ABSTRACT
Objective: Obesity is an important global public health problem that is associated with many chronic diseases and 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.

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
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, and liver disease (12-15). 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), and glutathione reductase (GR), some trace elements and vitamins A, E, and 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 pathophysiology 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 peroxidation 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 important 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 low 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

<table>
<thead>
<tr>
<th>Group</th>
<th>Diet</th>
<th>PO2</th>
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<tbody>
<tr>
<td>1.</td>
<td>SD/NO2</td>
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<tr>
<td>2.</td>
<td>SD/LO2</td>
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<tr>
<td>3.</td>
<td>HFD/NO2</td>
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<td>4.</td>
<td>HFD/LO2</td>
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**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 anesthetized 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 SONOPHAT). 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 Uchiniyama 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
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 tissue it was seen that there was an important rise in HFD/LO2 (p<0.05). There was a significant increase in liver tissue due to obesity and hypoxia (p<0.05). While there was no important rise between SD/NO2 and HFD/NO2 groups (p>0.05) (Figure 1).

When all groups were examined in terms of the amount of GSH in the rat liver tissue it was seen that the most important rise was in HFD/LO2 and the second important rise was in SD/NO2-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

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.
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).

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

**DISCUSSION**

In our study it was determined that the amount of MDA was raised 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 sulphide 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 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).
investigated to foretell the outcome of in vitro fertilization. Fallopian tubes were collected in the study. Biochemical analyses 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 fallopian 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.

**Conflict of Interest:** No conflict of interest was declared by the authors

**Ethics:** This study was conducted with the Approval of Inonu University Faculty of Medicine Experimental Animals Ethics Committee (Research Protocol No: 2016/A-71)

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