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Original Article

Evaluation of the relationship between weight loss and oxidative stress in obese, type 2 diabetic patients by thiol/disulfide balance

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ABSTRACT

Aims: Diabetes mellitus (DM) and obesity continue to be an important problem in all world. It is known that there is a close relationship between DM and obesity and that both diseases have a course intertwined with inflammatory processes. In order to determine the clinical severity of these two diseases and to predict complications, clinical tools are needed to evaluate patients in terms of inflammation and oxidative stress. Anthropometric measurements and various known and novel inflammatory biomarkers are being tested for this purpose. Thiol/disulfide is a biomarker whose role in inflammatory processes and defense against oxidative stress is known and whose clinical value is still under investigation. In this study, we aimed to determine the relationship between body weight change and thiol/disulfide balance under clinical follow-up in obese patients with type 2 DM.

Methods: Our study was conducted in a single center with a prospective design between December 2023 and April 2024. The study was completed with a total of 46 patients. Age, comorbidities, height, body weight, waist and hip circumference measurements were performed; glycated hemoglobin, albumin, leukocyte, neutrophil and c-reactive protein values were recorded. After 3 months, the same data were obtained again. Blood samples obtained during these two examinations were analyzed for native thiol, total thiol, and disulfide.

Results: Of the 46 patients, 30 patients lost more than 5% weight during the study period (group 1) and the remaining 16 patients were defined as group 2. There was no difference between groups 1 and 2 in terms of age (p=0.211) and comorbidities (p=>0.005) at the beginning of the study. Inflammatory markers and thiol markers were similar between the groups at the beginning of the study (p=>0.05). Native thiol (263.20-316.51; p=0<0.001), total thiol (296.91-355.63; p=<0.001) and disulfide (9350.84-10845.39; p=0.024) were increased in group 1 cases during the study period. Disulfide/native thiol (6.41-6.19; p=0.199), disulfide/total thiol (5.67-5.50; p=0.207), native/total thiol (88.65-88.99; p=0.206) did not change. In group 2 cases, native thiol (272.81-289.59; p=0.135), total thiol (307.22-322.18; p=0.173), disulfide (3916.43-10609.44; p=0.059), disulfide/native thiol (6.51-6.23; p=0.178), disulfide/total thiol (5.75-5.54; p=0.2187), native/total thiol (88.49-89.92; p=0.188) did not change.

Conclusion: There were no differences between patients who lost and gained weight during the study period in terms of comorbidities, age and anthropometric measurements at the beginning of the study. There was a statistically significant change in thiol markers in patients who lost weight.

Keywords: Diabetes mellitus, obesity, inflammation, oxidative stress, thiol/disulfide balance

INTRODUCTION

The incidence of diabetes mellitus (DM) and obesity are increasing rapidly together, often leading to significant complications, morbidities and numerous deaths.[1] In obesity, the reasons for increased oxygen consumption are mechanical load and increased myocardial metabolism. Consequently, the formation of superoxide, hydroxyl radical and hydrogen peroxide resulting from mitochondrial respiration increases. The relationship between obesity and increased oxidative stress is known, although not fully elucidated.[2] As BMI increases, the percentage of adipose tissue increases and correlated with this, oxidative stress increases. It can be hypothesized that the prevalence of metabolic diseases increases due to this inflammatory dominant process. We hypothesize that oxidative stress decreases with sugar regulation and weight loss in diabetic patients. This change can be detected by looking at thiol/disulfide balance. Based on this situation, in this study, we aimed to show that oxidative stress decreases with weight loss in obese diabetic patients.

METHODS

The study, which had a single-arm design without a prospective control group, was conducted between December 15, 2023 and April 15, 2024 at Kırıkkale University Faculty of Medicine, Department of Internal Medicine. The study was approved by the Kırıkkale University Faculty of Medicine Non-interventional Clinical Researches Ethics Committee (Date: 14.12.2024, Decision No: 25/04). All patients signed and free and informed consent form. All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Patients between the ages of 18 and 65 with type 2 DM, obese and insulin users were included in the study. Inclusion criterias were being between the ages of 18-65, having a diagnosis of type 2 DM, being followed with any subcutaneous insulin regimen and body-mass index (BMI) of 40 kg/m^2 and above. Exclusion criterias were patients with DM other than type 2, patients not using subcutaneous insulin, patients with a body-mass index less than 40 kg/m^2, pregnancy, patients who participated in the study but did not attend the control examination, refusing to participate in the study, withdrawing from the study.

Patients' age, additional chronic diseases and routine laboratory values (WBC, neutrophils, albumin, HbA1c) were obtained from the hospital information system. Anthropometric measurements were obtained as described and recorded in the case report form. The venous blood sera obtained from the participants during the outpatient clinic examination were separated and stored in clean and dry eppendorf tubes at -80° in a deep freezer until the time of the study. Participants were given a written case number on leaving the hospital and were asked to present this number at the 3rd month examination if they wished to continue working. The same process was repeated for the patients who applied to the 3rd month follow-up examination and volunteered to continue the study, and data and blood were collected. The data obtained were recorded on case report forms. The admission and control data of the patient group were tabulated by paying attention to patient anonymity and confidentiality.

Blood samples taken from the participants in a biochemistry tube with an 8-hour fasting condition were centrifuged at 1500 rpm for 10 minutes. The samples obtained were divided into ependorf tubes. These samples were stored at -80°C. All samples were run simultaneously once the target number of cases was reached. From these samples, native thiol, total thiol and disulfide measurements were performed by Erel and Neşelioğlu[3] using the methodology described by them.

By examining the weights of the patients at the time of initial admission and control examination, patients who lost more than 5% weight during the study period were classified as "group 1" and the other patients as "group 2". The categorical variables in the study were presented as frequency (n) and percentage (%) and analyzed with Pearson chi-square and Fisher's Exact test). In independent groups, independent sample t analysis was applied when both groups to be compared met the normality assumption. Mann-Whitney U was applied in cases where both groups did not meet the normality assumption. In the data that met the assumption of normal distribution, dependent groups t test was applied, otherwise Wilcoxon Signed Ranks test was applied. Data analysis was performed with IBM SPSS 27.0 package program (IBM Corp., Armonk, NY). p values less than 0.05 were considered statistically significant.

RESULTS

A total of 46 patients were analyzed in the study; all of the participants were obese, female patients with type 2 DM who were being followed with any subcutaneous insulin regimen. The age of the patients ranged from 34 to 66 years with a median value of 58.5 (SD: 8.65) (Table 1).

Table 1. Parameters of insulin-using diabetic morbidly obese patients at admission

	Mean	Min-Max	SD
Height (cm)	154.37	140.00-166.00	4.95
Weight (kg)	103.87	80.00-146.00	13.67
BMI (kg/m^2)	43.55	39.95-64.03	5.11
Waist circumference (cm)	124.54	105.00-152.00	11.42
Hip circumference (cm)	133.76	115.00-189.00	13.96
Age (years)	56.15	34.00-66.00	8.65

Min: Minimum, Max: Maximum, BMI: Body-mass index, SD: Standard deviation

Anthropometric measurements of the patients were analyzed at the time of initial presentation and during the follow-up examination. The 30 patients who were found to have lost more than 5% weight at the control examination were categorized as group 1. The remaining 16 patients were categorized as group 2.

The mean native thiol values in group 1 subjects were 263.20 (11.50-352.90) at the beginning of the study and 316.51 (228.00-466.60) at the end of the study. The final native thiol values were statistically significantly higher than the initial values in group 1 patients (z=-4.288, p=<0.001; Figure 1).

The mean total thiol values in group 1 subjects were 296.91 (124.30-370.75) at the beginning of the study and 355.63 (253.10-517.75) at the end of the study. The final total thiol values were significantly higher than the initial values in group 1 patients (z=-4.330, p=<0.001; Figure 2).

The mean disulfide values in group 1 subjects were 29350.84 (6.40-23225.00) at the beginning of the study and 10845.39 (12.55-27425.00) at the end of the study. The final disulfide values were statistically significantly higher than the initial values in group 1 patients (z=-5.027, p=<0.001; Figure 3).

The mean disulfide/native thiol values in group 1 patients were 6.41 (4.89-7.96) at the beginning of the study and 6.19 (5.07-7.40) at the end of the study. These values were not statistically significant (t=1.315, p=0.199).

The mean disulfide/total thiol values in group 1 patients were 5.67 (4.46-6.86) at the beginning of the study and 5.50 (4.60-6.45) at the end of the study. These values were not statistically significant (t=1.290, p=0.207).

In group 1 patients, the mean native thiol/total thiol values were 88.65 (86.27-91.09) at the beginning of the study and 88.99 (87.10-90.80) at the end of the study. These values were not statistically significant (t=-1.294, p=0.206, Table 2).

The mean disulfide/native thiol values in group 2 patients were 6.51 (45.21-7.68) at the beginning of the study and 6.23 (5.13-7.01) at the end of the study. These values were not statistically significant (t=1.415, p=0.178; Figure 4.10).

In group 2 patients, the mean disulfide/total thiol values were 5.75 (4.72-6.66) at the beginning of the study and 5.54 (4.65-6.15) at the end of the study. These values were not statistically significant (t=1.384, p=0.187).

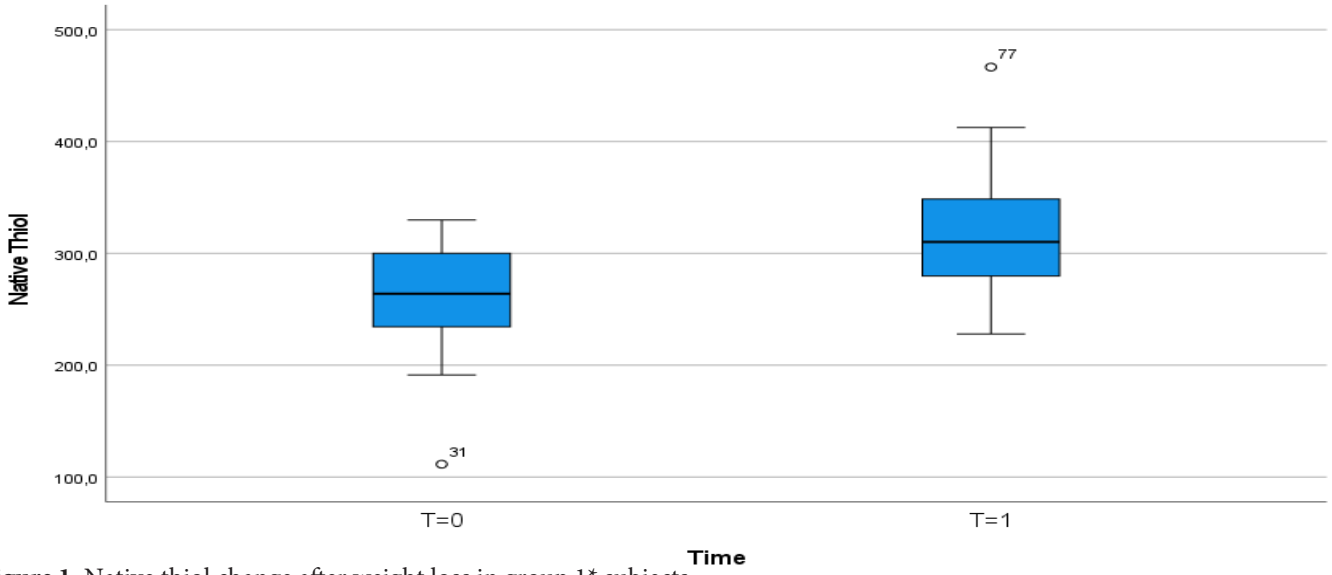

Figure 1. Native thiol change after weight loss in group 1* subjects

*Morbidly obese diabetic patients who lose more than 5% weight on follow-up

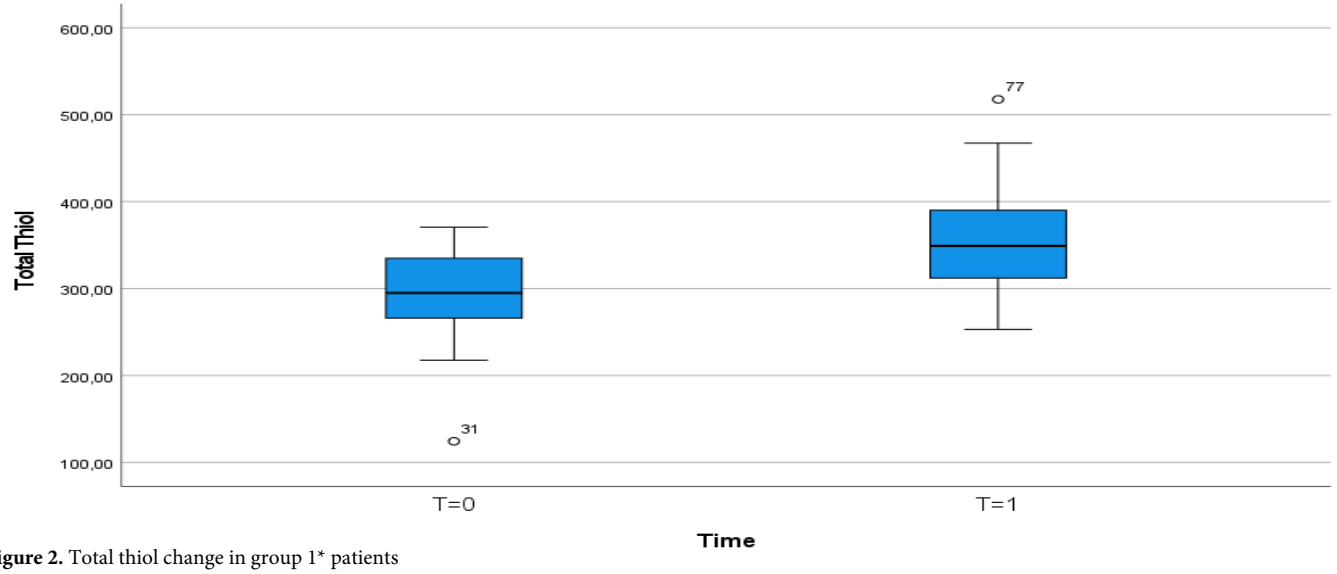

Figure 2. Total thiol change in group 1* patients

*Morbidly obese diabetic patients who lose more than 5% weight on follow-up

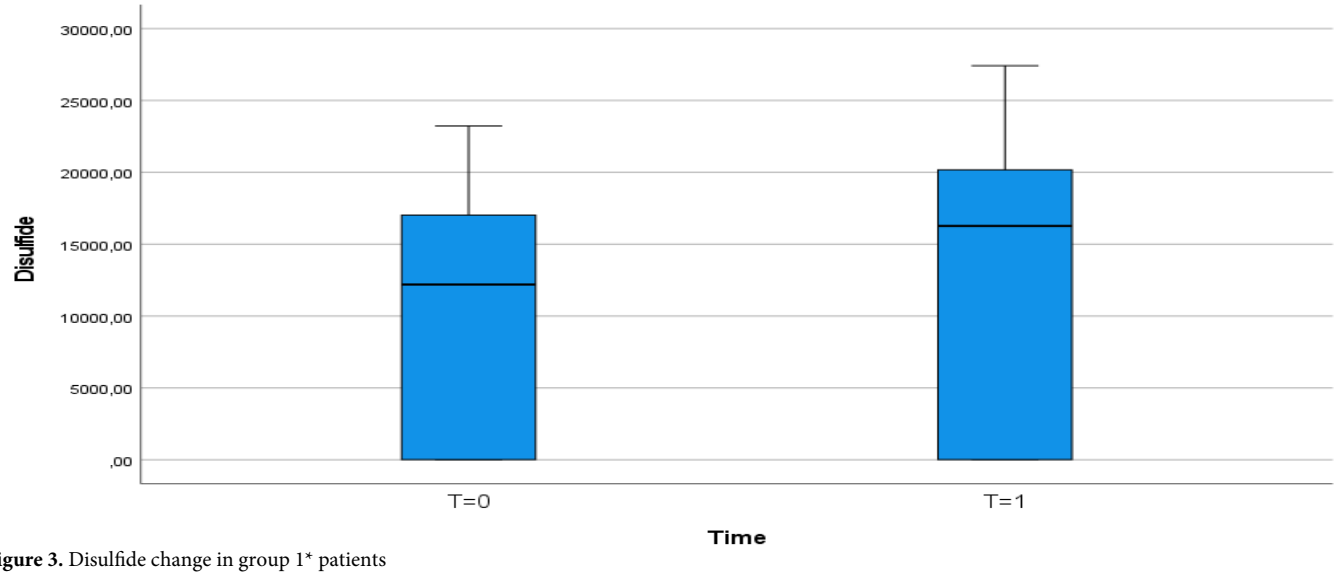

Figure 3. Disulfide change in group 1* patients

*Morbidly obese diabetic patients who lose more than 5% weight on follow-up

Table 2. Changes in thiol parameters in group 1 patients.

	T	Mean	min	max	SD	p (t/z)
Native thiol	**0**	263.20	111.50	329.90	47.80	<0.001
	1	316.51	228.00	466.60	50.70	(-4.288)
Total thiol	**0**	296.91	124.30	370.75	53.64	<0.001
	1	355.63	253.10	517.75	56.42	(-4.330)
Disülfide	**0**	9350.84	6.40	23225.0	9190.40	0.024
	1	10845.39	12.55	27425.0	10545.3	(-2.262)
Disülfide/ native thiol	**0**	6.41	4.89	7.96	0.79	0.199
ive thiol	**1**	6.19	5.07	7.40	0.52	(1.315)
Disülfide	**0**	5.67	4.46	6.86	0.62	0.207
Total thiol	**1**	5.50	4.60	6.45	0.41	(1.290)
Native thiol	**0**	88.65	86.27	91.09	1.24	0.206
Total thiol		88.99	87.10	90.80	0.82	(-1.294)

The mean disulfide values in group 2 patients were 3916.43 (12.40-16775.00) at the beginning of the study and 10609.44 (13.50-23275.00) at the end of the study. These values were not statistically significant (t=-2.039, p=0.059).

Table 3. Relationship between thiol parameters at the beginning of the study

	GR	Mean	min	max	SD	p (t/U)*
Native thiol	**1**	263.20	111.50	329.90	47.80	0.508
	2	272.81	200.80	352.90	43.82	(-0.668)
Total thiol	**1**	296.91	124.30	370.75	53.64	0.520
	2	307.22	225.60	391.60	46.68	(-0.648)
Disulfide	**1**	9350.84	6.40	23225.0	9190.40	0.189
	2	3916.43	12.40	16775.0	7002.61	(183.00)
Disülfide/ native thiol	**1**	6.41	4.89	7.96	0.79	0.682
ative thiol	**2**	6.51	5.21	7.68	0.71	(-0.413)
Disulfide/ total thiol	**1**	5.67	4.46	6.86	0.62	0.670
Tal thiol	**2**	5.75	4.72	6.66	0.56	(-0.429)
Native thiol/ total thio	**1**	88.65	86.27	91.09	1.24	0.676
Thiol/total thiol	**2**	88.49	86.68	90.57	1.11	(0.420)

*The test for disulfide measurements was performed with Mann-Whitney U and the U score is presented. For the remaining parameters, t scores are presented, min: Minimum, max: Maximum, SD: Standard deviation

In group 2 patients, the mean native thiol/total thiol values were 88.49 (86.68-90.57) at the beginning of the study and 88.92 (87.70-90.69) at the end of the study. These values were not statistically significant (t=-1.379, p=0.188).

Thiol/disulfide parameters at the beginning of the study and their differences between the groups were analyzed. The mean native thiol values at the beginning of the study were 263.20 (115.50-329.90) for group 1 and 272.81 (200.80-352.90) for group 2. There was no significant difference between the groups in native thiol values at the beginning of the study (p=0.508; t=-0.668).

The mean total thiol values at the beginning of the study were 296.91 (124.30-370.75) for group 1 and 307.22 (225.60-391.60) for group 2. There was no significant difference between the groups in total thiol values at the beginning of the study (p=0.520; t=-0.648).

The mean disulfide values at the beginning of the study were 9350.84 (6.40- 23225.00) for group 1 and 3916.43 (12.40-16775.00) for group 2. There was no significant difference between the groups in disulfide values at the beginning of the study (p=0.189; U=183.00).

The mean disulfide/native thiol values at the beginning of the study were 6.41 (4.89-7.96) for group 1 and 6.51 (5.21-7.68) for group 2. There was no significant difference between the groups in disulfide/native thiol values at the beginning of the study (p=0.682; t=-0.413).

Disulfide/total thiol values at the beginning of the study were 5.67 (4.46-6.86) for group 1 and 5.75 (4.72-6.66) for group 2. There was no significant difference between the groups in disulfide/total thiol values at the beginning of the study (p=0.670; t=-0.429).

The mean native thiol/total thiol values at the beginning of the study were 88.65 (86.27-91.09) for group 1 and 88.49 (86.68-90.57) for group 2. There was no significant difference between the groups in native thiol/total thiol values at the beginning of the study (p=0.676; t=0.420; Table 3).

Other Findings

The difference in the comorbidities of group 1 and group 2 patients was investigated. In group 1, 16 patients had hypertension, 3 had CAD, 4 had CKD and 9 had hyperlipidemia. In group 2 patients, 6 had hypertension, 3 had CAD, 3 had CKD and 2 had hyperlipidemia. Chi-square analysis revealed no significant difference between the groups in terms of having hypertension (p=0.475), CAD (p=0.342), CKD (p=0.681) and hyperlipidemia (p=0.282) and no significant difference in terms of age (p=0.211) at the beginning of the study.

We aimed to determine whether the patients were similar in terms of additional infectious and inflammatory pathologies at initial presentation. For this purpose, CRP, WBC, neutrophil and albumin levels were analyzed. No difference was found between the groups in terms of these four parameters (p=>0.05).

Anthropometric measurements taken at the beginning of the study and HbA1c values and their differences between the groups were analyzed. Body weight (p=0.212), BMI (p=0.062), waist circumference (p=0.404), hip circumference (p=0.229), HbA1c (p=0.525) values did not differ between the groups at the beginning of the study.

DISCUSSION

The most important result of our study is the correlation between weight loss and inflammatory markers with varying statistical power. These inflammatory markers are anthropometric measurements that have been well characterized by cohort studies and finally thiol mechanism related markers that have been recently studied in the literature. The parameters we defined in these two categories moved in the same direction and at different rates in obese diabetic patients who achieved weight loss.

Today, it is possible to talk about obesity as a chronic inflammatory disease and there is strong evidence of a relationship between pathologically increased adipose tissue and increased inflammation in obese patients.[4,5] This situation is similar in terms of diabetes. Studies indicate a higher

incidence of type 2 diabetes in healthy people with elevated inflammatory markers.[6] Aouacheri et al.[7] did not detect a correlation between the duration of diabetes and the severity of oxidative stress,but found a significant relationship between HbA1c and the severity of oxidative stress. It is also possible to mention a relationship between parameters associated with inflammation in diabetes and hyperglycemia. In a study of 70 people in Indonesia, a significant relationship was found between waist circumference and HbA1c in type 2 diabetes patients.[8] In our study, we found a significant difference in patients who achieved weight loss, similar to theother parameters we evaluated.

Clinical evidence suggests that the association of diabetes with central obesity is stronger than the association with total body adipose tissue.[9] Central obesity has been associated with decreased glucose tolerance, alterations in glucose-insulin homeostasis, reduced metabolic clearance of insulin and decreased insulin-stimulated glucose excretion.[10] In obese people, oxidative damage develops in the increased visceral adipose tissue itself before metabolicdisorders occur.[11]

Stevens et al.[12] found that waist circumference had better discriminatory power for diabetes than body mass index. Kulak et al.[13] reported that diabetes risk score increased significantly with increasing waist circumference. In a study conducted in Iran to determine the risk of developing diabetes, Fahrimeh et al.[14] observed that BMI and waist circumference were significant risk factors for diabetes in a 10-year follow-up.

We believe that our findings are similar to these outcomes. In our patient group in whom thiol/disulfide biomarkers showed significant changes, we found a decrease in BMI and waist circumference in the same way and in the same direction.

There are many studies on the role of thiol group-containing molecules in the defense against oxidative stress. Thiol/disulfide reduction buffering mechanism, reducing activity on radicals, chelator-like activity profile have been proposed as physiological mechanism of action.[15] The relationship between disruption of thiol/disulfide homeostasis and chronic inflammatory processes has been hypothesized in the literature based on this mechanism. Strong evidence has been obtained for the presence of impaired thiol/disulfide homeostasis in many diseases ranging from common chronic diseases to some malignancies.[16] In a study on thiol/disulfide in prediabetic patients, a significant positive correlation was found between disulfide and fasting blood glucose (p=0.017) and HbA1c (p=0.011), and a negative correlation between native thiol and fasting blood glucose (p=0.004).[17] In a study conducted in type 1 DM patients, a significant relationship was found between CRP, fasting blood glucose, HbA1c,disulfide/native thiol and disulfide/total thiol.[18] Eryılmaz et al.[19] examined breast cancer patients with thiol-disulfide inoutpatient clinic conditions and reported that disulfide values were significantly higher than the control group. Jankovic et al.[11] found that glutathione (GSH) levels, an intracellular thiol, were significantly decreased in visceral and subcutaneous adipose tissues in obese women. They explained this with the increase in NADPH oxidase enzyme production and ROS mechanism in adipose tissue.

The findings that thiol/disulfide homeostasis correlates with increased inflammatory process have been discussed above. Although there are many publications in the literature in terms of biomarkers, findings for clinical practice are relatively limited. In 2019, a prospective study conducted by Schillern et al.[20] managed to associate serum thiol levels with disease severity in type 2 DM patients, but found its ability to predict long-term complications insufficient.

In addition to the aforementioned findings, our study suggests that this correlation continues with the dampening of the inflammatory process. In their study with middle-aged and obese mice, Wong et al.[21] reported that mice treated with β-mercaptoethanol (BME), an antioxidant involved in thiol metabolism, gained less fat mass and more lean mass than those that were not. They found that BME users had reduced plasma lipid peroxidation, abdominal adipose tissue inflammation, muscle and liver fat infiltration, and liver and plasma CRP and insulin resistance. Similarly, in another study, Hildebrandt et al.[22] showed that the use of N-Acetyl Cysteine (NAC), a thiol metabolism-associated antioxidant effective on reactive oxygen species, decreased fat mass but increased insulin resistance in obese people without diabetes. In a double-blind placebo-controlled 20-week study of alpha lipoic acid, another thiol antioxidant, 360 obese individuals were randomized to oral alpha lipoic acid 1.200 or 1.800 mg/day or placebo. The treatment resulted in significantly greater weight loss compared to the placebo group.[23] In a randomized trial conducted in Iran, type 2 diabetics were given capsules containing 300 mg lipoic acid or placebo daily for 8 weeks. There was a significant reduction in postprandial glucose and insulin resistance with lipoic acid.[24]

Based on the existing correlations and the outcomes of thiol-related medical therapies, one could argue that there is hope for medical therapies targeting the thiol mechanism. However, it should be noted that weight loss may not affect all inflammatory cytokines together. A study published by Rosc et al.[25] confirmed that weight loss decreased CRP in morbidly obese patients, but failed to detect this decrease in terms of TNF-a and IL-6. Therefore, it would be erroneous to consider every weight loss or decrease in a marker associated with inflammation as an indication of widespread and absolute anti-inflammatory efficacy in the whole system.

Today, society as well as the medical world is aware of obesity and the dangers it poses to health. However, in our experience, people and even health professionals tend to view old age and co-morbidities as barriers to weight loss and lifestyle changes towards weight loss.[26,27]

During our study, we found that the age of the patients who lost weight and gained weight did not differ at the beginning of the study. Likewise, we found that the existing additional chronic diseases of the patients were similar in the weight loss and weight gain groups. The body weight and composition of the patients were similar at the beginning of the study. In a study conducted by Leyden et al.[28] found that in patients followed with an approach centered on lifestyle changes, patients over 65 years of age achieved 7.2% weight loss with this approach and patients under 65 years of age achieved 6.9% weight loss, and reported that age did not affect weight loss success. A prospective study by Finucane et al.[29] went beyond these findings and associated higher age with better weight loss success. According to our clinical experience, patients often complain of being too overweight to lose weight and ignore exercise recommendations. In our study, we found that the initial weight and BMI values of the patients were not significant. When the literature on this subject is examined, it

is seen that BMI is often not correlated with successful weight loss.[30] However, it is important to note that there are contrary findings that weight loss is more successful with high or low BMI.[31,32]

The most important strength of our study, in our opinion, is that it was blinded by design. It was unclear which patient would be in which group until the patients presented for a follow-up examination and their final weight was determined. In addition, because our design included anthropometric and routine laboratory measurements, we consider our study to be more than a laboratory experiment, but a research focused on daily clinical practice and concrete outcomes.

Limitations

The most important limitation of our study is the limited number of patients and the imbalance between the groups. Although we are pleased that our obese patients lost weight, we obviously did not anticipate that the number of patients would be so much in favor of those who lost weight. We interpreted this imbalance as patients who were under strict physician supervision may have paid more attention to life changes due to the feeling of being under observation and being re-evaluated. Obviously, this imbalance should be seen as an outcome of the study.

Another limitation that we inferred from the study results is the lack of time. The majority of the inflammatory markers we evaluated in our study were similar in both groups at the beginning of the study, a significant change was found in the weight loss group during the study period and no significant change was found in the weight gain group during the study period. Under these conditions, it was expected that there would be a difference in the outcome between the groups, but we did not reach this result. We attributed this to the insufficient duration of the study. In a design with an equivalent start and moving in different directions, a cross-sectional measurement made prematurely may not detect the divergence, while a second cross-sectional observation after a while may notice this divergence. Therefore, more consistent results can be obtained with longer-term measurements.

CONCLUSION

We think that thiol antioxidant therapies may increase the anti-inflammatory effect in tissues in obese and diabetic patients. Thus, in addition to encouraging weight loss in obese and diabetic patients, antioxidant therapies may pave the way for studies to protect against the negative effects of obesity and diabetes by reducing the damage of oxidative stress in the early period.

ETHICAL DECLARATIONS

Ethics Committee Approval

The study was approved by the Kırıkkale University Faculty of Medicine Non-interventional Clinical Researches Ethics Committee (Date: 14.12.2024, Decision No: 25/04).

Informed Consent

All patients signed and 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 the authors declare that they have all participated in the design, execution, and analysis of the study and that they have approved the final version

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