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The Relationship Between Lipid Profile, Oxidative Stress, and Thiol-Disulfide Levels in Healthy, Naturally Overweight and Obese Cats

Doğal Olarak Kilo Alan veya Obezite Gelişen Kedilerde Lipid Profili, Oksidatif Stres ve Tiyol-Disülfür Düzeyleri Arasındaki İlişkiler

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ABSTRACT

This study aimed to evaluate systemic inflammation, oxidative stress and lipid profile in cats that had either naturally gained excess weight or had developed obesity. The following groups were examined in the study: ten obese cats with a body condition score of (BCS) >8 (the obesity group), ten overweight cats with a BCS score of >6 (the overweight group) and ten ideal weight cats with a BCS score of 4-5 (the control group). In the cats that had either gained too much weight or had become obese, the serum AST ($P < .001$), albumin ($P = .002$) and total protein (TP) ($P < .001$) levels were found to be significantly higher than the values determined in the control group cats. Furthermore, blood serum high-density lipoprotein (HDL) ($P = .009$) and triglyceride (TG) ($P < .001$) levels in cats that had developed obesity were found to be significantly higher than the values defined in the control group cats. In the obese cats, serum procalcitonin (PCT), paraoxonase-1 (PON-1), total thiol, native thiol and MDA levels were found to be significantly higher than in overweight cats ($P < .001$). As a result, it was concluded that it would be useful for veterinarians to consider significant changes in parameters related to liver function and lipid metabolism, as well as to emphasize systemic inflammation and oxidative stress in their clinical evaluations in cats that had either naturally gained excess weight or had developed obesity.

Keywords: Cat, lipid metabolism, liver enzymes, obesity, oxidative stress.

ÖZ

Bu çalışmada, doğal olarak fazla kilo almış olan veya obezitenin geliştiği kedilerde sistemik inflamasyonun, oksidatif stresin ve lipid profilinin değerlendirilmesi amaçlandı. Çalışmada, vücut kondisyon skoru (BCS) >8 olan on obez kedi (obezite geliştiren grup), BCS skoru >6 olan on fazla kilolu kedi (fazla kilolu grup) ve BCS skoru 4-5 olan on ideal kilolu kedi (kontrol grubu) incelendi. Aşırı kilo almış olan ve obezitenin geliştiği kedilerde, serum AST ($P <$,001), albumin ($P =$,002) ve toplam protein (TP) ($P <$,001) düzeylerinin kontrol grubundaki kedilerde tanımlanan değerlerden anlamlı derecede yüksek olduğu belirlendi. Ayrıca, obezitenin geliştiği, kedilerde kan serumu yüksek yoğunluklu lipoprotein (HDL) (P = ,009) ve trigliserit (TG) ($P <$,001) düzeyleri kontrol grubundaki kedilerde belirtilen değerlerden anlamlı derecede yüksek bulundu. Obezitenin geliştiği kedilerde, serum prokalsitonin (PCT), paraoksonaz-1 (PON-1), toplam tiyol, doğal tiyol ve MDA düzeylerinin aşırı kilolu kedilere göre anlamlı derecede yüksek olduğu saptandı ($P <$,001). Sonuç olarak veteriner hekimlerin klinik değerlendirmelerinde, doğal olarak fazla kilo almış olan veya obezitenin geliştiği kedilerde, karaciğer fonksiyonları ve lipid metabolizması ile ilgili olan ve sistemik inflamasyon ve oksidatif stresi gösteren parametrelerdeki anlamlı düzeydeki değişiklikleri dikkate almalarının yararlı olacağı kanısına varıldı.

Anahtar Kelimeler: Karaciğer enzimleri, kedi, lipit metabolizması, obezite, oksidatif stress.

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INTRODUCTION

The most common health problems of domestic cats brought to the vets of many countries are energy metabolism issues that are related to excessive weight gain or obesity. Excess weight or obesity as a result of increased accumulation of fat tissue often give rise to mechanical (increased stress on joints and muscles) issues, as well as having other metabolic consequences. It is also reported that excess weight creates various other health problems in a cat's later life.[1-3]

As the body condition score (BCS) increases, the balance of antioxidants and oxidants in the body is disrupted in favor of oxidants, which means the body is under the intense influence of oxidative stress. This, in turn, means that various metabolic and organic disorders may occur in the organism, or there is increased risk of their occurrence. The process means that an animal's quality of life is reduced. It has been shown that elevated liver enzymes in overweight cats are associated with lipid metabolism disorders and oxidative stress potentiation.[4,5]

Since total thiol and native thiol molecules contain sulfhydryl groups with antioxidant/prooxidant properties in their structures, it is crucial to evaluate the thiol and disulfide status in order to determine the level of oxidative stress in patients. If oxidative stress increases and persists for a sufficient length of time, the lipid matrix of the biological membranes become inflamed or are destroyed due to oxidation, and so there is clearly a strong link between oxidative stress and systemic inflammation in the body.[6] Medical monitoring of serum CRP and procalcitonin levels are important biomarkers in the evaluation of systemic inflammation in feline sepsis patients.[7,8] The severity of oxidation caused by reactive oxygen species in the lipid matrix of biological membranes can be assessed by monitoring the changes in serum malondialdehyde (MDA) levels.[9] It has been shown that excessive weight gain in cats is associated with hepatic lipidosis and oxidative stress, and that reactive oxygen species can oxidize the lipoid matrix of biological membranes. Oxidative damage in membrane phospholipids begins with lipid peroxidation. The hydrolyzing capabilities of phospholipids after peroxidation suggest that paraoxonase-1 (PON-1) protects lipids from oxidation.[4,9] PON-1 is an extracellular hydrolase produced in the liver and bound to high-density lipoprotein (HDL). In animals with a high BCS score, it is important to monitor the serum PON-1 level in order to evaluate difficulties in the metabolic process.[10-12]

This study examines blood serum PCT and CRP levels to determine whether systemic inflammation has developed. The degree of oxidative stress is also evaluated by considering blood serum PON-1, total thiol, natural thiol and MDA levels. Blood serum HDL, TG, and CHOL levels are also considered to evaluate levels of lipid profile and the blood serum AST, ALT, albumin and the total protein. The overall aim is to examine the metabolic processes related to liver functions in naturally overweight or obese cats.

MATERIALS AND METHODS

Ethical approval was obtained from the Animal Experiments Local Ethics Committee (Date:14.12.2022 Number:2022-22-197) of Ankara University.

The animals examined in this study consisted of 30 neutered cats, aged 7-11 years, from different breeds and of both sexes. The animals were being kept as domestic pets in the homes of owners aged over 18 years and living in Ankara, Turkey. According to the information received from the owners of all of the cats used in this study, the animals were fed with commercial food in the amount recommended by the manufacturer. It was also stated that the cats began to be more than the ideal weight from the age of 2–3 years.

A general health check-up of the cats in the study was performed, and no medical issue was detected in 20 cats besides them being overweight (ten cats had a BCS >6 and ten a BCS >8). No medical condition was detected in clinical examination of the ten cats (BCS = 4–5) used as the control group.

In scoring the BCS of the animals, a system ranging from 0 to 9 points was used which took into account the physical appearance of the body and breed-specific body measurements. In this assessment system, cats with a BCS of >6 were considered overweight, and those with a BCS of >8 were considered to have developed obesity.[13]

Blood samples were collected from the vena cephalica antebrachii of all of the cats used in the study. Serum was extracted from blood samples from tubes without an anticoagulant, and CBC analysis was conducted from tubes with anticoagulant. CBC parameters were determined in the blood samples with anticoagulant using an automatic blood count device (Mindray BC 5000, Nanshan, China). The serum samples were placed in godets and kept at –20°C until biochemical analysis. ALT, AST, TP, albumin, urea, and creatinine were determined spectrophotometrically with an autoanalyzer (Mindray BS-120, Nanshan, China) placed in the blood serum samples, which were brought to

room temperature for melting before analysis. CRP level measurements were performed using the "Fuji film Nx 500" analyzer. Procalcitonin levels was measured spectrophotometrically by the ELISA method using relevant ELISA kits (BT Lab, Bioassay Technology Laboratory, Cat No: E0065Cat, Zhenjiang, China).

The blood serum MDA level was calculated according to the method specified by Draper and Hadley[14]. For this analysis, trichloroacetic was prepared at 10%, 2.5 mL of which was mixed in a test tube with 0.5 mL of the serum sample during the first stage of measurement. The next stage was for the mixture to be placed in a bath of boiling water, left to cool for 15 minutes, and then centrifuged at 400 g for 10 minutes. Two milliliters were taken from the supernatant and placed in a separate tube; precisely 1.0 mL of 0.67% thiobarbituric acid was added, gently mixed, and the resulting mixture incubated in a bath of boiling water for 15 minutes. To evaluate the reaction when the solution reached room temperature, absorbance values were measured at 532 nm in the spectrophotometer device (Tecan Sunrise RS 232, Grödig, Austria).

The method developed by Eckerson et al.[15] was used to measure PON-1 activity. This method is based on the formation of diethyl phosphate and p-nitrophenol metabolites caused by the action of a paraoxon (diethyl p-nitrophenyl phosphate) (1 mM) and $CaCl_2$ (1 mM) in 0,05 M glycine buıffer molecules as a result of the enzyme activity of paraoxonase in the serum. Measurement and calculation of the resulting p-nitrophenol was determined using a spectrophotometer with an absorbance of 412 nm.

During the determination of the thiol-disulfide levels, the thiol [-SH] and disulfide [-S-S] levels were determined. Measurements were made spectrophotometrically according to the method described by Erel and Neselioglu.[16] NaBH4 was used to reduce sulfide bonds in the serum samples and form free functional thiol groups, while formaldehyde was used to remove the unused portion of NaBH4 from the reaction medium. The DNTB and thiol groups were determined spectrophotometrically at 415 nm as total thiol and total thiol/native thiol.

Disulfide levels were calculated using the formula: (µmol/L) = (total thiol-native thiol)/2.

Statistical Analysis

Before proceeding to hypothesis testing, the data was analyzed in terms of normal distribution and homogeneity of variance for assumption controls. After the Shapiro-Wilk and Levene tests; control, overweight and obese groups were compared using One-way ANOVA test or Kruskal-Wallis test, as appropriate. The results were summarized as mean±standard error (Mean±SE). In order to determine significance difference, Tukey and Dunn's tests were used as for multiple comparisons. The differences between the groups were expressed as superscripts (a b c). In all analyses, P value of < .05 was accepted as significance criterion. The data was analyzed using IBM SPSS Statistics 26.0 (SPSS®, IL,USA).

RESULTS

Among the cats used in the study, the mean age of the animals in the control group was 9.3 ± 1.3 years, the overweight group 9.1 ± 1.4 years, and the obese group 9.2 ± 1.3 years.

The hematological and biochemical data of the cats in the control, overweight, and obesity groups are given in Tables 1, 2, and 3.

Table 1. Hematological parameters.

Groups	WBC (10^9/L)	NEU (10^9/L)	LYM (10^9/L)	MONO (10^9/L)	EOS (10^9/L)	BAS (10^9/L)	PLT (10^9/L)
Control (10)	9.78 ± 0.39	6.88 ± 0.48	2.33 ± 0.11	0.155 ± 0.03	0.497 ± 0.13	0.007 ± 0.001	251 ± 27.9
Overweight (10)	9.92 ± 0.35	6.54 ± 0.48	2.56 ± 0.1	0.205 ± 0.03	0.604 ± 0.14	0.019 ± 0.01	310 ± 45.4
Obesity (10)	9.81 ± 0.22	6.91 ± 0.37	2.36 ± 0.35	0.201 ± 0.02	0.283 ± 0.07	0.012 ± 0.001	228 ± 27.1
P	.949	.809	.371	.398	.125	.419	.243

Values in the table are given as arithmetic mean ± standard deviation (X ± SD).***** denotes differences between groups in the same column.

Table 2. Biochemical parameters.

Groups	PCT (mL/L)	PON-1 (U/L)	Total thiol (µmol/L)	Native thiol (µmol/L)	CRP (mg/L)	MDA (nmol/g protein)
Control (10)	9.78 ± 0.39	6.88 ± 0.48	2.33 ± 0.11	0.155 ± 0.03	0.497 ± 0.13	0.007 ± 0.001
Overweight (10)	9.92 ± 0.35	6.54 ± 0.48	2.56 ± 0.1	0.205 ± 0.03	0.604 ± 0.14	0.019 ± 0.01
Obesity (10)	9.81 ± 0.22	6.91 ± 0.37	2.36 ± 0.35	0.201 ± 0.02	0.283 ± 0.07	0.012 ± 0.001
P	.949	.809	.371	.398	.125	.419

The values in the table are given as arithmetic mean ± standard deviation (X±SD); ^{a,b,c} indicate differences between groups in the same column.

Table 3. Biochemical parameters.

Groups	AST (U/L)	ALT (U/L)	ALB (g/dL)	TP (g/dL)	UREA (mg/dL)	CREA (mg/dL)	HDL (mg/dL)	TG (mg/dL)	CHOL (mg/dL)
Control (10)	106±3.06^{c}	46.8±6.73	3.05±0.15^{b}	6.44±0.33^{b}	28.1±1.41	0.805±0.04	45.7±6.12^{a}	49.8±1.92^{b}	59.4±3.32
Overweight (10)	121±2.37^{b}	47.6±4.43	3.5±0.06^{a}	7.66±0.12^{a}	25.8±1.01	0.717±0.01	58.2±1.71^{ab}	53.2±1.4^{b}	63.3±1.51
Obesity (10)	132±1.31^{a}	52.7±2.9	3.62±0.06^{a}	8.06±0.28^{a}	27.4±1.26	0.755±0.02	65.5±2.01^{b}	62 ± 1.29^{a}	62.8±1.81
P	< .001	.325	.002	< .001	.403	.071	.009	< .001	0.452

The values in the table are given as arithmetic mean ± standard deviation (X ± SD); ^{a,b,c} indicate differences between groups in the same column.

There were no statistically significant differences in the levels of hematological parameters between the cats with an ideal BCS score, the cats with naturally gained excess weight and the cats with naturally developed obesity.

It was determined that the PCT and MDA, total thiol, and native thiol levels were higher in both overweight cats compared to the cats with ideal BCS score, and in cats with obesity compared to the cats with excess weight ($P < .001$). The PON-1 and CRP levels were lower in overweight cats compared to cats with an ideal BCS score cats, as well as in obese cats compared to overweight cats ($P < .001$).

DISCUSSION

A BCS value increase above the ideal score in cats is caused by a decrease in daily activity, neutering, aging, living in a domestic environment and being fed high-calorie food. [2,17] If the BCS score exceeds the ideal values, lipid metabolism deterioration, systemic inflammation and increases in oxidative stress lead to long-term organ function deterioration. In order to detect and monitor the damage that an increase in BCS may cause in the body, changes in the levels of various biochemical parameters related to metabolic processes are evaluated. For this purpose, firstly, serum triglyceride, cholesterol, HDL, AST, ALT, total protein, albumin, CRP, urea and creatinine levels are measured. Blood serum paraoxanase-1 (PON-1) level is also determined to evaluate the metabolic interaction of HDL and low-density lipoprotein levels.[18]

In this study, serum AST, TG and HDL levels measured for the purpose of evaluating liver function in overweight and obese cats were found to be statistically significantly higher than those reported in cats with ideal BCS (control group), which is consistent with the findings of various authors. [6,19,20] Okada et al.[21] found that serum triglyceride levels in obese cats were statistically significantly higher than those determined in the control group. Other studies on obese cats have shown that serum HDL and triglyceride levels were significantly higher than those determined in control cats. [6,22] In studies conducted by Bauer[23] on cats with ideal BCS, and Hoenig et al.[24] on obese cats, it was stated that the high HDL levels in both groups of cats may be due to cholesteryl ester transfer protein deficiency. Similarly, it was noted in this study that serum HDL and triglyceride levels in cats with obesity were significantly higher than the values determined in the control group cats. In addition, it was noted that in cats with increased BCS values, serum total protein and albumin values were higher than the values determined in the control group cats. This result shows that metabolic pathways related to protein production and degradation were affected in cats that gained excessive weight and developed obesity. Considering that oxidative stress and body fluid distribution are also effective in protein production and degradation, it is obvious that changes in serum total protein and albumin levels should be investigated in more detail on a pathway basis.

PON-1 is synthesized in the liver[25] and is directly related to serum HDL and triglyceride levels.[26] The level of blood serum PON-1 is strongly correlated with the degree of oxidative stress and inflammation states in the body.[9] In this study, serum PCT, PON-1, total thiol, native thiol and MDA levels were found to be significantly higher in overweight cats, as compared to the values determined in the control group, and in obese cats compared to the values determined in overweight cats ($P < .001$). According to these results, it can be seen that in cats exceeding the ideal BCS score, primarily fat metabolism is impaired in the body leading to gradual increases in systemic inflammation and oxidative stress. It has also been reported that as PON-1 protects lipids from peroxidation, it is an important parameter in evaluating the level of MDA, one of the lipid peroxidation metabolites of PON-1. Okada et al.[21] found that serum MDA levels were statistically significantly higher in obese cats compared to the control group. Rossi et al.[8] indicated that the serum PON-1 level in healthy cats is 58–154 U/L. Dağ and Şahinduran[27] reported that the PON-1 values in overweight patients were statistically lower than in the control group. In their study conducted on obese cats that then lost weight, and those that did not, Tvarijonaviciute et al.[28] determined that the PON-1 levels in the group that could not lose weight were significantly lower than the values determined in the other group.

The molecules in the plasma thiol pool have antioxidant

effects. The status of the plasma thiol pool, which reflects thiol/native thiol homeostasis, is evaluated by looking at the levels of serum albumin and thiol, which are low molecular weight proteins and compounds.[29,30] Mengen et al.[31] determined that the disulfide/total thiol level in obese individuals was significantly higher than the value determined in healthy individuals. Tursun et al.[29] stated that the antioxidant thiol level determined in obese rats was lower than the value in the control group. Giannuzzi et al.[30] found that the measurement of the level of thiol groups was significant in evaluating the liver functions of dairy cattle. In our study, it was determined that total thiol and native thiol levels were significantly higher in overweight cats and cats with developed obesity, as compared to the control group. This was interpreted as an increase in oxidative stress due to weight gain.

Various studies have shown that obesity causes a proinflammatory state in humans[32,33], dogs[34], and cats[35] due to an increase in inflammatory cytokines. It has been shown that neutrophil and monocyte counts are high in both obese children[32,33] and in obese dogs.[34] In our study, no statistically significant difference was found in terms of WBC values in overweight and obese cats compared to the values in the cats in the control group.

This study provides an insight into the metabolic pathways on which organic and metabolic disorders that occur in cats due to excessive weight gain or obesity are based, as well as on the pathogenesis of the disorders.

As a result of this study, statistically significant differences were found between the changes in the levels of parameters related to liver function and lipid metabolism, as well as in the levels of parameters indicating systemic inflammation and oxidative stress in overweight cats and obese cats compared to the values in the control group. These results both indicate that health problems begin in cats which exceed the ideal BCS values. It also demonstrates that it would be useful for veterinarians to take into account both significant changes in parameters related to liver function and lipid metabolism, and to indicate systemic inflammation and oxidative stress in the control and treatment of the health of such cats.

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