The Effects of High-Fat Diets on The Oxidative Stress in Adipose Tissue in Rats, Whose Retroperitoneal Adipose Tissues are Denervated

Retroperitoneal Yağ Dokusu Denerve Edilmiş Sıçanlarda Yüksek Yağlı Diyetin Yağ Dokusundaki Oksidatif Strese Etkisi

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Abstract

Objective: White adipose tissue is innervated by both the autonomic nervous system and the sensory nervous system. In this study, the aim is to investigate the effect of the high-fat diet on the superoxide dismutase (SOD), catalase (CAT) and malondialdehyde (MDA) levels in denervated retroperitoneal adipose tissue.

Material-Method: All nerve fibers were denervated from retroperitoneal adipose tissues of 16 male Sprague-Dawley rats aged 3-5 weeks old. Two groups were randomly formed from these rats which consist of control and high-fat diet groups. The rats were fed for 70 days with the diet specific to their groups. Retroperitoneal adipose tissues were collected and SOD, CAT, and MDA measurements were performed on these tissues.

Results: SOD enzyme activity was lower and CAT enzyme activity was higher in the group fed with high-fat diet (p=0.002, p=0.006, respectively) than the control group. The MDA level of the high-fat diet group was higher than the control group (p=0.015).

Conclusions: A situation of oxidative stress arises in the group fed on a high-fat diet, although the CAT activity is high; because CAT is not able to inhibit oxidative stress alone if SOD enzyme activity is low since the CAT substrate is produced by the SOD enzyme. In conclusion, our study showed that feeding a high-fat diet increases oxidative stress in adipose tissue in rats, whose retroperitoneal adipose tissue is denervated.

Keywords: Catalase, Denervation, High-Fat Diet, Retroperitoneal Adipose Tissue, Superoxide Dismutase

Özet

Amaç: Beyaz yağ dokusu hem otonom sinir sistemi hem de duysal sinir sistemi tarafından innerve edilir. Yağ dokusunu innerve eden sinirlerin yağ dokusunda lipoliz başta olmak üzere çok sayıda önemli rolleri bulunur. Bu çalışmada yüksek yağlı diyetin denerve edilmiş retroperitoneal yağ dokusundaki süperoksit dismutaz (SOD), katalaz (CAT) aktivitelerine ve lipid peroksidasyon markırı malondialdehit (MDA) düzeyine etkisinin incelenmesi amaçlandı.

Materyal-Method: Çalışmada 16 adet 3-5 haftalık erkek Sprague-Dawley sıçanların retroperitoneal yağ dokularındaki tüm sinir lifleri denerve edildi. Bu sıçanlardan rastgele kontrol ve yüksek yağlı diyet grubu olmak üzere iki grup oluşturuldu. Sıçanlar gruplarına özgü yemle 70 gün boyunca beslendi. Beslenme süresinin bitiminde retroperitoneal yağ dokuları toplandı ve bu dokularda SOD, CAT ve MDA ölçümleri yapıldı.

Bulgular: Yüksek yağlı diyetle beslenen grupta kontrol grubuna göre SOD enzim aktivitesi daha düşük, CAT enzim aktivitesi ise daha yüksek olduğu tespit edildi (sırasıyla p=0,002, p=0,006). MDA seviyesi ise kontrol grubuna göre yüksek yağlı diyet grubunda daha yüksek bulundu (p=0,015).

Sonuç: Yüksek yağlı diyetle beslenen grupta katalaz aktivitesi yüksek olmasına rağmen oksidatif stres görülmektedir. Bunun nedeni, katalazın substratı SOD enzimi tarafından üretildiği için SOD enzim aktivitesinin düşük olması durumunda katalazın tek başına oksidatif stresi engelleyememesidir. Sonuç olarak, çalışmamız retroperitoneal yağ dokusu denerve edilmiş sıçanlarda yüksek yağlı diyetle beslenmenin yağ dokusundaki oksidatif stresi artırdığını gösterdi.

Anahtar kelimeler: Katalaz, Yüksek Yağlı Diyet, Retroperitoneal Yağ Dokusu, Süperoksit Dismutaz

Introduction

In the body, white adipose tissue (WAT) is found around numerous internal organs such as the kidney, stomach, and beneath the skin (subcutaneous fat) (1). WAT is innervated by both the autonomic and sensory nervous systems (2-4). The metabolic and excretion capacities of the adipose tissues are tightly controlled by the autonomic nervous system (5). Studies have shown that the WAT is equipped with sympathetic fiber ends of the autonomic nervous system (5). The sympathetic nervous system controls lipolysis through secreting norepinephrine and neuropeptide Y by activating different receptors in fat cells (3, 6) and it is also the main trigger for the lipolysis (7). The effects of the nerves that innervate the adipose tissue on the lipolysis are well known

(2, 8, 9), while they have a significant role in the apoptosis, differentiation, and control of the reproduction (5). Nerves that innervate the adipose tissue also play an important role in the regulation of the anabolic pathways (8, 9).

The adipose tissue is named with special names according to the regions where it is found in the body. The retroperitoneal adipose tissue is found both in humans and in rats as a white adipose tissue pad located behind the peritoneum (1). These nerve fibers enter the adipose tissue from superior to the adipose tissue, which is close to the diaphragm. The retroperitoneal adipose tissue denervation is performed by surgically cutting these nerve fibers (10).

There are various antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) which inactivate superoxide and hydrogen peroxide that form in various ways in living creatures. SOD converts superoxide into hydrogen peroxides, and CAT converts hydrogen peroxide into water, thus, making these molecules harmless. The reactions of SOD and CAT were presented in Figure 1 (11). SOD has 3 isoenzymes. In particular, the expressions of SOD 2 and SOD 3 are the highest in adipose tissue, except for a few tissues when all tissues are compared and while SOD 1 and CAT are expressed in moderate levels (12). This fact proves the importance of SOD and catalase for the adipose tissue. Oxidative stress occurs in tissues in situations where antioxidant enzymes are inadequate (11). Lipid peroxidation status in the tissues is determined by measuring tissue malondialdehyde (MDA) (13).

In this study, the aim was to investigate the effects of high-fat diet on SOD and CAT activities and MDA levels, which is a biomarker of the oxidative stress in the denervated retroperitoneal adipose tissue.
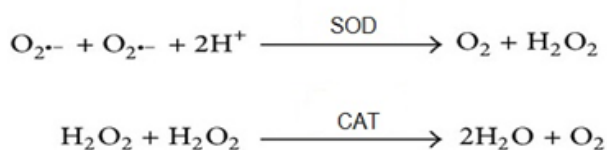
$$O_2^{\cdot-} + O_2^{\cdot-} + 2H^+ \xrightarrow{\text{SOD}} O_2 + H_2O_2$$

$$H_2O_2 + H_2O_2 \xrightarrow{\text{CAT}} 2H_2O + O_2$$

Figure 1: The reactions of SOD and CAT

Material-Method

Animals and Experimental Design

All of the experiments and research were applied with the permission of Karadeniz Technical University Animal Experimentation Ethics Committee protocol no. 2017/30.

Rats used in this experiment were provided from Karadeniz Technical University Surgical Application and Research Center. The rats were fed in the feedlot in a 12-hour flight, 12-hour darkness cycle, temperature (22±1ºC), relative humidity (55-60%). In the study, 16 male Sprague-Dawley rats aged 3-5 weeks old, weighing between 100 and 150 g, were used.

All nerve fibers in retroperitoneal adipose tissue on both sides of the rats (right and left) were denervated and a single dose of penicillin was injected into the abdomen before the abdomen was closed.

The rats were chosen in a random manner, and they were separated into two groups (control group and high-fat diet group) consisting of eight rats in total. All of the feed used in the experiments for the animals was purchased from Research Diets (diet code D12450J and D12492). The composition of the feed is introduced in Table 1. All of the rats were fed with feed and water in an ad libitum order. At the end of the feeding stage, which lasted 70 days, the rats were slaughtered under the anesthesia (ketamine hydrochloride 90 mg/kg (Alfamine, Alfasan, Woerden-Holland, intramuscular) and xylazine hydrochloride10 mg/kg (Alfazyne, Alfasan, Woerden-Holland, intramuscular). The retroperitoneal adipose tissue of all rats was resected and afterward, these tissues were frozen on dry CO_2. The specimens were placed into Eppendorf tubes immediately, and afterward, they were stored at -80ºC until analysis.

Table 1. Control diet and high-fat diet ingredients

Product	Control diet		High-fat diet	
	g%	kcal %	g%	kcal %
Fat	4.3	10	34.9	60
Protein	19.2	20	26.2	20
Carbohydrate	67.3	70	26.3	20
Total	-	100	-	100
kcal/g	3.89	-	5.24	-

Tissue Homogenization for Sod and Catalase Activity Measurement

Approximately 150 mg of fat tissue was weighed on a precision laboratory scale. The tissue was placed in 4mL of cold homogenization buffer (500 µL/L Tritonx100, 50 mM Tris-HCl, pH: 7.4), and homogenized with a homogenizer (Ultra-Turrax T25 homogenizer, IKA Labortechnik, Germany) for 10 seconds in a cold environment. Homogenized tissue was centrifuged at +4ºC, 3 000 rpm for 10 minutes (Allegra 64R Centrifuge). The supernatant under the fat layer was used for both SOD and catalase measurements. 0.8 mL of supernatant was added to 400 µL of ethanol chloroform mixture (2: 3 ratio, respectively) for SOD measurement. The mixture was vortexed and centrifuged at 10 000 g for 30 minutes at +4°C. After centrifugation supernatant was used for SOD measurement [by using the method of Sun and colleagues (14)]. 1 mL of supernatant was added to 300 µL of chloroform for catalase measurement. The mixture was vortexed and centrifuged at +4°C for 15 minutes at 10 000 g. After centrifugation supernatant was used for catalase measurement [by using the method of Aebi (15)].

Tissue Homogenization for MDA Measurement

Approximately 150 mg of fat tissue was homogenized in 2 mL of cold homogenization solution (0.15 M KCl, 1 mL/L Triton X-100). Afterwards, the homogenization homogenates were centrifuged at 3 000 rpm for 10 minutes. The oil layer collected at the top of the tube was removed, and 0.8 mL of the supernatant under the oil layer was transferred to a

clean tube. 400 µL of ethanol chloroform mixture (3:2 ratio, respectively) was added to the supernatant transferred tube. The mixture was vortexed and centrifuged at +4ºC 15 000 g for 10 minutes. After centrifugation, the supernatant phase and the bottom organic phase were transferred to a clean tube. This transferred solution was vortexed and used for MDA measurement [by using Uchiyama and Mihara's method (16)].

When the remaining retroperitoneal adipose tissue was examined, the activities of total superoxide dismutase (SOD) and catalase (CAT), protein concentrations, and MDA levels were identified. The activities of SOD were examined by using the method of Sun and colleagues, with slight modifications to the method (14). The results that were obtained regarding the proteins were expressed in U/mg protein. In this method, 0-10 U/mL SOD standard (Sigma), 167 U/L xanthine oxidase (Sigma), 0.3 mM xanthine (Sigma), 0.6 mM ethylenediaminetetraacetic acid (Carlo Erbo Reagent), 150 µM nitro blue tetrazolium (Sigma), 0.4 M Na2CO3 (Lancaster), 0.1% bovine serum albumin (Sigma), 2M (NH4)2SO4 for dilution of xanthine oxidase (Merck), 0.8 mM CuCl2 (Lancaster) and sample (homogenate) were used. The solutions were pipetted as shown in the Table 2, and results were calculated by using the method of Sun and colleagues (14).

Table 2. The reaction mixture for the measurement of SOD activity

Solutions	Volume (mL)
Sample (Standard, Sample, Blank solution)	0.25
SOD reaction mixture	
(0.3mM xanthine, 0.6mM ethylenediaminetetraacetic acid, 150µM nitro blue tetrazolium, 0.4M Na₂CO₃, 0.1% bovine serum albumin)	1.25
Xanthine oxidase	0.025
Incubation for 20 minutes in the dark, at 25ºC	
CuCl2	0.5
Measurement at 560 nm (Molecular Devices Versa Max)	

CAT activities were identified by using the method of Aebi, with slight modifications to the method (15), and the results regarding the proteins were expressed in U/mg protein.

In this method, 50 mM phosphate buffer pH: 7.00 (KH₂PO₄ and Na₂HPO₄.2H₂O, Merck) and 0.216% H₂O₂ (Sigma) and sample (homogenate) were used. The solutions were pipetted as shown in the Table 3, and results were calculated by using the method of Aebi after absorbance measurement at 240 nm (Beckman-Coulter) (15).

Table 3. The reaction mixture for the measurement of CAT activity

Solutions	Blank solution (mL)	Sample (mL)
Phosphate buffer	0.25	-
Sample	0.50	0.50
H₂O₂	-	0.25

The MDA levels of the tissues were examined by using Uchiyama and Mihara's method with slight modifications to the method (16). The MDA levels were expressed as wet tissues in nanomoles/mg protein. In this method, 1% H₃PO₄ (Merck), 0.6% Thiobarbituric acid,   0-5 nmoles/mL standard solutions (1,1,3,3-Tetramethoxypropane, Sigma) and sample (homogenate) were used. The solutions were pipetted as shown in the Table 4, and results were calculated by using the Uchiyama and Mihara's method after absorbance measurement at 532 nm (Molecular Devices Versa Max) (16). The concentrations of the proteins were identified by using the method of Lowry (17).

Table 4. The reaction mixture and applications for the MDA measurement

Solutions	Applications	Volume (mL)
Sample (Standard, Sample, Blank solution)	-	0.5
1% H₃PO₄	-	3
Thiobarbituric acid	-	1
Vortex		
Incubation for 45 minutes at 100ºC		
Centrifuge at 4 000 rpm for 10 minutes		
300 µL loading on the plate		
Measurement at 532 nm		

Statistical Analysis

The results of our study were expressed as mean (±) standard deviation (SD). The values obtained from the groups were compared using the nonparametric Mann-Whitney U-test. Statistical analyses were carried out using the SPSS 16.0 program pack. The level of statistical significance was determined to be $P < 0.05$.

Results

Significant differences between the groups were found after examining the SOD levels in the retroperitoneal adipose tissues of the control and high-fat diet groups (Figure 2). When the SOD levels of control and high-fat diet groups were compared, it was determined that the SOD levels of the high-fat diet group were lower than that of the control group in the retroperitoneal adipose tissue (p=0.002) (Figure 2).
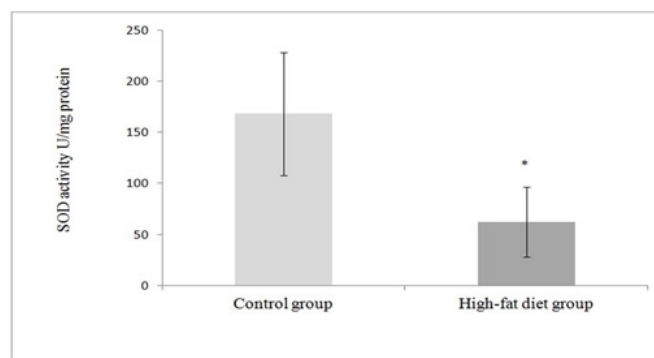

Figure 2: SOD activity in the retroperitoneal adipose tissue
Control group 168±60 U/mg protein, high fat diet 62±34 U/mg protein
*Statistically significant with respect to Control group p<0.05

When the CAT levels in the retroperitoneal adipose tissue of the high-fat diet group and control groups were examined, significant differences were found between the groups (Figure 3). When the CAT levels of the high-fat diet group and the control group were compared, the high-fat diet group's was found to be higher that of the control group in the retroperitoneal adipose tissue (p=0.006)
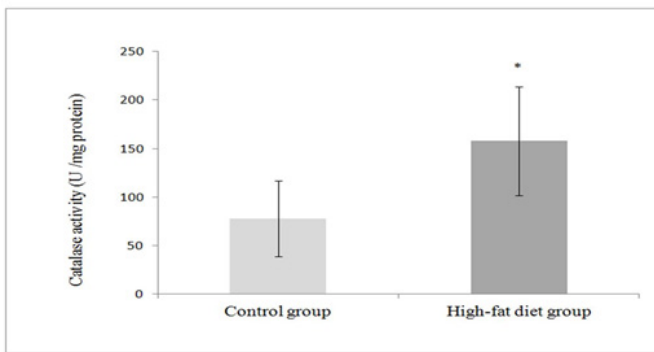

Figure 3: Catalase activity in the retroperitoneal adipose tissue
Control group 78±39 U/mg protein, high fat diet 158±56 U/mg protein
*Statistically significant with respect to Control group p<0.05

When the MDA levels were examined, the high-fat diet group's levels were higher than that of the control group in the retroperitoneal adipose tissue (p=0.003) (Figure 4).
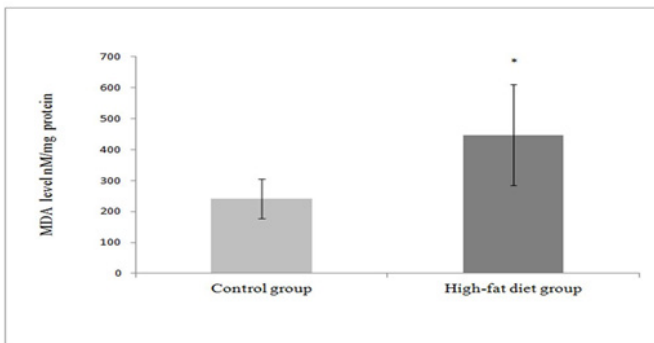

Figure 4: MDA levels in the retroperitoneal adipose tissue
Control group 242±64, high fat diet 447±163 nanomoles/mg protein
*Statistically significant with respect to Control group p<0.05

Discussion

The main metabolic pathways of adipose tissue are lipogenic (synthesis of triglycerides) and lipolytic pathways (degradation of triglycerides into free fatty acid and glycerol) (18). These lipogenic pathways were primarily controlled by insulin (5). As for lipolytic pathways, they are mostly under the dependency of perilipin A, adipose triglyceride lipase, and hormone-sensitive lipase (18).

Adipose tissue has various physiological functions with adipokines, such as leptin, adiponectin, synthesized and secreted apart from triglyceride storage (5). The nervous system plays an important role in the synthesis and secretion of some adipokines. In one study it was found that the concentration of leptin decreased with the stimulation of beta-

adrenergic receptors (19). In another study conducted with 3T3L1 adipocytes, it was determined that leptin secretion decreased in the presence of sympathetic neurons (20). Denervation of adipose tissue in Siberian hamsters causes a significant increase in adipocytes (21). This situation indicates that nervous system affects adipose tissue metabolism.

As the result of the transcriptomic studies on the rats that were fed with normal and high-fat diets, it was revealed that the high-fat diets changed the expression of 16,699 genes at the end of the second week and 5,111 genes at the end of 24th week of feeding (22).

Both the transcriptomic studies (22, 23) regarding the species of the feed and the studies on the innervation effect of the nerves (2) show that these two factors affect the metabolic activity of the adipose tissue to a considerable extent. It is possible that this metabolic activity change will affect the oxidant-antioxidant balance in the adipose tissue.

In a study conducted with both young and elderly rats where they were fed high-fat diets, no significant difference in liver SOD and CAT enzyme activities were found when compared to the control group (24). Contrary to that study, a decrease in SOD enzyme activity and an increase in CAT enzyme activity was found in the high-fat diet group in our study. The contradiction between these studies can be explained in two ways. First, this may be due to metabolic differences between the liver and retroperitoneal adipose tissue. The differentiation of SOD enzyme activities in various tissues of the body supports the above-mentioned possibility (25). Second, in our study, retroperitoneal fat tissue was denervated. However, in the literature study, fat tissue was not denervated. Different results between these two studies may be due to a different application (denervation).

In our study, SOD activity was found to be lower and MDA level was found to be higher in the high-fat diet group than in the control group. When the SOD and CAT reactions are taken into consideration (11), the decrease in SOD activity in the high-fat diet group causes the inability to remove the superoxide radical, which is formed due to metabolism. As a result of that, it is highly possible that the superoxide radical will attack the lipids and cause the level of MDA to increase.

In the high-fat diet group, CAT activity was higher than in the control group. At first glance, since CAT is an antioxidant enzyme, there might be the expectation of CAT's subduing the hydrogen peroxide which may lead to a decrease in MDA level in the high-fat diet group. However, this expectation is not correct. Since CAT enzyme use the product of SOD enzyme (hydrogen peroxide) as a substrate, a substrate shortage occurs for CAT, resulting from low SOD enzyme activity. This results in CAT not being able to have a sufficient effect, due to the lack of substrate, even though CAT enzyme activity is high. As a result, the excess CAT activity alone in the cell will not be able to remove the superoxide in the cell. Therefore, although the CAT enzyme level is high in our study, CAT might not have been able to prevent the oxidative stress condition in the high-fat diet group, due to the low SOD enzyme activity.

As a result, our study showed that the high-fat diet increased the oxidative stress in the adipose tissues of the rats, whose retroperitoneal adipose tissues were denervated.

Acknowledgments

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