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Original Article

The Significance of Thiol/Disulfide Homeostasis and Ischemia-Modified Albumin Levels in Assessing the Oxidative Stress in Obese Children and Adolescents

Running Head: Oxidative Stress in Obese Children and Adolescents

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What is already known on this topic?
Although different mechanisms are proposed for the pathogenesis of complications associated with obesity, the most widely accepted hypothesis is that adipose tissue inflammation plays a critical role, and oxidant status appears in obese individuals.

What this study adds?
Chronic inflammation due to oxidative stress induced by impaired metabolic parameters in the metabolically unhealthy obese children causes impairment in thiol redox homeostasis. Our data suggest that the increased oxidant status in obese children increases with increasing degrees of obesity and metabolic abnormalities. We believe that thiol/disulfide homeostasis and high serum ischemia-modified albumin levels may be reliable indicators of oxidant-antioxidant status in the metabolically unhealthy obese children.

ABSTRACT
Objective: In this study, we analyzed thiol/ disulfide homeostasis and serum ischemia-modified albumin (IMA) levels for the first time in order to clarify and determine the oxidant/antioxidant balance in metabolically healthy and unhealthy children.
Subjects and methods: This study included 196 obese children and 105 healthy volunteers between 4-18 years of age. The obese patients were divided into 2 groups: metabolically healthy obese (MHO) (n=58) and metabolically unhealthy obese (MUO) (n=138). Blood samples were obtained to analyze biochemical parameters, thiol/disulfide homeostasis, and IMA level.
Results: This study included 301 cases, 168 (55.8%) were females and 133 (44.2%) were males. The mean age of the control group was 11.62±3.13 years, the mean age of the MUO's was 11.81±2.81years and the mean age of the MHO's was 11.99±3.30 years. Natural thiol, total thiol levels and natural thiol/total thiol ratio were significantly lower in the metabolically unhealthy obese group than those in the control group; disulfide, disulfide/natural thiol, disulfide/total thiol and IMA levels, were statistically significantly higher in the metabolically unhealthy obese group than those in the control group.
Conclusion: In conclusion, chronic inflammation due to oxidative stress induced by impaired metabolic parameters in the metabolically unhealthy obese children causes impairment in thiol redox homeostasis. Our data suggest that the increased oxidant status in obese children increases with increasing degrees of obesity and metabolic abnormalities. We believe that thiol/disulfide homeostasis and high serum ischemia-modified albumin levels may be reliable indicators of oxidant-antioxidant status in the metabolically unhealthy obese children.
Keywords: obese**,** children and adolescents, thiol/disulfide homeostasis, ischemia-modified albumin

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Introduction

Childhood obesity is one of the most important health problems of the 21st century. This is a global problem and continuously affects urban populations of many low and middle-income families. The prevalence is increasing at an alarming rate (1). A recent study proposed that 1.48 billion adults in the World are overweight, 502 million adults are obese, and 180 million children are overweight or obese (2). Prevalence of overweight and obesity has shown a dramatic increase in the adult Turkish population, reaching figures as high as 30-40% (3).
Although different mechanisms are proposed for the pathogenesis of complications associated with obesity, the most widely accepted hypothesis is that adipose tissue inflammation plays a critical role, and oxidant status appears in obese individuals (4). Oxidative stress (OS) is the loss of normal homeostatic balance between reactive oxygen species (ROS) and antioxidant substances; it is toxic to cells by causing membrane lipid peroxidation and membrane damage (5). Thiols are important antioxidants which play an important role in non-enzymatic elimination of reactive oxygen species. Thiol/disulfide homeostasis is necessary for detoxification. Parameters of this homeostasis are native thiol, total thiol, disulfide,

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disulfide/native thiol, native thiol/total thiol, and disulfide/total thiol ratios. Dynamic thiol/disulfide homeostasis has critical roles such as antioxidant protection, detoxification, signal transduction, apoptosis, regulation of enzymatic activity, transcription factors and cellular signal mechanisms (6, 7). Moreover, dynamic thiol/disulfide homeostasis plays roles in many disorders (8-17).

Ischemia-modified albumin (IMA) occurs due to albumin modification caused by reactive oxygen species which occur during ischemia. High IMA levels are used to predict the cardiovascular risk in obese children and to evaluate subclinical vascular disease in patients with diabetes mellitus (18, 19).

Based on similar studies in the literature, we aimed to evaluate the antioxidant status in obese children. Unlike similar studies, we measured thiol / disulfide homeostasis and serum IMA levels in metabolically healthy and unhealthy obese children for the first time in order to clarify and determine the oxidant / antioxidant balance. In addition, we evaluated the effect of obesity in metabolically unhealthy children on biomarkers of oxidative stress.

Materials and Methods

Study design and patient selection

This case-control study was conducted in Ankara Pediatric Hematology and Oncology Research and Education Hospital between May-2018 and July 2018 and included 196 obese children and 105 healthy controls whose ages were between 4-18 years. Exclusion criteria for obese patients were the presence of any hepatic, renal, cardiac, autoimmune, infectious, musculoskeletal, or malignant diseases, take any from of vitamin supplementation, or drug use that lead to obesity or the presence of chromosomal, endocrine and genetic syndromes. The control group included 105 healthy children without any known chronic or acute disease. The control group consisted of sex- and age-matched healthy subjects who were normal weight and whose did not have obesity and overweight. In addition, none of the control group had insulin resistance, impaired fasting glucose, dyslipidemia, hypertension or hepatosteatosis.

Obese patients were divided into two groups. The patients who didn't have dyslipidemia, impaired fasting glucose, insulin resistance, hepatosteatosis, or hypertension were accepted as metabolically healthy obese (MHO), and those who had at least one of these conditions were accepted as metabolically unhealthy obese (MUO) (20). Clinical and laboratory findings of the obesity and the control groups were compared.

The weight measurement was performed with thin clothes, without shoes, using an electronic weighing device (SECA digital weighing device; sensitive to 0.1kg difference). Height measurement was performed with a Harpenden stadiometer (sensitive to 0.1 cm difference) in the upright position with bare feet. Using the weight and the height measurements, body mass index (BMI) was calculated with this formula: [Weight (kg)/ Height^2 (m^2)]. For statistical evaluation, BMI standard deviation score (BMI SDS) was used. Patients whose BMI SDS were over 2 were accepted as obese (21). The BMI standard deviation score (SDS) values were calculated using the reference values developed by Neyzi et al (22).

Routine physical examinations of all cases were performed. Puberty gradings of all cases were performed by Tanner staging system. In girls, stage 2 breast development and in boys 4 ml testis volume were accepted as puberty beginning (23, 24).

Blood pressure measurement was performed from the right arm at sitting position after 15 minutes of resting, using a mercury sphygmomanometer (ERKA, Germany). If the blood pressure was above 95th percentile according to age, gender, and height, two more measurements were obtained. Hypertension was accepted if two of the three measurements were at or above the 95th percentile (25).

The study was performed in accordance with the ethical rules based on the principles of the Helsinki Declaration. Written informed consent forms were obtained (when appropriate) from the parents and the children.

Ethical approval: The study was approved by Ankara Children's Hematology Oncology Training and Research Hospital's Ethics Committee (Approval number: 2018-70).

Laboratory analysis

Blood samples were obtained by intravenous route after 8-10 hours of fasting. From these blood samples, fasting plasma glucose (FPG), fasting plasma insulin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), total cholesterol (TC), triglyceride (TG), and high-density lipoprotein (HDL-C) cholesterol levels were measured. Low-density lipoprotein cholesterol (LDL-C) levels were measured using Friedewald formula (26). Serum glucose and lipid profile measurements were performed using Roche modular system/integra 800 device and kit. Fasting plasma glucose between 100-125 mg/dL was accepted as "impaired fasting glucose" (27). If total cholesterol≥200 mg/dl, triglyceride≥150 mg/dl, HDL-C≤35 mg/dl, and LDL-C≥100 mg/dl the children were accepted as dyslipidemia (28).

Fasting insulin, thyroid-stimulating hormone (TSH) and free T4 (fT4) levels were measured using enzymatic immunoassay method with Beckman Coulter DXI 800 device. Reference values for Beckman Coulter TSH and fT4 kits used in our hospital were 0.7-5.69 µIU/mL for TSH and 0.65-1.06 ng/dL for fT4. Insulin resistance was calculated using the Homeostasis Model Assessment of fasting Insulin Resistance (HOMA-IR) method with the following formula: fasting plasma glucose (FPG) (mmol/L) X Fasting Insulin (mIU/mL) / 22.5 (29). HOMA-IR cut-off value for prepubertal patients was taken as 2.5 and for pubertal patients it was taken as 4 (30).

Hepatosteatosis was evaluated in our radiology clinic with upper abdominal ultrasonography using a Toshiba Xaria I Style Ultrasound device.

Measurement of serum ischemia-modified albumin level

Serum samples were obtained after 5 minutes centrifuge at 3500 rpm using anticoagulant-free tubes. Samples to measure IMA blood concentrations were pipetted into Eppendorf tubes and stored at -80 ° C. Serum IMA Levels were measured by the colorimetric method described by Bar-Or et al (31). Measurement results were reported as Absorbance Units (ABSU).

Measurement of thiol/disulfide homeostasis parameters

To detect thiol/disulfide homeostasis parameters, blood samples were obtained at 8:00-10:00 after 8-10 hours fasting. The samples were then centrifuged at 1500 rpm for 10 minutes to separate plasma and serum. Separated serum samples were immediately frozen and stored at -80 °C until the thiol/disulfide homeostasis was analyzed. All thiol/disulfide parameters were studied from the same samples. Serum levels of native and total thiol and ratios of disulfide, and native and total thiol

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were determined by a spectrophotometric method using automatic clinical chemical analyzer by Erel and Neselioglu (Roche, Cobas 501, Mannheim, Germany) (32).

Statistical Method

A sample size calculation was performed considering detection of 0.20 effect size, α=0.05 and a power of %88.0 using Variance Analysis (On-way ANOVA). The result of the power analysis the minimum number of patients was required to be 303. Data obtained from this study were analyzed using SPSS statistical software package program (Version 23.0 for Windows; Armonk, NY: IBM Corp.) (33). Descriptive statistics were presented as mean ± standard deviation, median and interquartile ranges for continuous variables. Frequency distributions and percentages were given for categorical variables.For continuous variables, assumption of normality was tested by visual (histogram and probability plots), and analytic methods (Kolmogorov-Smirnov / Shapiro – Wilk Test). Equality of variances was controlled with the Levene test. One-way ANOVA was used to measure the difference among three groups if parametric test conditions were met, and the Bonferroni test among post hoc tests was used to make binary comparisons. Kruskal-Wallis test was used when parametric test conditions were not met. Student's t-test was used to determine whether a difference existed between two groups when parametric test conditions were met, and Mann Whitney U tests was used when conditions were not met Chi-square ($\chi 2$) test was used for the analysis of categorical variables. For MUO group, cut-off values of native thiol, total thiol, disulfide, disulfide/native thiol, disulfide/total thiol, native thiol/total thiol and IMA were determined by using Receiver Operating Characteristic Curve (ROC) analysis. Significance level of the tests was accepted to be $p<0.05$.

Results

This study included 301 cases, 168 (55.8%) were females and 133 (44.2%) were males. Within the group, 138 (45.85) were MUO, 58 (19.3%) were MHO and there were 105 healthy volunteers. No statistically significant difference could be found in ages and genders of the patients among all groups ($p>0.05$, for all). BMI SDS, glucose, insulin, HOMA-IR, TC, LDL-C and TSH values of the obese individuals were higher than those of the controls. The Bonferroni test was performed to determine the group which caused the difference among the groups and p values reflecting the difference between the groups were given in the second lines. The demographics and clinical and laboratory characteristics of the participants are displayed in Table 1.

In our study, the obese patients had lower native thiol (SH), total thiol (SH+SS) level, and native thiol/total thiol (SH/SH+SS) rate than controls ($p<0.001$, $p=0.002$, and $p=0.013$) respectively. Also, the obese patients had higher disulfide level (SS), disulfide/native thiol (SS/SH) and disulfide/total thiol (SS/SH+SS) than controls ($p=0.005$, $p<0.001$, and $p<0.001$; respectively). In addition, serum IMA level was found to be higher in the obesity group ($p<0.001$). Thiol/disulfide homeostasis parameters and the comparison of IMA between the control and the obese groups are given in Table 2.

Native thiol (SH), total thiol (SH+SS), and native thiol/total thiol (SH/SH+SS) ratio were statistically significantly lower in the MUO group than the control group ($p<0.001$, $p=0.005$, and $p=0.005$, respectively). Disulfide (SS), disulfide/native thiol (SS/SH), disulfide/total thiol (SS/SH+SS) and IMA levels were statistically significantly higher in the MUO group than the control group ($p=0.002$, $p<0.001$, $p<0.001$, and $p=0.001$, respectively). Comparison of dynamic thiol/disulfide homeostasis and IMA among the groups are given in Table 3.

Metabolically healthy and unhealthy obese patients, BMISDS> 3 were classified as subgroups. All parameters were similar in both groups ($p>0.05$, for all). Comparison of dynamic thiol/disulfide homeostasis and IMA between the groups are given in Table 4.

The ROC curve was drawn for MUO group native thiol, total thiol, disulfide, disulfide/native thiol, disulfide/total thiol, native thiol/total thiol and IMA. The area under the curve (AUC) of these parameters was calculated to be 0.611%95 CI [0.54;0.68] ($p=0.003$), 0.590 %95 CI [0.52;0.66] ($p=0.037$), 0.561 %95 CI [0.49;0.64] ($p=0.103$), 0.582 %95 CI [0.51;0.66] ($p=0.029$), 0.582 %95 CI [0.51;0.66] ($p=0.029$), 0.582 %95 CI [0.51;0.66] ($p=0.029$) and 0.719 %95 CI [0.66;0.78] ($p<0.001$) respectively. Only disulfide/total thiol of cut-off value was not statistically significant ($p> 0.05$). The values for sensitivity, specificity values were shown in Table 5.

Discussion

Evidence of oxidative stress due to obesity in adults has appeared over the last few years, and more recently, evidence has been shown in children (34). Obesity creates oxidant conditions that promote the development of comorbid diseases. Energy imbalance leads to storage of excess energy in adipocytes, resulting in both hypertrophy and hyperplasia. These processes are related with abnormalities of adipocyte function, especially with mitochondrial stress and impaired endoplasmic reticulum function (35, 36). Oxidative stress can also be induced by adipocyte-associated inflammatory macrophages (37). There is a close link among obesity, chronic low-level inflammation, and oxidative stress. In addition, the dysfunction of adipocytokines secreted by adipose tissue and induced by oxidative stress acts synergistically in metabolic abnormalities associated with obesity. Evaluation of the oxidative status is believed to allow the identification of patients with high risk of complications (34).

Thiol-disulfide balance has vital importance. The new method developed by Erel and Neselioglu can measure both variables separately and in total and allows both individual and integral evaluations (32). Until now, many studies have evaluated the oxidant-antioxidant status and reported various results for obese children. However, to the best of our knowledge, no previous study has reported thiol/disulfide homeostasis in MUO and MHO children. Elmas et al first evaluated thiol / disulfide homeostasis in obese children. In this study, antioxidant parameter levels were low in obese patients, while oxidant parameters were found to be higher (38). Different this study, we measured thiol / disulfide homeostasis in metabolically healthy and unhealthy obese children for the first time in order to clarify and determine oxidant/antioxidant balance.

In our study, the levels of native thiol, total thiol and native thiol/total thiol ratio were lower, disulfide level, and disulfide/native thiol and disulfide/total thiol ratios were higher in obese children than the healthy control group. This suggests a shift in thiol/disulfide homeostasis towards disulfide production. Oxidant parameters were high and anti-oxidant parameters were low in obese children. When obese children were divided into MUO and MHO groups, the oxidative stress level in the MUO group was higher compared to the healthy control group. This increased oxidant status in obese children was due to an increase in the metabolically unhealthy group. Development of chronic inflammation due to oxidative stress

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induced by metabolically impaired parameters in obese children has been shown to lead to disruption of thiol redox homeostasis. Our data suggest that the increased oxidant status in obese children augments with increasing obesity and metabolic abnormalities.

Many previous studies have found increased oxidant status in obese individuals similar to our study. Vehapoğlu et al. found that antioxidant capacity was significantly lower in prepubertal obese children (39). Karamouzis et al. demonstrated that loss of normal homeostatic balance between oxidant-antioxidant status led to increased oxidative stress with decreased antioxidant capacity in obese prepubertal and adolescent girls (40). Patlaoğlu et al. found that childhood obesity was associated with aseptic inflammation and oxidative stress (41). In another study investigating the changes in the oxidant / antioxidant status in obese children with and without metabolic syndrome, a significant impairment in the oxidant / antioxidant status was documented in obese children with metabolic syndrome (42). Another study also showed that children were more susceptible to oxidative stress than adults and the authors suggested that this was probably due to the incomplete development of the antioxidant system (43). The results of our study were consistent with those reported in studies on obesity and excessive oxidative stress. We believe that the thiol/disulfide homeostasis in metabolically unhealthy obese children may be a reliable indicator of oxidant-antioxidant status.

Rising degree of obesity has been shown to predict increased metabolic risk in obese children and adolescents (44). When compared to their moderately obese peers, children with severe obesity are at greater risk for adult obesity, early atherosclerosis, hypertension, type 2 diabetes, metabolic syndrome, fatty liver disease and premature death (45). In our study, there was no differences of thiol/disulfide homeostasis parameters and IMA between the MUO and MHO between obes children with >3SD. This result may be significant for metabolic risk that may develop in metabolically healthy but seriously obese children and adolescents.

Many previous studies assessed serum IMA levels in adult obesity patients and found correlations between some anthropometric and laboratory measurements. Piva et al. and Kazanis et al. reported that serum IMA level was significantly high in obese adults and overweight/obese postmenopausal women and this was associated with oxidative stress. In addition, they found an association between serum IMA level and BMI (46, 47). Baysal et al. studied serum IMA levels for the first time in obese children and found higher levels in children with metabolic syndrome (18). Similarly, in our study, serum IMA level was higher in the obese group than the control group. But we couldn't detect a difference between MUO and MHO. We found positive correlations between serum IMA level and BMI, fasting blood glucose, insulin, and HOMA-IR levels.

When Table 5 is examined, the IMA variable has the highest AUC value. This parameter has the highest distinguishing value according to other parameters. According to the cut-off value (0.665), the sensitivity and specificity were 74% and 66%, respectively. The sensitivity and specificity of the native thiol parameter relative to the cut-off value (439.2) were 51% and 74%, respectively. The sensitivity and specificity of the total thiol parameter relative to the cut-off value (477.5) were 52% and 71%, respectively. The high specificity values in these two parameters indicate that they are likely to differentiate between healthy individuals. The sensitivity and specificity of the disulfide parameter relative to the cut-off value (23.18) were 79% and 43%, respectively. Here, the sensitivity values are high, which means that it is a parameter that can distinguish between high and possibly unhealthy.

Oxidative stress may be the unifying mechanism underlying the development of comorbidity in obesity. Evidence suggests that there is a set of oxidative stress sources in obesity. Hyperglycemia, insulin resistance, dyslipidemia, increased blood pressure, insufficient antioxidant defenses, increased free radical formation rates, enzymatic sources in endothelium and chronic inflammation mediate the development of comorbidity.

Study Limitations

Important limitation is that this study is cross-sectional. Furthermore, thiol/disulphide parameters were not compared with other enzymatic and nonenzymatic oxidative stress parameters.

Conclusion

In conclusion, free radicals in metabolically unhealthy obese children may produce disulfide bonds by causing oxidation of thiol groups of sulfur-containing amino acids in proteins. Again, the imbalance between free radicals and antioxidant defenses in obese people contribute to the structural and functional changes in some proteins, such as human serum albumin, which plays a vital role in the effective antioxidant defense of the organism. This study demonstrated the loss of normal homeostatic balance between oxidant and antioxidant status in MUO children. The main goal in obese children and adolescents is to reduce weight and increase antioxidant status in order to prevent future cardiovascular complications. Our study provides an idea about these issues; however, future in-depth studies are warranted.

Author contributions: All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

Ethics

Ethics Committee Approval: The study was approved by Ankara Children's Hematology Oncology Training and Research Hospital's Ethics Committee (Approval number: 2018-70).

Informed Consent: Written informed consent forms were obtained (when appropriate) from the parents and the children.

Authorship Contributions

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Conflict of Interest: No conflict of interest

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Table 1. Demographics and clinical and laboratory characteristics of participants.

	Control group (n=105)		**MUO group (n=138)**		**MHO group (n=58)**		**p-Value**
	Mean±SD	**Median(IQR)**	**Mean±SD**	**Median(IQR)**	**Mean±SD**	**Median(IQR)**	
Age (years)	11.62±3.13	11.60 (4.50)	12,80±2.81	13.11(3.89)	11.99±3.30	11.90 (5.33)	0.609
Sex (Female)	57 (%54.3)		77 (%55.8)		34 (%58.6)		0.867**
BMI, SDS	0.24±0.78	0.30 (1.54)	2.59±0.55	2.45 (0.77)	2.42±0.57	2.15 (0.57)	<0.001* <0.001[a],<0.001[b],0.284[c]
Fasting blood glucose, mg/dL	88.03±6.62	90 (10)	98.01±8.00	98 (11)	92.67±4.79	93 (7)	<0.001* 0.001[a],<0.001[b],<0.001[c]
Insulin, mcIU/mL	6.83±3.13	6.30 (5.01)	16.45±9.54	13.74 (10.04)	9.55±3.38	9.08 (5.13)	<0.001* <0.001[a],0.049[b],<0.001[c]
HOMA-IR	1.43±0.71	1.27 (1.08)	4.14±2.71	3.53 (2.47)	2.24±0.83	2.13 (1.22)	<0.001* <0.001[a],0.030[b],<0.001[c]
Total cholesterol, mg/dL	143.09±25.86	139 (43)	171.80±34.56	171 (48.5)	156.90±22.46	157 (35)	<0.001* <0.001[a],0.014[b],0.004[c]
Triglyceride, mg/dL	94.26±27.29	79 (49)	140.48±69.09	122 (87.75)	93.16±22.78	93.5 (29.25)	<0.001* <0.001[a],0.99[b],<0.001[c]
HDL-C, mg/dL	52.43±9,15	47 (7)	46.28±9.89	44.5 (12.25)	51.28±9.40	49 (12.25)	<0.001* <0.01[a],0.0,99[b],0.001[c]
LDL-C, mg/dL	72.81±17.35	74.20 (27.80)	98.76±26.62	95.90 (32.75)	83.90±14.76	87.70 (22.08)	<0.001* <0.001[a],0.006[b],<0.001[c]

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ALT, U/L	15.80±6.08	13 (7)	24.38±16.09	18.50 (15)	17.74±12.49	16 (7.25)	<0.001* <0.001[a],0.99[b],0.003[c]
TSH, µIU/mL	2.54±1.04	2.50 (1.40)	3.15±1.71	2.70 (1.77)	3.35±2.11	2.58 (2.22)	0.002 0.011[a],0.007[b],0.99[c]
fT4, ng/dL	0.89±0.12	0.89 (0.15)	1.52±7.59	0.87 (0.16)	0.88±0.12	0.88 (0.17)	0.564
SBP, mmHg	102.57±10.77	100 (20)	111.77±17.62	110 (20)	102.53±16.49	100 (20)	<0.001* <0.001[a],0.99[b],<0.001[c]
DBP, mmHg	63.86±10.61	60 (15)	73.22±14.16	70 (20)	65.26±9.62	65 (10)	<0.001* <0.001[a],0.99[b],<0.001[c]

*Significance in analysis of variance (comparison among three groups). IQR: Interquartile Range
**Chi-square test.
[a]Significance between control group and MUO group (pairwise comparison).
[b]Significance between control groupand MHO group (pairwise comparison).
[c]Significance between MHOgroup and MHO group (pairwise comparison).

Table 2. Thiol/disulfide homeostasis parameters and the comparison of IMA between the control and the obese groups

	Control group (n=105)		**Obese group (n=196)**		**p-Value**
	Mean±SD	**Median(IQR)**	**Mean±SD**	**Median(IQR)**	
Native thiol, µmol/L	455.43±34.60	450.40 (42.55)	437.98±41.97	442.65 (56.93)	<0.001
Total thiol, µmol/L	492.48±36.77	492.00 (48.80)	477.29±43.34	480.85 (62.95)	0.002
Disulphide, µmol/L	17.69±6.23	18.05 (11.63)	19.65±4.50	19.59 (6.05)	0.005
Disulphide/native thiol, %	3.92±1.44	3.83 (2.52)	4.52±1.11	4.51 (1.52)	<0.001
Disulphide/total thiol, %	3.61±1.28	3.71 (2.17)	4.13±0.93	4.14 (1.28)	<0.001
Native thiol/total thiol, %	92.50±2.77	92.35 (3.73)	91.74±1.86	91.73 (2.56)	0.013
IMA (ABSU)	0.57±0.06	0.56 (0.07)	0.61±0.11	0.62 (0.12)	<0.001

Significance between control and obese group (Student's T-Test).IQR: Interquartile Range

Table 3. Comparison of dynamic thiol/disulfide homeostasis and IMA among the groups

	Control group (n=105)		**MUO group (n=138)**		**MHO group (n=58)**		**p-Value**
	Mean±SD	**Median(IQR)**	**Mean±SD**	**Median(IQR)**	**Mean±SD**	**Median(IQR)**	
Native thiol, µmol/L	455.43±34.60	450.40 (42.55)	435.45±41.20	440.75 (56.15)	444.01±43.52	449.70 (58.68)	0.001* <0.001[a],0,235[b],0.500[c]
Total thiol, µmol/L	492.48±36.77	492.00 (48.80)	475.39±43.15	478.80 (59.78)	481.82±43.86	485.35 (55.75)	0.006* 0.005[a],0,344[b],0.959[c]
Disulphide, µmol/L	17.69±6.23	18.05 (11.63)	19.97±4.52	20.16 (5.87)	18.90±4.42	18.19 (5.54)	0.003* 0.002[a],0.460[b],0.560[c]
Disulphide/native thiol, %	3.92±1.44	3.83 (2.52)	4.62±1.10	4.55 (1.41)	4.30±1.11	4.29 (1.69)	<0.001* <0.001[a],0.165[b],0.317[c]
Disulphide/total thiol, %	3.61±1.28	3.71 (2.17)	4.21±0.92	4.17 (1.18)	3.95±0.93	3.95 (1.44)	<0.001* <0.001[a],0.162[b],0.340[c]
Native thiol/total thiol, %	92.50±2.77	92.35 (3.73)	91.58±1.84	91.66 (2.37)	92.11±1.87	92.10 (2.88)	0.006* 0.005[a],0.855[b],0.386[c]
IMA (ABSU)	0.57±0.06	0.56(0.07)	0.61±0.12	0.62 (0.14)	0.61±0.09	0.63 (0.11)	0.001* 0.001[a],0.021[b],0.99[c]

*Significance in analysis of variance (comparison among three groups).IQR: Interquartile Range
[a]Significance between control groupand MUO group(pairwise comparison).
[b]Significance between control groupand MHOgroup (pairwise comparison).
[c]Significance between MUO groupand MHO group(pairwise comparison).

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Table 4. Comparison of dynamic thiol/disulfide homeostasis and IMA between the MHO and the MUO subgroups (BMI SDS>3)

	MHO group (BMI SDS>3) (n=14)	**MUO group (BMI SDS>3) (n=24)**	**p-Value**
Native thiol, µmol/L	420.08±41.70	417.34±33.25	0.825
Total thiol, µmol/L	461.19±43.29	455.68±35.66	0.673
Disulphide, µmol/L	20.55±4.45	19.17±4.26	0.356
Disulphide/native thiol, %	4.93±1.12	4.60±1.03	0.371
Disulphide/total thiol, %	4.47±0.93	4.20±0.86	0.376
Native thiol/total thiol, %	91.06±1.86	91.60±1.72	0.376
IMA (ABSU)	0,61±0,85	0.64±0.10	0.299

Significance between control and obese group (Student's T-Test).

Table 5. The cut-off values of parameters in predicting MUO group.

	Native Thiol	**Total Thiol**	**Disulfide**	**Disulfide/Native thiol (%)**	**Disulfide/Total thiol (%)**	**Native thiol/Total thiol (%)**	**IMA**
Cut-off value	439.2[L]	477.5[L]	23.185[s]	5.1025[s]	4.63[s]	90.7395[L]	0.665[s]
Sensitivity (%)	51	52	79	71	71	71	74
Specificity (%)	74	71	43	46	46	46	66
AUC (%95CI)	0.611 [0.54;0.68]	0.590 [0.52;0.66]	0.561 [0.49;0.64]	0.582 [0.51;0.66]	0.582 [0.51;0.66]	0.582 [0.51;0.66]	0.719 [0.66;0.78]
p	0.003	0.016	0.103	0.029	0.029	0.029	<0.001

[s]Smaller test result indicates more positive test, [L]Larger test result indicates more positive test, AUC: Area under curve. CI: Confidence Interval.

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