The Intercontinental Journal of
Internal Medicine

DOI: 10.51271/ICJIM-0002

Original Article

Evaluation of the effect of obesity on oxidative stress with thiol/disulphide balance

Kübra Öklü[1], Aydın Çifci[1], Aşkın Güngüneş[2], Şenay Durmaz Ceylan[2], Özcan Erel[3]

[1]Department of Internal Medicine, Faculty of Medicine, Kırıkkale University, Kırıkkale, Turkey
[2]Division of Endocrinology, Department of Internal Medicine, Faculty of Medicine, Kırıkkale University, Kırıkkale, Turkey
[3]Department of Medical Biochemistry, Ankara Bilkent City Hospital, Faculty of Medicine, Yıldırım Beyazıt University, Ankara, Turkey

Cite this article: Öklü K, Çifci A, Güngüneş A, Durmaz Ceylan Ş, Erel Ö. Evaluation of the effect of obesity on oxidative stress with thiol/disulphide balance. *Intercont J Int Med* 2023; 1(1): 8-14.

Corresponding Author: Kübra Öklü, kubra.ozde@hotmail.com

Submit Date: 31/01/2023 **Accept Date:** 20/02/2023

ABSTRACT

Aims: As the body mass index (BMI) increases, the percentage of body fat increases and oxidative stress increases accordingly. This change can be determined by looking at the thiol/disulphide balance which is a biochemical test. We aim to emphasize the importance of oxidative stress due to obesity. Thus, by protecting obese individuals from oxidative stress (with antioxidant support treatments, etc.), studies to minimize the impact of obesity can be opened the front.

Methods: The study group of our study was selected from 18-55-year-old female obese or overweight female patients who applied to Kırıkkale University Medical Faculty Internal Medicine and Endocrinology (Obesity) Polyclinics for slimming. Acontrol group consisting of healthy and normal weighted women who were in the same age range and applied to our hospital for general control were included in the study. According to BMI; groups consisting of 45 women, normal weight (18,5-24,9 kg/m^2), overweight (25-29,9 kg/m^2), stage 1 (mild obese; 30-34,9 kg/m^2), stage 2 (moderately obese; 35-39,9 kg/m^2) and stage 3 (morbid obese; 40-49,9 kg/m^2), were formed. Participants were premenopausal women with no additional disease. Demographic data and routine investigations were obtained from the hospital system. Blood samples were studied with thiol/disulphide balance measurement tests.

Results: We analyzed the five groups that were graded as normal weight, overweight, obese (mild, moderate, morbid) and the positive correlation between BMI and waist circumference andbody fatpercentage was significant in all groups ($r = 0.936$, $p<0.001$; $r = 0.857$, $p<0.001$, respectively). In all groups, the levels of native thiol and total thiol were decreased as BMI increased. There is a relatively lower difference between the normal thiol and total thiol levels of the normal weight and overweight group; there was a significant decrease in these values when passing from overweight to any stage of obesity. Negative correlation between all groups with native thiol ($r = -0.473$, $p<0.001$), total thiol ($r = -0.472$, $p<0.001$) and SH/total SH values ($r = -0.296$, $p<0.001$) were significant. The positive correlation between SS/SH ($r = 0.296$, $p<0.001$) and SS/total SH ($r = 0.296$, $p<0.001$) was significant in all groups. The positive correlation between disulphide and all groups ($r = 0.103$, $p=0.25$) was not significant.

Conclusion: Increased fat tissue inflammation in obesity is associated with oxidative stress. Thiol-containing organic compounds are antioxidants for defense against oxidative stress. Measures should be taken in the early period to reduce oxidative stress in the management of obesity.

Keywords: Obesity, body mass index, oxidative stress, thiol/disulphide balance

INTRODUCTION

Obesity, one of the biggest health problems of today, is defined by the World Health Organization (WHO) as an abnormal or excessive amount of fat tissue accumulation that poses a health risk.[1] Obesity is associated with endocrinological diseases (type 2 diabetes mellitus and insulin resistance, metabolic syndrome, etc.) and cardiovascular diseases (hypertension, hyperlipidemia, etc.), cerebrovascular diseases, cholecystitis, sleep apnea, osteoarthritis, hyperuricemia and gout, endometrial, breast, gallbladder cancers in women, and colon, rectum, prostate cancer in men. Obesity was treated when it caused any disease in the past. Today, along with preventive health policies, it is aimed to reduce mortality by treating obesity without causing any chronic diseases.[2]

The mechanism of obesity associated with diet is one of the most common causes of obesity. Dietary intake of free fatty acids in excess of antioxidant capacity leads to lipid peroxidation and may cause oxidative stress.[3]

Free radicals or reactive oxygen species (ROS) are products of normal cellular metabolism and are essentials for low levels of biological functions and enzymatic reactions. Free radicals have been described as having one or more unconjugated electrons, small molecular mainly, and highly unstable in molecular structure, and tend to damage molecules such as nucleic acids, proteins, carbohydrates, and lipids.The formation of free oxygen radicals is an ongoing metabolic event. Organisms have developed antioxidant systems that

reduce the damage of free radicals or ROS.Disruption in molecular and cellular functions as a result of loss of balance between ROS production and the body's antioxidant system is defined as "oxidative stress".[4-6]

Oxidative stress causes necrosis and death of the cell as a result of the high amount of free radicals produced during metabolism and cannot be rendered harmless by antioxidants, thus causing tissue damage and chronic diseases.[5] Thiol is an organic compound containing sulfhydryl (-SH) group that has a role in preventing any oxidative stress from occurring in cells. The main intention of ROS is the thiol groups of sulfur-containing amino acids in proteins. Thiol groups turn into reversible disulfide bonds after oxidation with ROS. Thus, the dynamic thiol/disulfide balance moves towards the disulfide form.[6] This transformation is the earliest sign of radical-mediated protein oxidation. The resulting disulfide bond structures can be reduced to functional thiol groups again and thus the dynamic thiol/disulfide balance is maintained.[7]

Dynamic thiol/disulfide balance has important roles in many mechanisms such as antioxidant defense, apoptosis, enzyme regulation, and cellular signal transduction in the organism.[8] It is examined as an indicator of oxidative stress in some metabolic diseases such as obesity and cardiovascular diseases; thiol/disulfide balance cancers; neurological diseases such as Parkinson's and Alzheimer's.[9] Therefore, investigating the dynamic thiol/disulfide balance can provide valuable information about the diagnosis, treatment, and mortality/morbidity processes of some common diseases in the society.

In this study, our aim is evaluating the relationship between oxidative stressand biochemical parameters and thiol/disulfide levels in obese people. Thus, with the antioxidant support treatments etc. that can be applied, studies to minimize the effect of obesity by protecting people from oxidative stress can be paved.

As the body mass index increases, the percentage of body fat increases, and in parallel with this, oxidative stress increases. This change in oxidative stress can be determined by looking at the thiol/disulfide balance, which is a biochemical test.

METHODS

Selection of the Study Group

This speciality thesis study was approved by the Kırıkkale University Medical Faculty Clinical Researches Ethics Committee (Date: 31.10.2017, Decision No: 20/02). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. Written consent was obtained from the patient participating in this study.

The study group of our study was selected from obese or overweight female patients aged 18-55 years who applied to internal diseases and endocrinology (obesity) outpatient clinics for weight loss between 15.12.2017-15.03.2018. A control group consisting of healthy and normal weight women who were in the same age range as the study group and who applied to our hospital for general control was included in the study. Informed consent form was obtained from all of the 225 volunteers.

Inclusion Criteria for Research

- Being older than 18 years old
- Being younger than 55 years old
- The study group will be overweight or obese, and the control group will be those with a BMI of <25 kg/m²
- Persons who apply to the internal diseases and obesity outpatient clinic for slimming purposes and undergo the routine examinations
- Those without known systemic disease
- Those without malignancy
- Those who are unpregnant
- Those who do not currently have an acute pathology (such as upper respiratory tract infection)
- Agree to participate in the study

Exclusion Criteria for Research

- Being younger than 18 years old
- Being older than 55 years old
- Not agreeing to participate in the study
- Patients who take medication (oral contraceptives, vitamin supplements, antioxidant drugs, people taking medication that affects platelet function)
- Malignancy
- Patients with a history of other endocrinological diseases: Hypothyroidism, hypogonadism, Polycystic Ovary Syndrome, Cushing's syndrome
- People who are followed up with anemia, thrombocytopenia
- Being a member of another study
- People treated for obstructive sleep apnea
- Patients with previous myocardial infarction, cerebrovascular accident, peripheral artery disease
- People treated for DM
- People treated for hypertension
- People treated for dyslipidemia
- Pregnant women
- People with a history of liver and kidney failure
- History of arrhythmia
- Those who smoke and drink alcohol
- Patients treated for acute or chronic infection

Table 1. Rating of obesity in adults according to BMI (kg/m²)

Slim	<18.50
Normal-weight	18.5-24.99
Overweight	25.00–29.99
Obese	≥30.00
Slightly Obese	30.00–34.99
Moderately Obese	35.00–39.99
Morbid Obese	40.00-49.99
Super Obese	≥50.00

Population and Sample of the Research

Our study was grouped as normal weight, overweight, stage 1 (mild) obese, stage 2 (moderate) obese, stage 3 (morbid) obese, and a total of 225 female volunteers between the ages of 18-55, 45 from each group, were included in our study.

Data Collection Methods

Demographic data of the patients, results of laboratory analysis (biochemical and hormone tests) , bioelectrical impedance measurement results and native thiol (-SH), total thiol [(-SH+SS-)], disulfide (-SS-), disulfide/native thiol [-SS-/-SH)], disulfide/total thiol [-SS-/(-SH+SS-)], native thiol [-SH/(-SH+SS-)] results were used for our study. Demographic data and routine examinations of the participants were obtained from the hospital system. Demographic data and routine examinations of our study group consisting of premenopausal

women without known additional disease were taken from the hospital system. All volunteers participating in the study were physically examined in the outpatient clinic. Blood pressure was measured with a sphygmomanometer from the brachial artery for 5 minutes after resting, with the patients in the sitting position, at 2-minute intervals, and their averages were recorded. Participants with pathological ECG, blood pressure, pulse values, and smoke and/or alcohol users were not included in the study.

Bioelectrical Impedance Analysis (BIW) and Measurement of Waist Circumference

Bioelectrical impedance analysis was performed using the "Tanita-BC 420 MA® device. Impedance is the resistance of the tissue to electric current and is inversely proportional to conductivity. The device has a weak electrical and 1.5 Ampere current feature at a frequency of 50-60 KHz. It is a method based on the difference in permittivity of tissues. The difference in impedance between lean tissue and adipose tissue is measured.[10]

Thiol /Disulfide Balance Measurement Tests (Erel Panel)

The blood samples taken from the antecubital vein to the biochemistry tube between 08:00 and 09.00 in the morning, following 8-12 hours of night fasting, were centrifuged at 1500 rpm for 10 minutes. Separated serum samples were placed in ependorphs and stored for a maximum of 3 months at-80°C to study the serum thiol/disulfide balance until the target number was reached. Serums that separated from the blood were sent to Ankara Yıldırım Beyazıt University Atatürk Training and Research Hospital Biochemistry Laboratory, where thiol/disulfide balance measurement method developed byErelet al.[9] was used and thiol/disulfide KİTs were studied. Measurements were presented using an automated clinical chemistry analyzer (Cobas 501, Roche) and results were presented in µmol/L. In this method, dynamic disulfide bonds(-SS-) are reduced to functional thiol groups (-SH) with sodium borohydriate (NaBH4). Unused NaBH4 is completely removed with formaldehyde. The total amount of thiol is calculated by spectrophotometric measurement of the chromogen compound formed by the modified Ellman's reagent at a wavelength of 415 nm. Native Thiol is measured using a modified Ellman's reagent (The Classic Ellman's reagent was modified by adding a formaldehyde solution). The Native Thiol value is subtracted from the total thiol and the amount of disulfide bond is found by dividing the difference obtained by two.This newly developed method is an easy, inexpensive, practical, automatic and manual spectrophotometric test to determine the plasma dynamic thiol/disulfide balance. With this method, native thiol (-SH), total thiol [(-SH) + (-S-S-)], and dynamic disulfide (-SS-) values were determined by measuring. With the ratios of these values, disulfide/native thiol [(-SS-/-SH)], disulfide/total thiol [-SS-/(-SH+SS-)], native thiol/total thiol [-SH/(-SH+SS-)] parameters are obtained.

Statistical Analysis

Statistical analyses of the study were performed using the "Statistical Package for the Social Sciences" (SPSS) version 20.0 package software. Descriptive statistics are summarized as number, percentage, average and standard deviation. Variables that fit the normal distribution are given with mean ±standard deviation and those that do not fit with the median (minimum-maximum) values. The suitability of the variables for normal distribution was examined using analytical methods (Shapiro-Wilk Tests). Kruskal Wallis test was used for numerical variables that did not show normal distribution in comparisons between multiple groups, and One-way Analysis of Variance (ANOVA) test was used to compare more than two independent groups for numerical variables with normal distribution. Pothoc Tukey Test was used for normally distributed groups and Kruskal Wallis Posthoc Test was used fornot normally distributed groups. In the comparisons between the two groups, Mann Whitney-U Test was used for numerical variables that did not show normal distribution, and T Test was used for numerical variables that showed normal distribution in independent samples between the two groups. In the determination of the relationships between variables, when both variables were normally distributed, correlation coefficients and statistical significance were calculated with Pearson Test, and correlation coefficients and statistical significance were calculated with Spearman Test for the relationships between variables at least one of which was not normally distributed. Results with a p value of <0.05 were considered statistically significant.

RESULTS

The study was evaluated for all parameters for the general population (225 people) who applied to internal medicine and endocrinology (obesity) outpatient clinics at Kırıkkale University Faculty of Medicine Hospital and met the inclusion criteria. 90 non-obese women (group 1 and group 2) and 135 obese women (group 3, group 4, group 5) were compared among themselves. In addition, sub-analyses were performed to determine the relationships between all groups, including 45 normal weight (group 1) and 45 overweight (group 2), 45 mildly obese (group 3), 45 moderately obese (group 4), and 45 morbid obese (group 5).

The average values of the general age and anthropometric measurement parameters of the participants are given in Table 2.

Table 2. Average anthropometric measurement and age values of all participants, p values according to the differences between groups 1, 2, 3, 4, 5 (n =225)

Age (years)	33.0 (18.0-52.0)	p =0.051*
BMI (kg/m²)	31.80 (18.50 -49.80)	p =0.08*
Waist Circumference (cm)	96.0 (62.0-134.0)	p <0.001*
Body Fat Percentage (%)	38.70 (11.30-53.90)	p <0.001*
Height (cm)	162.0±6.88	p =0.015**
Weight (kg)	82.13±20.54	p <0.001**

p <0.05 was considered statistically significant
* The significance level between the groups was given according to the K.Wallis Test.
** Significance level was given according to ANOVA Test.

The average values of the thiol/disulfide balance parameters examined in the serum plasma samples of the participants are shown in Table 3.

Table 3. Comparison of average thiol/disulfide balance variables of the all participants, and p values according to the between differences of the groups (groups 1, 2, 3, 4, 5) (n=225)

Native thiol (SH) (µmol/l)	296.60 (65.0-480.50)	p <0.001*
Disulfide (SS) (µmol/l)	13.70 (2.0-55.30)	p =0.011*
SS/SH (%)	4.60 (0.61-62.62)	p <0.001*
SS/Total SH (%)	4.21 (0.60-27.80)	p <0.001*
SH/Total SH (%)	91.57 (44.0-98.79)	p <0.001*
Total thiol (Total SH) (µmol/l)	330.01 ±67.77	p <0.001**
Albumin (g/dl)	4.45 ±0.31	p <0.001**

p <0.05 was considered statistically significant
* The significance level between the groups was given according to the K.Wallis Test.
** Significance level was given according to ANOVA Test.

In the comparison of the significance of age, anthropometric measurements, and BFP values between the paired groups, sub-analyses were performed for anthropometric measurements among the five groups we classified as normal weight, overweight, obese (mild, moderate, morbid), and it was found that weight, BMI, WCM, and BFP values showed a linear increase in all groups. All paired group comparisons were statistically significant ($p < 0.001$).

It is seen that there is a serious increase in waist circumference, especially when passing from the overweight stage to any stage of obesity. This increase was found to be statistically significant ($p<0.001$).

There was no significant difference between all groups for age ($p = 0.78$).

The level of thiol/disulfide balance parameters by groups is given in Table 4.

In all groups (as the body mass index increased), native and total thiol levels decreased.

While there is a relatively small difference between the native thiol and total thiol levels of the normal weight and overweight groups; a significant decrease was detected in these values when passing from overweight to any stage of obesity (BMI >30 kg/m^2). This relationship was statistically significant. However, no linear relationship was found between disulfide level and groups (Table 4).

There was no significant difference between all parameters of thiol/disulfide balance and albumin levels when the normal weight (group 1) and overweight group (group 2) were compared. Similar results were also valid for the comparison of moderately obese (group 4) and morbid obese (group 5) (Table 5).

In the comparison of group 1 with group 4 and group 1 with group 5, the difference between oxidant (disulfide/native thiol, disulfide/total thiol) and antioxidant (total thiol, native thiol, native thiol/total thiol) parameters was significant. However, there was variability in the analysis for disulfide level and albumin. A significant difference was found for oxidant-antioxidant parameters in between group 2 and group 4,and in between group 2 and group 5 comparisons. There was no significant difference for all ratios of balance (disulfide/native thiol, disulfide/total thiol, native thiol/total thiol) when group 1 was compared with group 2, and group 1 with group 3, and group 2 with group 3, and group 4 with group 5. A statistically significant difference was found in the comparisons of all other groups (Table 5).

Pairwise comparisons were made between non-obese groups of 90 premenopausal women formed with normal weight and overweight and obese groups (mild, moderate, morbid) of 135 premenopausal women. Both groups were similar for age factors and there was no significant difference between them ($p=0.09$). In addition, there was an increase in all anthropometric measurements in obese patients compared to non-obese patients, and this was statistically significant ($p<0.001$). Antioxidant values (native thiol, total thiol, native thiol/total thiol) and albumin values of non-obese groups were significantly higher than those of obese groups ($p<0.001$). Oxidant values (disulfide, disulfide/native thiol, disulfide/total thiol) were lower in the non-obese group compared to the obese group. However, there was no statistically significant difference in these comparisons for disulfide ($p=0.35$) (Table 6).

Table 4. Average and median values of thiol/disulfide balance variables by groups

	Normal-weight (n:45)	Overweight (n:45)	Slightly Obese (n:45)	Moderately Obese (n =45)	Morbid Obese (n:45)
Total SD (µmol/l)	379.36±76.34	362.83±59.66	320.59±52.80	307.96±60.33	289.33±60.48
SH (µmol/l)	350.70 (236.50-483.10)	327.50 (227.30-467.10)	280.80 (191.0-194.0)	276.30 (97.20-399.70)	257.0 (65.0-370.9)
SS (µmol/l)	16.50 (3.50-32.30)	13.20 (2.0-28.50)	12.60 (3.30-34.60)	15.0 (5.50-55.30)	17.45 (3.70-43.20)
SS/SH (%)	4.83 (0.73-11.21)	4.0 (0.61-10.07)	4.42 (1.02-12.89)	5.82 (1.71-56.89)	6.13 (1.63-62.62)
SH/Total SH (%)	4.40 (0.72-9.16)	3.70 (0.60-8.38)	4.06 (1.0-10.25)	5.21 (1.65-26.61)	5.46 (1.58-27.80)
SS/Total SH (%)	91.19 (81.68-98.55)	92.60 (83.24-98.79)	91.88 (79.50-98.0)	89.57 (46.78-96.69)	89.07 (44.40-96.83)
Albumin (g/dl)	4.51±0.4	4.49±0.26	4.34±0.27	4.40±0.23	4.25±0.26

Table 5. Significance comparison of thiol/disulfide balance parameters between paired groups

Case (n:225)	Significance Levels						
	Albumin (g/dl)	Total SH µmol/l)	SH (µmol/l)	SS (µmol/l)	SS/SH (%)	SH/Total SH (%)	SS/Total SH (%)
Group 1							
Group 2	p =0.99	p =0.71	p =0.97	p =0.05	p =0.09	p =0.083	p =0.091
Group 3	p =0.03	p <0.001	p =0.002	p =0.041	p =0.66	p =0.66	p =0.67
Group 4	p =0.56	p <0.001	p <0.001	p =0.76	p=0.034	p =0.04	p=0.034
Group 5	p <0.001	p <0.001	p <0.001	p =0.56	p =0.002	p =0.002	p =0.002
Group 2							
Group 3	p =0.10	p =0.01	p =0.002	p =0.94	p =0.21	p =0.199	p =0.209
Group 4	p =0.80	p <0.001	p <0.001	p =0.023	p <0.001	p <0.001	p <0.001
Group 5	p =0.002	p <0.001	p <0.001	p =0.011	p <0.001	p <0.001	p <0.001
Group 3							
Group 4	p =0.67	p =0.87	p =0.30	p =0.019	p =0.011	p =0.011	p =0.011
Group 5	p =0.65	p = 0.12	p =0.008	p =0.009	p <0.001	p <0.001	p <0.001
Group 4							
Group 5	p= 0.055	p =0.61	p =0.104	p =0.009	p =0.35	p =0.353	p =0.351

p <0.05 was considered statistically significant

Table 6. Results of thiol/disulfide parameters of obese and non-obese groups

	Non-obese (n = 90)	Obese (n=135)	p-value
Total SH µmol/l)	370.60±68.63	305.10±58.97	<0.001**
SH (µmol/l)	340.73 (227.3-483.1)	271.89 (65.0-399.7)	<0.001*
SS (µmol/l)	15.19 (2.0-32.30)	17.04 (3.30-55.30)	0.35*
SS/SH (%)	4.57 (1.0-11.0)	7.58 (1.0-63.0)	<0.001*
SH/Total SH (%)	91.76 (81.68-98.79)	88.16 (44.40-98.0)	<0.001*
SS/Total SH (%)	4.12 (0.60-9.16)	5.92 (1.0-27.80)	<0.001*
Albumin (g/dl)	4.51±0.34	4.30±0.26	<0.001**

Non-obese: It consists of normal weight and overweight (BMI = 18.5-29.99 kg/m²) groups.
Obese: It consists of mildly obese, moderately obese, and morbid obese groups (BMI ≥30 kg/m²).
*The significance level between the groups was given according to the Mann-Whitney U-Test.
* * Significance level was given according to T Test in independent groups.
p <0.05 was considered statistically significant

The correlation relationships between BMI, WCM, BFP parameters and each other, all groups, and thiol/disulfide balance were examined. The positive correlation between BMI and waist circumference (WCM) (r =0.936, p < 0.001) and between BMI and body fat percentage (BFP) (r =0.857, p < 0.001) was significant (**Figure 1**).

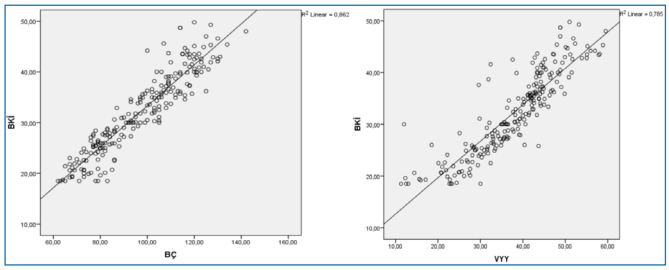

Figure 1. The relationship between BMI and WCM, BMI and BFP

The positive correlation relationship between all groups and BMI, WCM and BFP was significant (((r =0.980, p < 0.001), (r =0.932, p < 0.001), (r =0.887, p < 0.001), respectively) (**Figure 1**).

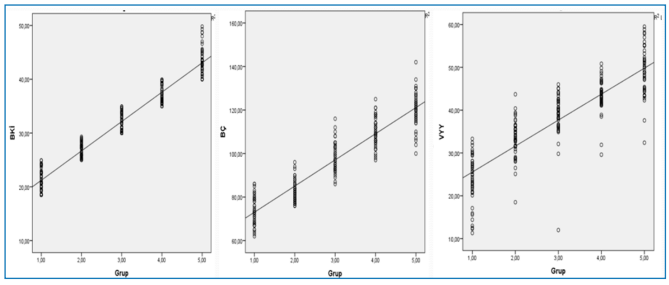

Figure 2. Relationship between all groups and BMI, WCM and BFP

A negative correlation was found between BMI and native thiol (r = -0.479, p<0.001), total thiol (r=-0.450, p<0.001), SH/Total SH values (r=-0.280, p<0.001), and the analysis was significant. A positive correlation was found between BMI and SS/SH (r =0.280, p< 0.001), SS/Total SH (r =0.280, p<0.001), and the analysis was significant. A positive correlation was also found between disulfide and BMI (r=0.08, p=0.25); however, no significant difference was found. A negative correlation was found between all groups and native thiol (r=-0.473, p<0.001), total thiol (r=-0.472, p<0.001), SH/Total SH values (r = -0.296, p<0.001), and this relationship was significant.

A positive correlation was found between all groups and SS/SH (r=0.296, p<0.001), SS/Total SH (r=0.296, p<0.001), and this relationship was significant. There was also a positive correlation between disulfide and all groups (r=0.103, p=0.25); but no significant difference was found. A negative correlation was found between WCM and native thiol (r = -0.456, p=0.002), total thiol (r = -0.429, p<0.001), SH/Total SH values (r=-0.240, p<0.001), and the analysis was significant. A positive correlation was found between WCM and SS/SH (r=0.280, p<0.001), SS/Total SH (r=0.280, p<0.001), and the analysis was significant.A positive correlation was also found between disulfide and WCM(r=0.08, p=0.25); however, no significant difference was found.

A negative correlation was found between BFP and native thiol (r=-0.479, p<0.001), total thiol (r= -0.450, p<0.001), SH/Total SH values (r=-0.280, p<0.001), and the analysis was significant. A positive correlation was found between BFP and SS/SH (r =0.280, p<0.001), SS/Total SH (r =0.280, p<0.001), and the analysis was significant.A positive correlation was also found between disulfide and BFP(r=0.08, p=0.25); however, no significant difference was found.

In the comparison with albumin level, the negative correlation relationship between BMI (r=-0.297, p<0.001), WCM (r=-0.208, p<0.001), BFP (r=-0.298, p<0.001), and all groups (r=-0.269, p<0.001) was significant, respectively. There was a negative correlation between non-obese and obese groups and albumin and it was statistically significant (r=-0.273, p<0.001). The negative correlation between all groups and albumin was also statistically significant (r=-0.264, p<0.001).

DISCUSSION

Elmas et al.[11] found that thiol/disulfide balance deteriorated as BMI increased in their study with 75 obese and 64 normal weight children who were similar in terms of age and gender and did not have any endocrinological risk factors.

In all of our sub-analyses, we found that thiol/disulfide balance deteriorated as BMI, WCM, and BFP increased. A negative correlation was found between BMI, WCM, BFP and antioxidant parameters (native thiol, total thiol, native thiol/total thiol) and the analysis was significant.

Waist circumference measurement is associated with the amount of intraabdominal (visceral) luposis.[11] In our study, it is seen that there is a significant and serious increase in waist circumference as BMI increases, especially when passing from overweight to any stage of obesity.

While there is a relatively small difference between the native thiol and total thiol levels of the normal weight and overweight groups; a significant decrease was detected in these values when passing from overweight to any stage of obesity (BMI >30 kg/m²). In our study, when BMI was >30 kg/m², we think that this significant change in WCM and the increase in visceral adipose tissue were strong factors in disrupting the thiol/disulfide balance.

We found that the disulfide level, another oxidant parameter, was also higher in obese patients. However, this correlation did not make sense. In the study of Elmaset al.[11] the disulfide level was higher in obese children and the relationship was not significant, which is similar to our study. Since plasma thiols are composed of many molecules such as albumin and glutathione, this can be explained by the presence of other unmeasured parameters and enzymatic/

nonenzymatic activities while looking at components such as native thiol, total thiol, and disulfide.

In the study of Du Nan et al.[12] showed that the risk of urinary albumin excretion increased in individuals with increased BMI and WCM, and stated that weight loss may be beneficial for reducing excretion.

According to our results, the albumin level decreased with the increase in BMI. Since plasma thiols are mostly composed of albumin, one of the reasons for the deterioration in the thiol/disulfide balance in obesity may be the decrease in albumin. We predict that the level of native thiol and total thiol may also have decreased due to this decrease.

Similar to our study, Jankovic et al.[13] conducted a study with premenopausal normal weight and obese women. It was found that glutathione (GSH) levels, an intracellular thiol, were significantly reduced in human visceral and subcutaneous adipose tissues in obese patients. They explained this with NADPH oxidase enzyme production and ROS variables in increased adipose tissue. They stated that antioxidant intracellular thiols are oxidized by activating to reduce reactive oxygen derivatives, thus measuring GSH level less in adipose tissue.

Brown et al.[14] similar toour study, investigated the relationship between BMI, WCM and BFP and some antioxidant parameters (lipid hydroperoxide, total antioxidant status, superoxide dismutase, and reduced glutathione) in groups classified as normal-weight, overweight and obese adults. In the sub-analyses, they showed differences between the groups in terms of obesity degrees and suggested that these differences in their study were related to the degree and duration of obesity development.

Wong et al.[15] in their study on obese and middle-aged mice, they found that β-mercaptoethanol (BME), which is a thiol antioxidant, had less increase in fat masses than those who did not. They suggested that the use of β-mercaptoethanol reduced inflammation through insulin resistance and lipid peroxidation.

Şimşek et al.[16] showed that thiol/disulfide balance was impaired regardless of age, gender and BMI in patients with familial hypercholesterolemia. They explained this situation with oxidized LDL, endothelial dysfunction and atherosclerosis.

Özler et al.[17] reported that thiol/disulfide balance was impaired in overweight adolescents with PCOS compared to normal weight patients with PCOS. Although this deterioration is associated with hyperandrogenism and insulin resistance, they stated that more weight and BMI increase are at the forefront.

In obesity, the release of free fatty acids from adipose tissue increases, causing insulin resistance by being stored in the liver and muscles.Metabolic dysfunction, which starts with insulin resistance, progresses to prediabetes and all these conditions are associated with oxidative stress. Obesity can be associated with many conditions related to increased oxidative stress such as insulin resistance, diabetes, dyslipidemia, atherosclerosis.[18,19]

In our study, we examined obese individuals without known comorbidities such as diabetes and dyslipidemia. Thus, we found that the balance was impaired in obese people regardless of other mechanisms. This only emphasizes the importance of increased BMI and the inflammatory effect of increased adipose tissue.

Limitations of the study: Thiol/disulfide balance is affected by many other factors such as dietary pattern and physical activity. Our study is a cross-sectional and descriptive study. Current thiol/disulfide values and anthropometric measurements of the individuals were used. Although many factors were tried to be excluded in our study, not all causes could be eliminated. Therefore, the cause-effect relationship has not been fully established. While the consumption of foods with antioxidant properties provides positive effects in antioxidant defense such as thiol/disulfide balance, high-calorie diets have the opposite effect. In our study, it is one of our important deficiencies not to take a story about the lifestyle of the participants such as nutritional characteristics and diet type.

One of our other limitations is that the participants' data on education, physical activity, income level, marriage, number of births, sleep patterns, and family history were not included in the study. In addition, the duration of the occurrence of obesity was not questioned by questioning the process of being obese or overweight throughout the life of the individuals (infancy, childhood, adolescence, etc.). Another limitation is that factors other than the parameters of native thiol, total thiol, disulfide, SH/total SH, SS/SH, SS/Total SH cannot be measured. In our study evaluating the thiol/disulfide balance; Obese and non-obese individuals according to BMI; multiple subgroups analyzes were performed for the first time, grading according to BMI as normal weight, overweight, mild, moderate, and morbid obese.

There are studies with fewer people investigating the thiol/disulfide balance in premenopausal adult women. In our study, it is our strength to create a large sample with more participants. Apart from obesity that may cause oxidative stress, factors such as additional diseases (CVD, DM, dyslipidemia, HT, OSAS, acute-chronic infections, etc.), menopause, drugs, smoking and alcohol use were excluded and only BMI and thiol/disulfide balance parameters were compared.

CONCLUSION

With weight gain, there is an increase in BMI, WCM, and BFP, and the formation of a subclinical inflammation with the effect of cytokines released from fat cells affects thiols. In order to prevent obesity-related morbidity and mortality, our main treatment goal should be to control weight and prevent abdominal obesity in people who are overweight. Therefore, we believe that the thiol/disulfide balance may be a guide for oxidant-antioxidant status in obese adults.

We think that thiol antioxidant treatments such as BME and NAC can increase adipose tissue and anti-inflammatory effect at the systemic level in obese and overweight patients.

We think that by looking at waist circumference, body mass index, body fat percentage measurements, and albumin levels, we can have an idea about the thiol/disulfide balance, which is related to oxidative stress. Thus, studies to minimize the effect of obesity by protecting people from oxidative stress with antioxidant support treatments, etc. that can be applied in addition to weight control can be paved. However, large-scale studies are needed to make more comments about these parameters.

ETHICAL DECLARATIONS

Ethics Committee Approval: This speciality thesis study was approved by the Kırıkkale University Medical Faculty Clinical Researches Ethics Committee (Date: 31.10.2017, Decision No: 20/02).

Informed Consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

REFERENCES

1. World Health Organization. Obesity: preventing and managing the global epidemic. 2000: World Health Organization.
2. Serter R. Obezite Atlası. Ankara, Karakter Color Basımevi, 2004.
3. Kılıç T. Obezite ile iliskili oksidatif stresin altında yatan mekanizmalar: leptin ve adiponektinin rolü/Mechanisms underlying obesity associated oxidative stress: the role of leptin and adiponectin. *Anadolu Kardiyoloji Dergisi: AKD*. 2010;10(5):397.
4. Mercan U. Toksikolojide serbest radikallerin önemi. *Yüzüncü Yıl Üniversitesi Veteriner Fakültesi Dergisi*. 2004;15(1):91-96.
5. Sezer K, Keskin M. Serbest oksijen radikallerinin hastalıkların patogenezisindeki rolü. *FÜ Sağ Bil Vet Derg*. 2014;28(1):49-56.
6. Cremers CM, Jakob U. Oxidant sensing by reversible disulfid bond formation. *J Biol Chem*. 2013:jbc. R113.462929.
7. Jones DP, Liang Y. Measuring the poise of thiol/disulfide couples in vivo. *Free Radical Biol Med*. 2009;47(10):1329-1338.
8. Biswas S, Chida AS, Rahman I. Redox modifications of protein–thiols: emerging roles in cell signaling. *Biochem Pharmacol*. 2006;71(5):551-564.
9. Erel O, Neselioglu S. A novel and automated assay for thiol/disulphide homeostasis. *Clin Biochem*. 2014;47(18):326-332.
10. Salmi JA. Body composition assessment with segmental multifrequency bioimpedance method. *J Sports Sci Med*. 2003;2(a00101s1):1-29.
11. Elmas B, Karacan M, Dervişoğlu P, Kösecik M, İşgüve ŞP, Bal C. Dynamic thiol/disulphide homeostasis as a novel indicator of oxidative stress in obese children and its relationship with inflammatory-cardiovascular markers. *Anatolian J Cardiol*. 2017;18(5):361.
12. Du N, Peng H, Chao X, Zhang Q, Tian H, Li H. Interaction of obesity and central obesity on elevated urinary albumin-to-creatinine ratio. *PloS One*. 2014;9(6):e98926.
13. Jankovic A, Korac A, Srdic-Galic B, et al. Differences in the redox status of human visceral and subcutaneous adipose tissues–relationships to obesity and metabolic risk. *Metabolism*. 2014; 63(5): 661-671.
14. Brown LA, Kerr CJ, Whiting P, et al. Oxidant stress in healthy normal-weight, overweight, and obese individuals. *Obesity*. 2009;17(3):460-466.
15. Wong S, Kirkland JL, Schwanz KA, et al. Effects of thiol antioxidant ß-mercaptoethanol on diet induced obese mice. *Life Sci*. 2014;107(1-2):32-41.
16. Şimşek Ö, Çarlıoğlu A, Alışık M, Edem E, Koca Biçer C. Thiol/disulfide balance in patients with familial hypercholesterolemia. *Cardiol Res Pract*. 2018. 2018.
17. Özler S, Oztas E, Erel O, et al. Impact of gestational diabetes mellitus and maternal obesity on cord blood dynamic thiol/disulfide homeostasis. *Fetal Pediatr Pathol*. 2017;36(1):8-15.
18. De Koning L, Merchant AT, Pogue J, Anand SS. Waist circumference and waist-to-hip ratio as predictors of cardiovascular events: meta-regression analysis of prospective studies. *Eur Heart J*. 2007;28(7):850-856.
19. Mozumdar A, Liguori G. Persistent increase of prevalence of metabolic syndrome among US adults: NHANES III to NHANES 1999–2006. *Diabetes Care*. 2011;34(1):216-219