ORIGINAL ARTICLE

Comparison of Malondialdehyde and Reduced Glutathione in Some Rat Tissues in Hypoxia and Obesity

Hipoksi ve Obezite Olgusunda Malondialdehit ve İndirgenmiş Glutatyon'un Bazı Sıçan Dokularında Karşılaştırılması

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ABSTRACT

Objective: *Obesity is an important global public health problem that is associated with many chronic diseases and is increasing day by day in our country as well as in the world. The aim of this study is to compare malondialdehyde (MDA) and reduced glutathione (GSH) in some rat tissues due to obesity and hypoxia.*

Material and Method: *In our study 24 male Sprague Dawley rats were used. Rats were divided into four groups (n:6) as standard diet/normal oxygen, standard diet/low oxygen, high-fat diet/normal oxygen, and high-fat diet/ low oxygen. For the study, a special cage with low oxygen level of 17-18% in the closed system was used. Weight gain of 20-25% was achieved in obese rats. MDA and GSH levels were measured in liver, kidney and brain organ tissues of rats.*

Results: *In our study it was determined that there were significant increases in the amount of MDA and GSH. It was observed that MDA and GSH had a protective effect against hypoxia and obesity in liver and brain tissue, but not in kidney tissue.*

Conclusion: *As a result of our research we think that MDA and GSH may support the current criteria in the diagnosis and/or treatment of obesity and will contribute greatly to more comprehensive analyzes to be made in the future.*

ÖZET

Amaç: *Obezite, günümüzde birçok kronik hastalıklarla bağlantılı olup dünyada görüldüğü gibi ülkemizde de gün geçtikçe artan, önemli küresel halk sağlığı problemidir. Bu araştırmanın amacı obezite ve hipoksiye bağlı olarak malondialdehit (MDA) ve indirgenmiş glutatyon (GSH)'un bazı sıçan dokularında karşılaştırılmasıdır.*

Gereç ve Yöntem: *Çalışmamızda 24 adet Sprague Dawley cinsi erkek sıçan kullanılmıştır. Sıçanlar standart diyet/normal oksijen, standart diyet/düşük oksijen, yüksek yağlı diyet/normal oksijen ve yüksek yağlı diyet/düşük oksijen olmak üzere dört gruba (n:6) ayrıldı. Çalışma için kapalı sistemde oksijen seviyesi düşük %17-18 olan özel kafes kullanılmıştır. Obez sıçanlarda %20-25 ağırlık artışı sağlanmıştır. Sıçanların karaciğer, böbrek ve beyin organ dokularında MDA ve GSH seviyeleri ölçülmüştür.*

Bulgular: *Çalışmamızda MDA ve GSH miktarı açısından anlamlı artışların olduğu saptanmıştır. MDA ve GSH'ın karaciğer ile beyin dokusunda hipoksi ve obeziteye karşı koruyucu etkisi olurken, böbrek dokusunda olmadığı görülmüştür.*

Sonuç: *Araştırmamız sonucu MDA ve GSH'ın obezitenin tanı ve/veya tedavisinde mevcut kriterlere destek sağlayabilir olabileceği ve gelecekte yapılacak olan daha geniş kapsamlı analizlere büyük ölçüde katkı sağlayacağı düşüncesindeyiz.*

INTRODUCTION

Hypoxia is a condition of the body where the arterial oxygen concentration is less than normal and is caused by inflammation, sepsis, hypertension, and also causes the release of hypoxia-inducible factor 1 (HIF-1) (1, 2).

Obesity is a chronic metabolic disease that results from inequality between energy intake and expenditure. Increased fat and lipid density feature is observed in the blood. One of the most important reasons for the development of obesity is lack of physical activity (3-5). In a study it was seen that the share of physical activity insufficiency in the onset of obesity is very important (67.5%) (3). Obesity has reached epidemic proportions,

contributing greatly to the global burden of some chronic diseases. Epidemiological studies have highlighted a tight link between excess fat deposition and oxidative stress (6, 7). Fat accumulation has also been recognized as a source of oxidative stress (8). Some studies suggest that oxidative stress may be a prerequisite for adipogenesis. It has been found that there is rise in the level of reactive oxygen species (ROS) during adipogenesis (9). Obesity is a very factor with syndromic and nonsyndromic variants. In 2011-2014 the prevalence of obesity was 36% among adults in the United States (10). Between 2015 and 2016, the prevalence of obesity in the United States was 39.8% among adults and 18.5% among teenagers. The

prevalence of obesity was higher among adults aged 40-59 years than adults aged 20-39 years overall and in both men and women (11). Obesity type II diabetes, hypertension, coronary heart disease, stroke, oiled liver, dementia, obstructive sleep apnea and a risk factor for metabolic disorderliness and illness that significantly increase the risk of developing various cancer species. As a result of studies it is estimated that the average life expectancy will decrease as a result of such diseases (12-15). In general the amount of fat in the body is determined using the body mass index (BMI) and calculation is made by body weight (kg/m²) (16). There are approximately 300 million obese people all over the world and this number is increasing gradually (17).

The adipose tissue is a very active endocrine organ secreted by a series of biologically active molecules called adipokines. The dysfunction of the adipose tissue is one of the primary flaws of obesity. The bad corrupt adipose tissue function is characterized by an atherogenic adipokin structure and proinflammatory secretion (14). Oxidative stress occurs with the increase of free radicals and reactive oxygen radicals and causes severe damage to biological macromolecules and causes disorders in metabolism and physiology. Cells manage to maintain their vital functions against oxidative damage with the help of a system. This system contains glutathione peroxidase (GSHPx), superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), some trace elements and vitamins A, E, C which counter oxidative damage. Recent studies have shown that superoxide formation is enhanced in obesity related disorders and SOD is inhibited by nonenzymatic glycation and furthermore hyperlipidemia increases endothelial superoxide production. Therefore superoxides are thought to play a key role in the physiopathology of the cardiovascular and metabolic effects of obesity (18).

MDA resulting from lipid peroxidation is an indicator of oxidative stress in tissues and cells. Lipid peroxidase is a derivative enzyme of the unsaturated fatty acid that emerged as a result of dissipating complex components (19). Due to the relatively short half lives of free radicals level detection is difficult (20).

GSH is an endogenous peptide that can be synthesized in the liver without the need for genetic data, consists of glutamic acid, cysteine and glycine amino acids and is an significant water soluble antioxidant. GSH plays a vital role in cells so that enzymes and other cellular components are not kept in a reduced state. Glutathion in very loud concentrations in many cells protects biological membranes opposite lipid peroxidation. GSH is mostly synthesized in the liver and approximately 40% is excreted in bile (21-23). Free radicals react with peroxidase to defend cells against oxidative damage (24). ROS's potentially deleterious impacts are controlled by the cellular antioxidant defense system. GSH is an significant component of intracellular preventive mechanisms opposite many deleterious stimuli, including oxidative stress (25).

The aim of our study is to research the effectiveness of MDA and GSH enzyme changes in the diagnosis and treatment stages, in addition to the criteria valid in the evaluation of obesity.

MATERIAL AND METHOD
Rats Used in the Study
5 month old male Sprague Dawley rats were used in the study. Rats were housed in special lattices for 12 clock in light/dark, ventilated room temperature at 24°C. Rats other than the obesity group were given standard diet and water, and the group in which obesity was desired was given high-fat diet and water. Obese rats were fed a high fat diet for 23 weeks. Weight gain of 20-25% was achieved in obese rats. Their average weights varied between 450-534 grams (g). Animal rights are protected in line with the principles of the 'Guide for the Care and Use Guide of Laboratory Animals' (ethics committee no: 2015/86).

Table 1: Rat groups used in the study, their numbers and nutritional content

1. Group (n:6)	Standard Diet/Normal PO2 (SD/NO2)
2. Group (n:6)	Standard Diet/ Low PO2 (SD/LO2)
3. Group (n:6)	High Fat Diet/Normal PO2 (HFD/NO2)
4. Group (n:6)	High Fat Diet/Low PO2 (HFD/LO2)

Retrieval of Tissues
A mixture of 1500 µl/kg ketamine and 500 µl/kg xylazine was administered intramuscularly (i.m.) as anesthetic agent. The abdomen of the anesthetic administered rats was cut, the thorax was opened and the vena cava vessels were cut. The perfusion process was completed by injecting 5 ml of saline into the right and left ventricles of the heart.

Collection and Homogenization of Working Tissues (liver, kidney and brain)

Each rat used in the experiment was euthanized by perfusion and removing the rat's heart. All liver, kidney and brain tissue were taken. It was reperfused with physiological saline and wrapped in labeled aluminum foils. Tissues were placed in liquid nitrogen immediately after collection. After the dissection procedures were completed, the tissues were removed from liquid nitrogen and stored at -40 °C.

The homogenization process was carried out quickly and rapidly in ice. The tissues were cut with a scalpel, weighed on a precision balance (ATX224) and taken into glass tubes with buffer solution. 4500 µl of 0.2 M pH: 7.2-7.6 phosphate buffer was added to 0.5 g tissue. Tissues were sonicated on ice for 30 to 60 seconds with an ultrasonicator (BANDELIN SONOPLUS). These tissues were centrifuged (MicroCL 21 centrifuge), separated into supernatant and homogenate parts, placed in 1000 µl eppendorf tubes and stored at -40 °C.

Measurement of MDA Amount: According to the method of Uchiyama et al. it was determined by spectrophotometric measurement of the supernatant extracted from the N-butanol phase of the pink colored product formed by the reaction of MDA with thiobarbituric acid at 95 °C at 520 and 535 nm wavelengths (26).

Measurement of GSH Amount: GSH analysis was performed according to the method described by Ellman. The amount of reduced glutathione was determined by the reaction of the glutathione in the analysis tube with 5,5'-dithiobis 2-nitrobenzoic acid to give a yellow greenish color and by measuring the light intensity of this
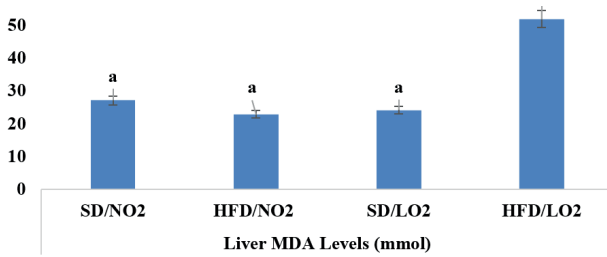
Figure 1: Amounts of MDA (mmol) in rat liver tissue. The differences between different letters in the pillars are statistically important. Results are given as ±SE.
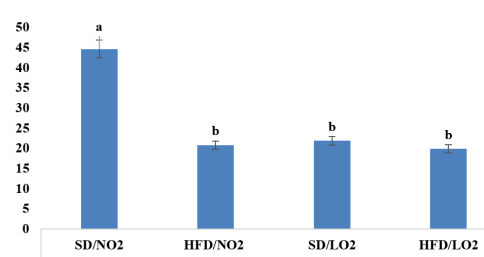

Figure 2: Amounts of MDA (mmol) in rat kidney tissue. The differences between different letters in the pillars are statistically important. Results are given as ±SE.
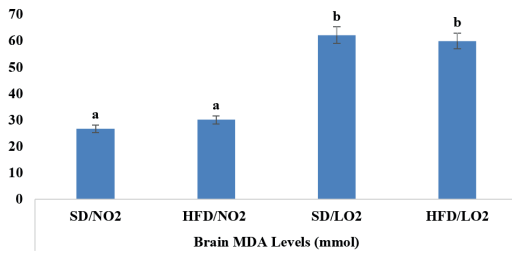

Figure 3: Amounts of MDA (mmol) in rat brain tissue. The differences between different letters in the pillars are statistically important. Results are given as ±SE.
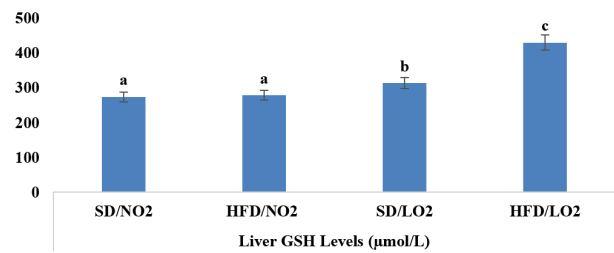

Figure 4: GSH amounts (µmol/L) in rat liver tissue. The differences between different letters in the pillars are statistically important. Results are given as ±SE.
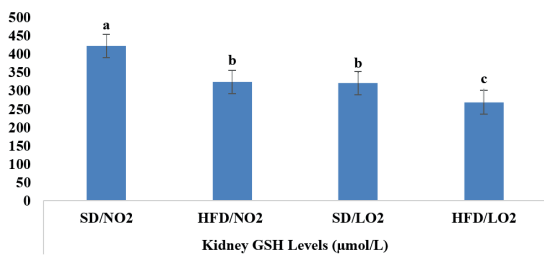

Figure 5: GSH amounts (µmol/L) in rat kidney tissue. The differences between different letters in the pillars are statistically important. Results are given as ±SE.
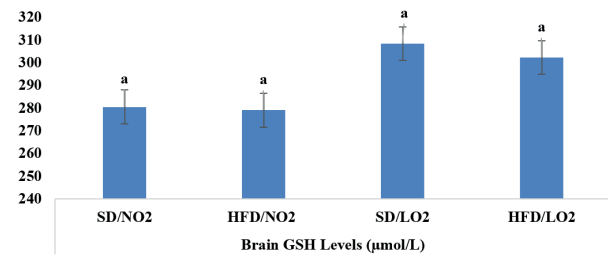

Figure 6: GSH amounts (µmol/L) in rat brain tissue. The differences between different letters in the pillars are statistically important. Results are given as ±SE.

color at a wavelength of 410 nm spectrophotometrically (27).

Statistical Analysis

Statistical evaluations were made with SPSS for Windows Version 15.0 package program. Data for measurable variables are given as mean ± standard error. The tukey test method was used to determine the differences between the groups. The value found was evaluated at the 5% significance level (95% confidence interval, $p < 0.05$).

RESULTS

In all working groups; In liver, kidney and brain, MDA was determined by Uchiyama et al. and GSH analysis was performed by spectrophotometric measurement according to the method described by Ellman. Graphical representations of MDA amounts measured in tissues are given in Figure 1, Figure 2 and Figure 3, and GSH amounts are given in Figure 4, Figure 5 and Figure 6.

When all groups were examined in terms of MDA amount in rat liver it was seen that there was a important rise in HFD/LO2 ($p < 0.05$). There was a significant increase in liver tissue due to obesity and hypoxia ($p < 0.05$). There was no significant increase between SD/NO2, HFD/NO2 and SD/LO2 groups ($p > 0.05$) (Figure 1).

When all groups were examined in terms of the amount of MDA in the rat kidney tissue it was observed that there was a significant increase in SD/NO2 ($p < 0.05$). There was no important rise between HFD/NO2, SD/LO2 and HFD/LO2 groups ($p > 0.05$)(Figure 2).

When the rat brains of all groups were analyzed for the amount of MDA it was seen that there was an important increase between SD/NO2-HFD/NO2 and SD/LO2-HFD/LO2 groups ($p < 0.05$). There was an important rise in brain tissue due to hypoxia ($p < 0.05$). While there was no important rise between SD/NO2-HFD/NO2 groups ($p > 0.05$), there was no significant increase between SD/LO2-HFD/LO2 groups ($p > 0.05$)(Figure 3).

When all groups were examined in terms of the amount of GSH in the rat liver it was seen that the most important rise was in HFD/LO2 and the second important rise was in SD/LO2 ($p < 0.05$). There was an important rise due to hypoxia ($p < 0.05$). When SD/LO2 and HFD/LO2 groups were compared it was observed that there was an important rise between these groups ($p < 0.05$). There was no important rise between SD/NO2 and HFD/NO2 groups ($p > 0.05$) (Figure 4).

When all groups were examined in terms of GSH amount in rat kidney it was seen that the most important rise was in SD/NO2, the second important rise was in HFD/NO2 and SD/LO2 ($p < 0.05$). There was no important rise between HFD/NO2 and SD/LO2 groups ($p > 0.05$). There was an

important rise between HFD/NO2-SD/LO2 and SD/NO2-HFD/LO2 groups (p<0.05) (Figure 5).

When all groups (SD/NO2, HFD/NO2, SD/LO2, HFD/LO2) were examined in terms of the amount of GSH in the rat brain, no significant difference was observed (p>0.05) (Figure 6).

DISCUSSION

In our study it was determined that the amount of MDA was rised in HFD/LO2 in the liver, SD/LO2 in the brain and in the HFD/LO2 groups compared to the other groups. It was observed that this increase was related to obesity and hypoxia. It was determined that the amount of GSH was increased in the liver and brain in the SD/LO2-HFD/LO2 groups compared to the other groups. In the kidney it was observed that the amount of MDA and GSH reduced in the other three groups compared to the control group. This makes us think that MDA and GSH play a role in maintaining body homeostasis in hypoxic conditions as well as protecting against hypoxia and obesity.

Erkasap S, Erkasap N, Aral E, et al. The protective effect of Epidermal Growth Factor (EGF) on wounds in the gastric mucosa of rats treated with ethanol was investigated. MDA, protein sulphuride groups (SH) and protein carbonyl values were measured in gastric tissue. In the ethanol+EGF group, ulcer symptoms, histamine, MDA and protein carbonyl values were decreased. When these values were compared with the values of animals without EGF they reported that EGF acted as an antioxidant as well as a protective effect on gastric mucosal injuries (28). In the presence of oxidative stress the lipid peroxidation indicator MDA level increases in various tissues and blood plasma/serum samples while the GSH and SOD enzyme activities which provide ROS elimination decrease (29, 30). In this study plasma MDA level increased and GSH and SOD enzyme activity decreased in rabbits who were injected with aglepristone for 2 consecutive days. From these results it was observed that abortion (low) and/or especially aglepristone had a negative effect on reducing antioxidant defense capacity and on oxidative stress parameters. In addition decreased antioxidant capacity was thought to play a role as a determining factor in the pathogenesis of infertility (31). Troudi et al. found a rise in MDA levels and a reduce in antioxidant enzymes such as SOD and GSH due to oxidative stress in rats in the early and late stages of pregnancy using a neurotoxic agent that triggers the formation of oxidative stress. However, no abortion occurred in either of these studies (29). In our study an increase was observed in liver tissue except kidney and brain tissue. Changes in oxidative stress parameters support our study.

It has been stated that oxidative damage accumulation in cells may play a role in the pathogenesis of some diseases due to deterioration in protein, lipid and membrane structure, as well as cell repair mechanisms (32, 33). Studies have found that there is a positive correlation between disease severity and oxidative stress markers and a negative correlation between antioxidant markers (34, 35). In our study we determined that the body tries to increase antioxidant enzyme activity against oxidant stress in order to protect itself.

In this study investigating the efficacy of free oxygen radicals in patients with head and neck malignant tumors; while the erythrocyte MDA levels and SOD activities of the patients were higher than the control group, their CAT activities decreased. It was observed that there was no statistically important difference between the GSH-Px activities in both groups. As a result of the research it was stated that erythrocyte MDA levels may play a significant role in tissue damage that leads to the development of head and neck malignant tumors and the addition of drugs with antioxidant effects may be beneficial to reduce the damage and carcinogenic effects of increased free oxygen radicals on the tissue (36). Doner et al. and Torun et al. showed that serum MDA levels were considerably increased in patients with head and neck malignant tumors compared to normal individuals. They also stated that while MDA levels increase in cancer patients, antioxidant enzyme activities may increase or decrease (37, 38). Solmaz et al. reported that CAT and SOD, enzyme activities in the tumoral tissue in head and neck epidermoid cancers gradually decrease as the stage progresses and the MDA level gradually increases (39). The increase in free oxygen radical level can cause changes to mutagenism, cytotoxicity and gene expression, it may lead to malignant tumor development and that a malignant development of this mutagenism can contribute to a malignant transformation of a malignant development (40). It has been stated that MDA which is the product of destruction by free oxygen radicals is also mutagenic and potentially carcinogenic (38).

The relationship between breast cancer and oxidative stress and the activities of some enzymes was investigated and MDA level was found to be higher in breast tumor tissue than in normal tissue. It has been thought that the increase in MDA level may be related to the formation of necrosis caused by insufficient vascularization in invasive ductal carcinoma and the increase in antioxidant enzymes may be due to the rise in enzyme expression in tumor cells (41).

Oxidative stress markers were investigated in liver, heart and kidney tissues of obese mice. The first group received HFD for 16 weeks and the second group (control group) received only SD for 16 weeks. Lipid profile measurement, tissue samples taken from the liver; blood samples were taken and checked for MDA, protein carbonyl (PCO), GSH levels and glutathione S-transferase activities (GST). Feeding with HFD has been shown to significantly rise body weight and induce dyslipidemia. In the study an important rise in MDA and PCO levels in the liver and heart tissues of obese mice and a decrease in the kidney were shown. GSH levels, reduce in kidney and liver tissues of obese animals, important rise in heart tissue were noted. A negative correlation was found between MDA-PCO levels and GSH levels in liver and kidney tissues. A positive correlation was found between GSH levels in heart tissues. It has been stated that the rise in MDA-PCO levels in obesity, being correlated with antioxidant enzyme activities and decrease in glutathione levels, accompanied by oxidative stress in liver, heart and kidney tissues, may possibly contribute to the progression of obesity-related problems (42).

In another study biochemical markers of nitric oxide (NO), MDA, GSH and the oxidative state of the follicle were

investigated to foretell the outcome of in vitro fertilization. Follicular cells were collected in the study. Biochemical analyzes of NO, MDA and GSH were performed in the collected cells. When successful and unsuccessful pregnant groups were compared in terms of NO, MDA and GSH, MDA was found to be high in follicular cells and low in the pregnant group. Correlation analysis between oxidative stress and IVF parameters revealed a weak correlation between MDA and fertilization rate. ROC curve analysis showed that MDA had a field below the 0.74 curve and could predict pregnancy with high precision. Since MDA is significantly different in pregnant and nonpregnant female and has a good sensitivity profile in predicting pregnancy it has been said that it can be considered as a marker to predict IVF success (43).

CONCLUSION
In our study it was observed that the amount of MDA increased in the liver and brain tissues, while the amount of GSH increased in the liver tissue and did not increase in the brain tissue. The most significant increase was observed in the hypoxia and obese groups of liver and brain tissues. We believe that these enzymes will have a positive effect in preventing obesity, adapting to the negative conditions that occur in hypoxia and in diagnosis and/or treatment. We think that investigating MDA and GSH enzymes in other tissues besides liver, kidney and brain tissues may yield useful results. Our research has shown that it will contribute to future comprehensive studies.

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