant difference between study groups by means of age and gender. Serum elements/minerals in obese and control groups with descriptive statics shown in Table 1. Obese group had significantly higher serum Fe levels were than control group (p: 0.007). There was no statistical significance in other analyzed elements/minerals among study groups (Table1). There was positive correlation between Cr-Ni (p=0.01), Cu-Mg (p=0.02), Cu-Se (p=0.01), Cu-P (p=0.09), Mg-Se (p=0.01), Mg-Ca (p=0.04), Mg-P (p=0.028), B-Co (p=0.01), B-Ca (p=0.04), Ni-Co (p=0.01), Co-Ca (p=0.001) and Ca-P (p=0.05) in obese dogs (Table 2). Furthermore, a negative correlation was found between Fe-Mg (p=0.01) in control group (Table 3).

Table 1. Serum elements/minerals levels in study groups

Parameters	Control group (5 F/5 M) (Mean± SD) (n: 10)	Std. Error	Obese group (9 F/11 M) (Mean± SD) (n: 20)	Std. Error	p value
Cu (µg/dL)	31.36± 8.93	2.98	30.15± 10.85	2.49	0.495
Fe (µg/dL)	103.70± 57.49	19.20	187.92± 102.02	23.4	**0.007**
Zn (µg/dL)	34.70± 10.45	3.49	31.45± 11.03	2.53	0.460
Se (µgdL)	29.90± 6.36	2.12	32.77± 10.56	2.42	0.567
Cr (µg/dL)	0.17± 0.06	0.02	0.19± 0.12	0.04	0.724
Ni (µg/dL)	4.62± 0.58	0.33	7.88± 4.00	0.89	0.181
Ca (mg/dL)	7.26± 0.51	0.17	6.73± 0.97	0.22	0.130
P (mg/dL)	17.09± 2.61	0.87	17.00± 4.01	0.92	0.524
Mg(mg/dL)	1.19± 0.42	0.14	1.05± 0.34	0.08	0.613
B (µg/dL)	2.35± 1.08	0.360	1.77± 1.09	0.251	0.192
Co (µg/dL)	0.46± 0.20	0.069	0.35± 0.19	0.045	0.175
Al (µg/dL)	5.96± 3.04	1.01	4.54± 3.25	0.747	0.276

Table 2. Correlation of serum elements/minerals in obese group (n=20)

	Mg	Se	Zn	B	Ni	Co	Ca	P
Cr	*-0.10*	*0.15*	*-0.27*	*0.17*	***0.56***	*0.21*	*-0.07*	*-0.09*
	0.65	0.50	0.23	0.47	**0.01**	0.35	0.75	0.68
Cu	***0.51***	***0.78***	*-0.20*	*-0.32*	*0.29*	*0.03*	*0.007*	***0.38***
	0.02	**0.01**	0.37	0.16	0.20	0.87	0.97	**0.09**
Fe	*0.07*	*-0.22*	*0.32*	*-0.04*	*-0.13*	*-0.02*	*0.36*	*0.23*
	0.76	0.33	0.15	0.98	0.56	0.90	0.10	0.32
Mg	-	***0.53***	*-0.04*	*-0.13*	*0.16*	*0.21*	***0.45***	***0.49***
		0.01	0.83	0.57	0.49	0.35	**0.04**	**0.028**
Se	-	-	*0.06*	*-0.22*	*0.26*	*0.02*	*-0.07*	*0.22*
			0.79	0.32	0.26	0.92	0.76	0.33
B	-	-	-	-	*0.17*	***0.54***	***0.45***	*-0.29*
					0.45	**0.01**	**0.04**	0.19
Ni	-	-	-	-	-	***0.52***	*0.29*	*0.21*
						0.01	0.20	0.36
Co	-	-	-	-	-	-	***0.67***	*0.22*
							0.001	0.33
Ca	-	-	-	-	-	-	-	***0.43***
								0.05

Numbers in Italics represent the "r value" and numbers written in normal characters represent the "p-value"

Table 3. Correlation of serum elements/minerals in control group (n=10)

	Mg	**Se**	**Zn**	**B**	**Ni**	**Co**	**Ca**	**P**
Cr	*-0.41*	*0.35*	*-0.60*	*-0.49*	*1.00*	*-0.29*	*0.15*	*0.18*
	0.41	0.29	0.06	0.13	0.33	0.40	0.65	0.58
Cu	*0.78*	*0.44*	*-0.73*	*-0.80*	*0.50*	*0.20*	*0.01*	*0.21*
	0.056	0.18	0.10	0.21	1.00	0.58	0.94	0.53
Fe	***-0.75***	*-0.31*	*0.58*	*-0.15*	*-1.00*	*0.35*	*0.45*	*-0.36*
	0.01	0.38	0.08	0.67	0.33	0.35	0.20	0.30
Mg	-	*0.35*	*-0.60*	*-0.49*	*1.00*	*-0.29*	*0.15*	*0.18*
		0.29	0.06	0.13	0.33	0.40	0.65	0.59
Se	-	-	*-0.21*	*-0.06*	*0.50*	*-0.74*	*-0.37*	*-0.03*
			0.53	0.84	1.00	0.09	0.27	0.91
B	-	-	-	-	*-0.50*	*-0.26*	*-0.13*	*-0.01*
					1.00	0.46	0.68	0.94
Ni	-	-	-	-	-	*-0.50*	*-0.60*	*0.50*
						1.00	1.00	1.00
Co	-	-	-	-	-	-	*0.33*	*-0.31*
							0.35	0.38
Ca	-	-	-	-	-	-	-	*-0.02*
								0.95

Numbers in Italics represent the "r value" and numbers written in normal characters represent the "p-value"

DISCUSSION

Since elements/minerals act as cofactors for many enzymes also including the antioxidant enzymes, they have vital roles in different metabolic processes in the body, especially enzyme and protein metabolism. It is stated that in case of deficiencies of these elements/minerals, oxidative stress (OS) related diseases may occur due to a decrease in antioxidants and increased lipid peroxidation. Obesity, being one of the OS related diseases, is an inflammation process due to multiple factors including genetic, environmental, and dietary issues (Cayir et al., 2014; Ates Alkan et al., 2019; Cihan and Tural, 2019). Obesity is an abnormal and/or excess fat accumulation in various sites of biological organisms including adipose tissue, visceral membranes, and organs such as liver and pancreas (Cedeno et al, 2020; Tinkov et al., 2017). The aim of study was to focus on the relationship between

a selected variety number of elements/minerals and obesity pathogenesis in dogs.

Copper

Copper is a component of antioxidant enzymes that protect the body against the effects of free radicals, especially in cardiovascular diseases (Azab et al., 2014; Karatela and Ward, 2016) Cu was reported to be related with fat tissue metabolism inducing fat accumulation in liver tissue resulting in fatty liver disease. Besides, has been determined to cause inflammation and OS via dysfunction in lipid metabolism (Yerlikaya et al., 2013). The mechanism of Cu uptake in obese patients is unclear, but Interleukin-1, one of the proinflammatory cytokines secreted from adipose tissue, is thought to increase intracellular Cu uptake and intracellular Zn accumulation (Demerdash, 2015). In two studies (Azab et al., 2014; Cayir et al., 2014), obese children and in one study (Yerlikaya et al., 2013) it was reported significantly higher Cu levels in adult obese women compared to the control group. On the contrary, there are studies that did not detect a significant difference in Cu levels in obese children and obese adult men compared to the control group (Ozata et al., 2002; Tascilar et al., 2010). In the present study, no statistical difference was found in serum Cu levels in obese dogs compared to the control group.

Iron

Most of the Fe in the body is included in heme biosynthesis in erythropoietic bone marrow and other heme-containing enzymes, while the remaining amount is found in hepatocytes and reticuloendothelial system cells (Demerdash, 2015). It is also involved in the expression of genes for ferritin, transferritin, and metallothionine receptors (Karatela and Ward, 2016). In addition, it is an important catalyst groove in the formation of strong prooxidant hydroxyl radicals that damage cellular membrane lipids, proteins and nucleic acids. Obesity is accepted to be related with decreased serum Fe levels in children and adults. Karatela and Ward (2016) reported that various mechanisms might play a role in lowering Fe levels in obese individuals. Unhealthy eating habits, which often lead to inadequate Fe consumption, decreased absorption and Fe binding due to chronic inflammation, have been reported in obese individuals (Karatela and Ward, 2016). Otherwise, pro-inflammatory cytokines and excessive adipose tissue cause Fe deposition in biological tissues initiating inflammation and OS process. Thus, obesity can be evaluated to be associated with both excess in serum Fe and Fe deficiency (Mc Clung and Karl, 2009; Demerdash, 2015). Several studies revealed that serum Fe levels were lower in overweight and obese children and adults compared with controls (Nead et al., 2004; Yerlikaya et al., 2013; Azab et al, 2014). However, it remains unclear whether the low serum Fe levels observed in obesity might have reflected a functional iron deficiency associated with an inflammatory process or whether obesity might have been caused by accurate Fe deficiency (Azab et al., 2014). In other studies (Ozata et al., 2002; Tascilar et al., 2010), no significant relationship was found between serum Fe levels in obese children and control groups. The present study concluded that serum Fe levels in obese group were significantly higher than the control group, which might show the beginning of the inflammation process. In addition, high Fe concentration in the serum has been associated with the habit of consuming excess food in obese dogs.

Zinc

Zinc is a trace element that has an important metabolic function in the metabolism of proteins, carbohydrates, lipids and nucleic acids (Zohal et al., 2019). Zn, which is also a metalloenzyme, is involved in the synthesis, storage, release, and appetite control of insulin (Karatela and Ward, 2016). Studies based on obese humans reported that low serum Zn levels were related with excess amount of adipose tissue and tendency to obesity and Zn deficiency leads to decreased leptin secretion from adipocytes and decreased serum leptin level resulting in increased appetite and obesity (Zohal et al., 2019). Furthermore, Zn deficiency in obese individuals augments inflammation and OS via high burden of radical oxygen species (ROS). Zn deficiency has been reported to be associated with obesity, since it causes insulin resistance and glucose intolerance leading to obesity related complications. (Tascilar et al., 2010; Karatela and Ward, 2016). Consistently with human studies, our serum Zn levels in obese group were lower than control group without any significancy. This result might indicate the consumption of Zn via defence mechanisms.

Selenium

Selenium plays an important role in many enzyme reactions, especially glutathione and thyroxine in antioxidant defence mechanism (Karatela and Ward, 2016; Ates Alkan et al., 2019). It has a pro-

tective effect against ROS and other harmful effects associated with metabolic complications of obesity (Azab et al., 2014). Stranges et al. (2010) reported that diets containing high Se might promote hyperglycemia by stimulating glucagon release or induce overexpression of enzymes related with Se, leading to balance insulin resistance and to prevent obesity. Se deficiency is associated with increased OS, impaired thyroid function and lipid peroxidation in obese individuals (Demerdash, 2015). When the studies on Se levels in obesity were evaluated, it was observed that there have been conflicting outcomes. Studies held on obese children Azab et al. (2014) and Ortega et al. (2012) determined that serum Se levels were lower in obese group compared to controls. Whereas another study with obese children reported higher serum Se levels in obese group than controls (Cayir etal., 2014). However, there have been some studies indicating no significant change in serum Se levels in obesity (Bougle et al., 2009; Tascilar et al., 2010). As far as we could reach, there have been limited studies based on Se in obese dogs (Van Zelst et al., 2016). In the present study, serum Se level was analyzed to be higher in obese group compared to control group, but there was no statistical difference. Higher serum Se levels in obese group might postulate the probable antioxidant effect of Se against the inflammatory process caused by obesity. Consistently, the positive correlation between serum Cu and Se might point out the scavenging of higher Se over Cu.

Chromium

Chromium, which is included in the chemical structure of insulin, is related with biomolecule metabolism, especially carbohydrate and lipid metabolism. Indeed, it has a close functional metabolism with insulin by controlling hunger. In a study conducted with obese dogs revealed higher serum cholesterol and triglyceride levels compared with controls (Cihan and Tural, 2019). Cr has been reported to contribute to balance appetite and to the reduction of adipose tissue and body weight in obesity (Yerlikaya et al., 2013; Karatela and Ward, 2016). Low serum Cr levels induce high lipid levels, secretion pro-inflammatory mediators and OS (Demerdash, 2015). In the case of a diet rich in fat and obesity process, Cr level of the adipose tissue decreases, leading to a more pronounced peripheral insulin resistance (Tinkov et al., 2017). In addition, Cr supplementation was reported to reduce body weight significantly (Onakpoya et al., 2013). On the other hand, a study conducted with children, Azab et al (2014) reported that there was no significant difference in serum Cr levels between obese children and controls. Yerlikaya et al. (2013) also revealed that there was no difference in serum Cr levels between obese women and controls. In this study, no statistical significancy was observed between obese group and controls by means of serum Cr levels. There is need for further studies to enlighten the relationship between Cr levels and obesity in dogs.

Nickel

Ni mostly taken orally or via inhalation has not been elucidated yet by means of its biological functions. The toxic effects of Ni related with ROS, OS and inflammation have been analyzed in many studies (Das et al., 2008). In a study conducted on rats, it was reported that Ni could alter glucose homeostasis by inducing pancreatic islet dysfunction and affecting insulin secretion (Gupta et al., 2000). Although serum Ni levels were lower in obese children, no significant difference was analyzed (Tascilar et al., 2010). In another study (Aguilar et al., 2007), serum Ni levels were found to be high in adults with diabetes, but no statistically significant difference was found among the groups. Similarly, in this study we conducted higher serum Ni levels in obese dogs compared with controls with no statistical difference. Therefore, obese dogs might be prone to diabetes because of deterioration of pancreatic metabolism. Indeed, the positive correlation between serum Cr and Ni in obese groups might indicate the close relationship between these two trace elements in obesity.

Calcium - Phosphorus

Ca is an essential mineral found in bones and teeth in high levels possessing a crucial role in many physiological functions in the body, including hormone and enzyme processes, regulation of lipid metabolism and insulin sensitivity, blood coagulation and neuro-muscular functions (Karatela and Ward, 2016; Zohal et al., 2019). Ca content of dairy products in a daily diet has been accepted to reduce fat formation (Heaney and Rafferty, 2009; Onakpoya et al., 2011). P plays a critical role in regulating many key biological processes, including carbohydrate metabolism, signal transduction, transcription and recording of genetic information, and repair of lipid membrane structure (Celik and Andiran, 2011; Zohal et al., 2019). In a study conducted in obese females, serum Ca levels were significantly lower than controls presenting the inhibiting effect of Ca in adipose tissue (Amin et al.,

2020). On the other hand, Bowen et al. (2005) and Heaney (2011) reported that the increase in dietary Ca intake was not found to be effective in prevention of obesity. However, no relationship was found for serum Ca and P levels between adult obese male and controls. In the same study, a negative correlation was reported in females between serum Ca and P levels with waist circumference linked to the metabolic syndrome (Zohal et al., 2019). Significantly lower serum P levels were reported in obese children compared to healthy group (Celik and Andiarn, 2011). Whereas, in another held on obese children, both serum and saliva P levels were found to be significantly higher than the control group (46). Our study revealed that both serum Ca and P levels were slightly lower in obese group presenting the synergistic effects of these minerals in defence mechanism of obesity.

Magnesium – Boron

Magnesium plays a critical role in regulating insulin metabolism, insulin-dependent glucose uptake, body temperature, muscle contraction and vascular tone (Ando et al., 2017; Zohal et al., 2019). Mg is a cofactor in many enzymatic reactions including carbohydrate metabolism, and functions as an antagonist of Ca (Demerdash, 2015; Karatela and Ward, 2016; Zohal et al., 2019). Although the exact reasons for the decreased serum Mg levels in obese individuals have not been explained yet, but it has been an accepted data that diet rich in Ca and P causes diminished absorption of Mg from the gastrointestinal system. Low serum Mg levels induces loss of appetite, weight gain and insulin dysfunction, being as a cofactor of insulin receptor. Thus, the sensitivity of peripheral insulin decreases resulting in fat accumulation in adipose tissue and muscle dysfunction (Mert et al., 2008; Demerdash, 2015). In animal studies, it was reported that decreased serum Mg levels caused fatty liver and, that there was a negative correlation between serum Mg with metabolic syndrome and insulin resistance (Palladini et al., 2010). Although the importance of B in human health has not yet been fully explained, it has metabolic activity in antioxidant systems through lipid and energy metabolism, bone structure, stability of the cell wall, enzymatic reactions, and scavenging of ROS (Ates Alkan et al. 2021). B was noted to prevent atherosclerosis by inhibiting fat accumulation in biological tissues. It has been shown that a daily diet rich in B have an improvement in lipid profile, and decreases fasting glucose, insulin levels and BMI (Kuru et al., 2019). Zohal et al. (2019) analyzed lower serum Mg levels in obese group compared with controls. Similarly, in a study of hypomagnesemic rats, it was concluded that peripheral insulin resistance augmented and glucose uptake into the cell deteriorated resulting in hyperglycemia (Suarez et al., 1995). In a study (Ando et al., 2017), a decreased serum Mg levels were observed in dogs fed with the same formula in the winter. In this study, it has been reported that mammals need to maintain their body temperature in winter, as Mg takes part in maintaining body temperature. Although it was reported that there was no relationship between serum B level and obesity (Tascilar et al., 2010), there have been studies indicating negative relationship between serum B levels and obesity (Hasbahceci et al., 2013; Karatela and Ward, 2016). Despite the fact that, effects of serum B on Ca, P and Mg metabolism were reported that serum Ca levels increased after B administration to hypocalcemic healthy and pregnant cows (Kabu et al., 2013), we have not encountered studies related with B metabolism in obese dogs. The present study concluded that both serum Mg and B levels were lower in obese group with no significancy. The positive correlation of serum Mg with serum Ca and P postulating that the physiologically known antagonism between Ca and Mg has been negatively effected. In addition, serum Mg was positively correlated with serum Cu and Se levels might be due to the protective effect of Se on inflammation process. However, there was a negative correlation between serum Fe and Mg levels in control group probably indicating the defense mechanism of Mg against pro-oxidant effects of Fe. The positive correlation between serum Ca and B might be related with the improving effect of B supplementation on Ca metabolism.

Cobalt

Cobalt is found in the structure of Vitamin B12 and is essential for erythropoiesis (Karatela and Ward, 2016; Tarhan et al., 2016). Co plays an important role in animal metabolism, as it has a wide distribution within the animal body (Mert et al., 2008). Kawakami et al. (2012) pointed out in their study related with high fat content feeded mice that serum Co levels were associated with reduction of adipose tissue in obese group. A study conducted with streptozotocin induced diabetic rats indicated the beneficial effects of serum Co in glycogen and insulin metabolism (Vasudevan and Mac Neill, 2007). However, there have been limited studies about serum Co in obese dogs. Our results showed that obese group had lower se-

rum Co levels than controls. Moreover, the positive correlation of serum Co with serum Ni might be due to the protective effect of Co on Ni by means of insulin metabolism. Serum Co was also a positively correlated with serum Ca and B probably indicating their synergistic effects in adipose tissue and glucose metabolism.

The present study reported significantly higher serum Fe levels in obese group, whereas the other discussed elements/minerals (Cu, Zn, Se, Cr, Ni, Ca, P, Mg, B and Co) presented no significant alterations among study groups. The limitations of the present study can be evaluated as the probably limited number of dogs and different breeds in each study group, and differences of elements/minerals concentration in dry commercial food.

CONCLUSION

There have been mainly detailed research in obesity pathogenesis in humans, animal studies have been limited with data based on rat, mice, rabbit and ruminants (Azab et al., 2014; Demerdash, 2015; Karatela and Ward, 2016). However, there have been scarce research held about the evaluation of elements/minerals in obese dogs. Thus, this study was established on the effect of multiple number of elements/minerals in the pathogenesis of obesity in dogs. Further studies are needed in a design of different breeds with a planned daily dietary intake as detailed calculations of energy intake by means of carbohydrates, proteins and lipids and levels of elements/minerals. Both trace elements and minerals might be as biomarkers for the determination of effects of obesity in dogs.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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