Dynamic thiol/disulphide homeostasis as a novel indicator of oxidative stress in obese children and its relationship with inflammatory-cardiovascular markers

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Abstract

Objective: Childhood obesity is an important cause of cardiovascular risk with chronic inflammation. Oxidative stress may contribute to the pathogenesis of obesity-related cardiovascular pathologies. We aimed to evaluate thiol/disulphide homeostasis as a novel and sensitive marker of oxidative stress and to evaluate its relationship with some inflammatory and cardiovascular markers in obese children.

Methods: In this case-controlled study, 65 children with exogenous obesity and 64 healthy children, as a control group, were included. In both groups, thiol/disulphide homeostasis parameters and inflammatory (white blood cells, platelets, mean corpuscular volume, neutrophil/lymphocyte ratio, and high-sensitivity C-reactive protein) and cardiovascular (epicardial adipose tissue thickness and left ventricular mass index) markers were studied. Correlation analyses of thiol/disulphide homeostasis parameters with body mass index standard deviation scores (BMI SDS) and inflammatory and cardiovascular markers were performed. Receiver-operating characteristic analysis was performed to determine the sensitivity, specificity, and optimal cut-off values of thiol/disulphide homeostasis parameters.

Results: Native thiol, total thiol, and native thiol/total thiol ratios (antioxidant parameters) were lower (p<0.05) and disulphide/native thiol and disulphide/total thiol ratios (oxidant parameters) were higher in the obese group than in the control group (p<0.01). A positive correlation of oxidant parameters with BMI SDS and inflammatory markers was found. However, a negative correlation of antioxidant parameters with BMI SDS and inflammatory markers was found. The specificities of disulphide/native thiol and disulphide/total thiol ratios were higher in the obese group.

Conclusion: The impairment in thiol/disulphide homeostasis, which is indicative of oxidative stress, is associated with inflammation in obesity. In addition, cardiovascular involvement may also contribute to this impairment. *(Anatol J Cardiol 2017; 18: 361-9)*

Keywords: cardiovascular risk, children, inflammation, obesity, oxidative stress, thiol/disulphide homeostasis

Introduction

Obesity is an increasing major health problem in both children and adults and is a multifactorial disorder (1). A recent study has suggested that an estimated 1.48 billion adults are overweight, 502 million adults are obese, and 180 million children are overweight or obese worldwide. Moreover, approximately 32% and 17% of children and adolescents in the United States are considered overweight and obese, respectively (2). Obese children and adolescents have a higher risk of being obese in adulthood, and adulthood obesity is associated with an increased risk of mortality and morbidity (3). Obesity is a low-grade (subclinical) systemic inflammatory disease. Overweight and obese children have elevated serum levels of white blood cells (WBCs), lymphocytes (L), high-sensitivity C-reactive protein (hs-CRP), interleukin 6, and tumor necrosis factor-alpha and increased neutrophil-lymphocyte ratio (NLR), which are known markers of inflammation and closely associated with cardiovascular risk factors (4, 5).

There is a significant direct relationship between the amount of epicardial adipose tissue thickness (EATT) and general body adiposity. There is substantial evidence that supports the role of EATT in the pathogenesis of coronary artery disease. There is a positive correlation of EATT with the presence of coronary pathology and the ability of adipose tissue to secrete hormones and cytokines that provoke coronary artery atherothrombosis. Thus, EATT may be an important risk factor for cardiovascular disease in obesity (6).

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Obese children exhibit changes in the left ventricular (LV) mass that are related to an increase in cardiac workload (7). Even with normal ventricular mass, overweight children exhibit subtle changes in LV systolic and diastolic functions that may have implications for their future cardiovascular health (8). LV mass has been established as an independent risk factor for cardiovascular morbidity and mortality (9, 10). The LV mass index (LVMI) has been proposed to be accountable for differences in body size (11).

Recent studies have highlighted the role of oxidative stress in the pathogenesis of obesity. It is emphasized that the coexistence of subclinical inflammation and oxidative stress plays a significant role as a pathophysiological mechanism in the development and progression of atherosclerotic process (12). Excess energy due to energy imbalance accumulates in adipocytes; this in turn leads to hypertrophy and hyperplasia. This situation causes hypoxia in adipose tissue and increases the secretion of inflammatory cytokines and chemokines (13). Adipocytokines are responsible for local and systemic inflammation associated with obesity. Inflammation causes mitochondrial oxidative stress and endoplasmic reticulum dysfunction in adipocytes. Additionally, adipocyte-related macrophages can trigger the oxidative stress (14, 15).

Thiols, as a major antioxidant, play an important role in the eradication of reactive oxygen molecules via nonenzymatic pathways. Thiols engage in oxidation reactions, forming disulphide bonds with oxidant molecules. Thiol/disulphide homeostasis is essential for detoxification. The parameters of this homeostasis include native and total thiol; disulphide; and disulphide/native thiol, native thiol/total thiol, and disulphide/total thiol ratios. These parameters have recently been studied as novel oxidative stress parameters in a wide range of diseases such as coronary heart disease (16, 17), isolated coronary artery ectasia (18), slow coronary flow (19), diabetes mellitus (20, 21), respiratory diseases (22), Alzheimer's disease (23), and pre-eclampsia (24). Nevertheless, to the best of our knowledge, no study has investigated thiol/disulphide homeostasis as a novel oxidative stress indicator in obese children and their association with inflammatory and cardiovascular markers until today. Therefore, we conducted this study to investigate thiol/disulphide homeostasis, a novel and sensitive oxidative stress marker, and its relationship with some inflammatory and cardiovascular markers in obese children.

Methods

Study design, participants, and blood samples

This case-control study included 139 children aged 5–17 years who were admitted to outpatient clinics of the Departments of Pediatric Endocrinology and Pediatric Cardiology of the Sakarya University, Research and Training Hospital between May 2015 and April 2016; the children were divided into obese and control groups. Obese group comprised 75 children with exogenous obe-

sity who were first referred to the Pediatric Endocrinology clinic for obesity. In all obese children, age- and gender-adjusted body mass indices were above the 95th percentile. They were asked to have obesity for at least a year. Body weight and height measurements were performed using a same tool. The body mass index (BMI) values were calculated as weight (kg) divided by height (m) squared. The BMI reference curves established by Bundak et al. (25) for Turkish children were used for determination of corpulence. As defined by the International Obesity Task Force, children with BMIs above 95th percentiles were accepted as obese, according to age and sex (26). BMI standard deviation score (BMI SDS) was used in statistical calculations because there was a wide age distribution in both obese and control groups. Patients with BMI SDS of 2 and above were accepted as obese. Children with obesity originating from secondary and genetic causes were excluded from the study after an evaluation by the pediatric endocrinologist. Children aged <4 years, children with suspected monogenic obesity and with syndromic obesity, and obese children with hormonal disorders were excluded from the study. Patients with impaired glucose tolerance, diabetes, dyslipidemia, and hypertension were also excluded. Furthermore, patients with early cardiovascular disease history, chronic illnesses, long-term medication for any reason, or infection with and without fever during the last 15 days and smokers were excluded. Age- and gender-matched 64 children who visited the outpatient clinic of the Department of Pediatric Cardiology with chest pain or murmur in whom pathology could not be identified by the pediatric cardiologist on physical examination, electrocardiography, and echocardiographic findings and in whom cardiac enzyme and hemoglobin levels and BMI were within the normal range (BMI values under 85th percentile; BMI SDS <2) were included in the control group. All control cases were healthy.

Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured twice from the right arm after a 10-min rest in the supine position using a same mark and model sphygmomanometer that was appropriate for ages of all participants in this study. All ultrasound studies were performed using a Philips iE33 ultrasound machine with 3 MHz phase transducer (Philips Ultrasound, Bothell, USA). Examinations were performed in the left lateral position with standard parasternal long-axis and apical four-chamber views. Two-dimensional targeted M-mode echocardiographic tracings were obtained in the parasternal long-axis view. LV mass was automatically calculated by the device using the current standardized formula, and height was used for indexing, and indexation of LV mass to height raised to an allometric exponent of 2.7 (LVMI=LV mass/height 2.7) (11, 27).

EATT was measured using two-dimensional echocardiography as an echo-free space over the pericardial layers, and its thickness was measured on the free wall of the right ventricle, perpendicular to the wall, using parasternal long-axis view at end-diastole (28). The measurements were performed by the same pediatric cardiologist.

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Table 1. Demographic data, clinical findings, inflammatory parameters, and echocardiographic data of study groups			
	Obese group (n=75)	**Control group (n=64)**	**P**
Demographic data and clinical findings			
Age, years	12.0 (5.0–17.0)	12.8 (5.0–17.0)	0.303
Gender, male/female	34/41	27/37	0.710
BMI SDS	2.63 (2.01–14.64)	-0.13 (-3.50–1.94)	<0.001
SBP, mm Hg	125 (100–139)	120 (71–137)	0.021
DBP, mm Hg	70 (50–95)	70 (58–95)	0.816
Inflammatory markers			
WBC, x10^3/mm^3	8.40 (4.88–15.00)	7.35 (2.50–16.10)	0.004
NLR	1.27 (0.63–14.06)	1.91 (0.78–47.67)	<0.001
N, x10^3/mm^3	3.92 (1.53–12.50)	3.93 (1.58–14)	0.884
L, x10^3/mm^3	3.18 (1.67–11.20)	2.27 (0.73–3.63)	<0.001
MCV, fL	81.2 (73.80–92.80)	85.4 (65.8–94.8)	<0.001
PLT, mm^3	354 (191–624)	268 (175–491)	<0.001
PLR	118.30 (28.84–230.15)	120.10 (63.35–294.52)	0.289
hs-CRP, mg/L	2.37 (0.10–15.50)	0.45 (0.16–10.10)	<0.001
Echocardiographic data			
EATT, mm	5.10 (3.90–5.70)	4.80 (4.20–5.60)	<0.001
LVMI, g/m$^{2.7}$	51.85 (26.86–80.76)	39.33 (18.20–94.87)	<0.001

Parameters were expressed as n and median (range). Mann–Whitney U test and χ^2 test were performed, and P value < 0.05 was considered significant. BMI SDS - body mass index standard deviation score; DBP - diastolic blood pressure; EATT - epicardial adipose tissue thickness; hs-CRP - high-sensitivity C-reactive protein; L - lymphocytes; LVMI - left ventricular mass index; MCV - mean corpuscular volume; N - neutrophils; NLR - neutrophil-to-lymphocyte ratio; PLR - platelet-to-lymphocyte ratio; PLT - platelets; SBP - systolic blood pressure; WBC - white blood cells

Peripheral venous blood samples were obtained using EDTA-containing blood collector tubes for determining complete blood count (CBC), including WBC, L, neutrophil (N), and platelet (PLT) counts as well as NLR and mean corpuscular volume (MCV). CBC was determined within 1 h. Blood samples for measuring serum thiol/disulphide homeostasis parameters and hs-CRP levels were collected in 5 mL to 16x100-mm tubes with red caps not containing gel (BD Vacutainer, New Jersey, USA). Serum samples were separated after centrifugation at 1500 rpm for 10 min and stored at –80°C until further analysis.

This study was conducted in accordance with the principles of the Declaration of Helsinki. The study protocol was approved by the Sakarya University Local Ethical Committee, and fully informed consents were obtained from the parents of all the children.

Measurement of thiol/disulphide homeostasis parameters and hs-CRP levels

Thiol disulphide parameters were determined using a novel spectrophotometric method previously described by Erel and Neşelioğlu (29). Briefly, reducible disulphide bonds were reduced to form free functional thiol groups. Formaldehyde was used to remove unused and consumed sodium borohydride. All thiol groups including native thiol groups and reduced thiol groups were measured after reaction with DTNB [5,5'-dithio-bis-(2-nitrobenzoic acid)]. The amount of dynamic disulphide was calculated using the half of the difference between the total thiol and native thiol. Disulphide/native thiol, disulphide/total thiol, and native thiol/total thiol ratios were calculated after determining dynamic disulphide, native thiol, and total thiol levels. An automated clinical chemistry analyzer (Cobas 501, Roche Diagnostics, Mannheim, Germany) was used, and the results were presented in μmol/L. Serum hs-CRP concentration was determined using the Siemens CardioPhase hs-CRP (Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany) particle-enhanced immune nephelometric assay on the BN II analyzer (Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany). Expected hs-CRP levels in healthy individuals were lower than 3.0 mg/L.

Statistical analysis

Descriptive statistics were conducted to inform the general features of participants. Kolmogorov–Smirnov test was used to determine the distribution of numerical variables. Numerical variables with normal distribution were calculated as mean±standard deviation, those with abnormal distribution median (range). Categorical variables were denoted as numbers (n) and percentages (%). Student's t-test was used to compare numerical variables with normal distribution. Mann–Whitney U test was used to compare numerical variables with abnormal

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distribution. Categorical variables were compared using χ^2 test. For establishing a relationship between numerical variables with normal distribution, Pearson correlation coefficient was calculated and for those with abnormal distribution, Spearman correlation coefficient was calculated. The cut-off value for thiol/disulphide homeostasis parameters was calculated using a receiver-operating characteristic (ROC) curve. A p value <0.05 was considered as statistically significant for all analyses.

Results

The median ages of 75 obese children and 64 healthy controls were 12.0 (5.0–17.0) years and 12.8 (5.0–17.0) years, respectively. No significant differences regarding age and gender were observed between the groups (p>0.05). There was no significant difference between two groups regarding DBP (p=0.816), but SBP values were significantly higher in obese group (p=0.021).

hs-CRP levels and L and PLT counts were significantly higher (p<0.001 and p<0.001, respectively) and NLR and MCV were lower (p<0.001 and p<0.001, respectively) in obese group. However, there were no significant differences regarding N count and platelet-to-lymphocyte ratio between the groups (p=0.884 and p=0.289, respectively). EATT and LVMI were significantly higher in obese group than in control group (p<0.001, both). Demographic data, clinical characteristics, inflammatory parameters, and echocardiographic data of subjects are summarized in Table 1.

Thiol/disulphide homeostasis parameters of the groups are summarized in Table 2. The native and total thiol levels and native thiol/total thiol ratios were lower (p=0.019, p=0.044, and p=0.001,

Table 2. Thiol/disulphide homeostasis parameters of study groups

	Obese group (n=75)	Control group (n=64)	P
Native Thiol, μmol/L	383.28 (52.07)	403.11 (45.68)	0.019
Total Thiol, μmol/L	419.82 (53.92)	437.53 (47.92)	0.044
Disulphide, μmol/L	18.27 (3.64)	17.21 (2.67)	0.051
Disulphide/Native thiol %	4.83 (1.09)	4.29 (0.67)	0.001
Disulphide/Total thiol %	4.38 (0.89)	3.94 (0.57)	0.001
Native Thiol/Total thiol %	91.22 (1.78)	92.10 (1.14)	0.001

Parameters are expressed as mean (SD). Student's t-test was performed, and *P* value <0.05 was considered significant

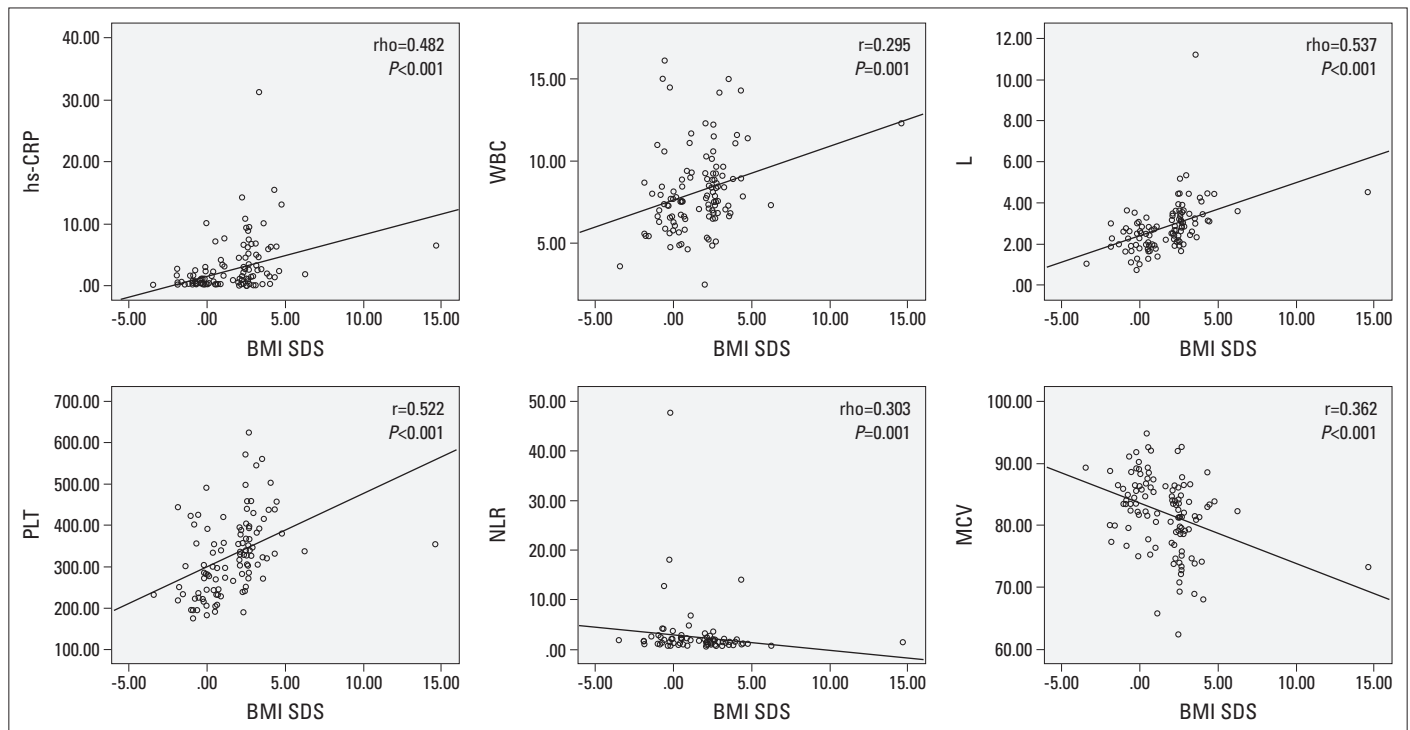respectively) and disulphide/native thiol and disulphide/total thiol ratios were higher (p=0.001 and p=0.001, respectively) in obese group than in control group. Although disulphide levels were higher in obese group, the difference was not significant (p=0.051).

The results of correlation analysis are summarized in Table 3. There was a positive correlation of BMI SDS with hs-CRP levels (r=0.482, p<0.001), WBC count (r=0.295, p=0.001), L count (r=0.537, p<0.001), and PLT count (r=0.406, p<0.001) and a negative correlation with NLR (r=–0.303, p=0.001) and MCV (r=–0.362, p<0.001) (Fig. 1). In addition, BMI SDS positively correlated with SBP (r=0.241, p=0.004), LVMI (r=0.428, p<0.001), and EATT (r=0.451, p<0.001) in obese group (Fig. 2). There was a negative correlation of BMI SDS with native thiol (r=–0.239, p=0.005), total thiol (r=–0.224, p=0.008), and native thiol/total thiol ratio (r=–0.205, p=0.015) and a positive correlation with disulphide/

Table 3. Results of the correlation analyses between thiol/disulphide homeostasis parameters and BMI SDS, inflammatory, and cardiovascular markers

	Native thiol		Total thiol		Disulphide		Disulphide/ Native thiol		Disulphide/ Total thiol		Native Thiol/ Total thiol		BMI SDS	
	R	P	R	P	R	P	R	P	R	P	R	P	R	P
BMI SDS[a]	-0.239	0.005	-0.224	0.008	0.053	0.533	0.206	0.015	0.205	0.015	-0.205	0.015		
SBP[b]	0.015	0.859	0.011	0.901	0.001	0.999	0.034	0.692	0.034	0.692	-0.034	0.692	0.241	0.004
DBP[a]	0.044	0.610	0.040	0.643	-0.020	0.814	-0.039	0.651	-0.038	0.655	0.038	0.655	-0.011	0.893
hs-CRP[b]	-0.134	0.115	-0.136	0.110	0.019	0.824	0.125	0.141	0.125	0.141	-0.125	0.141	0.482	<0.001
WBC[a]	-0.123	0.194	-0.137	0.148	-0.032	0.733	0.095	0.318	0.095	0.318	-0.095	0.318	0.295	0.001
N[b]	-0.008	0.931	-0.025	0.793	-0.100	0.296	-0.054	0.573	-0.054	0.573	0.054	0.573	0.086	0.369
L[b]	-0.311	0.001	-0.310	0.001	0.016	0.865	0.253	0.007	0.253	0.007	-0.253	0.007	0.537	<0.001
NLR[b]	0.213	0.024	0.197	0.037	-0.103	0.280	-0.231	0.014	-0.231	0.014	0.231	0.014	-0.303	0.001
PLT[a]	-0.102	0.285	-0.097	0.305	0.108	0.254	0.221	0.019	0.221	0.019	-0.221	0.019	0.522	<0.001
PLR[a]	0.277	0.003	0.262	0.005	-0.039	0.685	-0.216	0.023	-0.216	0.023	0.216	0.023	-0.102	0.286
MCV[a]	0.146	0.124	0.147	0.120	0.057	0.546	-0.055	0.565	-0.053	0.579	0.053	0.579	-0.362	<0.001
LVMI[b]	-0.174	0.041	-0.172	0.043	-0.021	0.805	0.096	0.260	0.096	0.260	-0.096	0.260	0.428	<0.001
EATT[b]	0.034	0.688	0.041	0.635	0.045	0.596	0.018	0.833	0.018	0.833	-0.018	0.833	0.451	<0.001

[a]Pearson correlation test and [b]Spearman correlation test were performed. BMI SDS - body mass index standard deviation score; DBP - diastolic blood pressure; EATT - epicardial adipose tissue thickness; hs-CRP - high-sensitivity C-reactive protein; L - lymphocytes; LVMI - left ventricular mass index; MCV - mean corpuscular volume; N - neutrophils; NLR - neutrophil-to-lymphocyte ratio; PLR - platelet-to-lymphocyte ratio; PLT - platelets; SBP - systolic blood pressure; WBC - white blood cells

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Figure 1. Scatter plot figures for correlations analyses of hs-CRP, WBC, L, NLR, PLT, and MCV with BMI SDS in obese group. Spearman correlation coefficient was calculated between BMI SDS and hs-CRP, L, and NLR, whereas Pearson correlation coefficient was calculated between BMI SDS and WBC, PLT, and MCV

BMI SDS - body mass index standard deviation score; hs-CRP - high-sensitivity C-reactive protein; L - lymphocytes; MCV - mean corpuscular volume; NLR - neutrophil-to-lymphocyte ratio; PLT - platelets; WBC - white blood cells
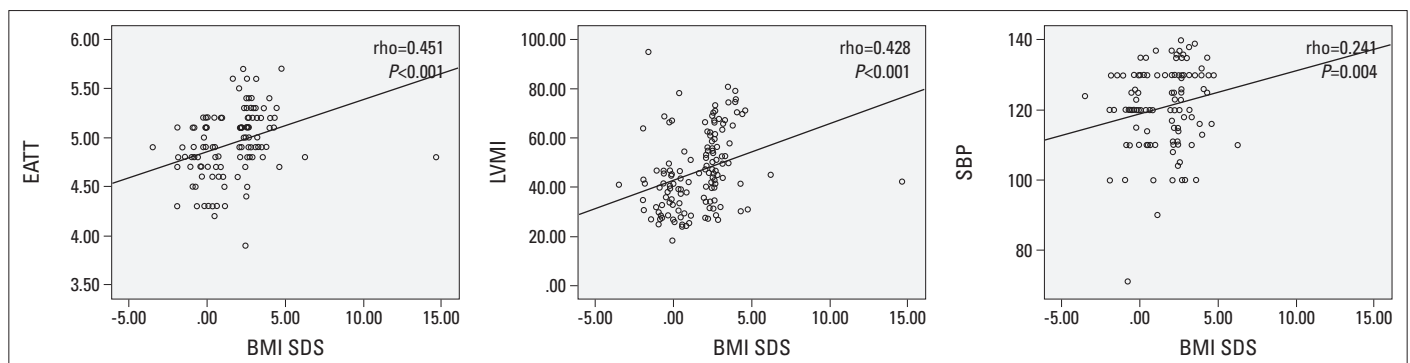
Figure 2. Scatter plot figures for correlations analyses of EATT, LVMI, and SBP with BMI SDS in obese group. Spearman correlation coefficient was calculated

BMI SDS - body mass index standard deviation score; EATT - epicardial adipose tissue thickness; LVMI - left ventricular mass index; SBP - systolic blood pressure
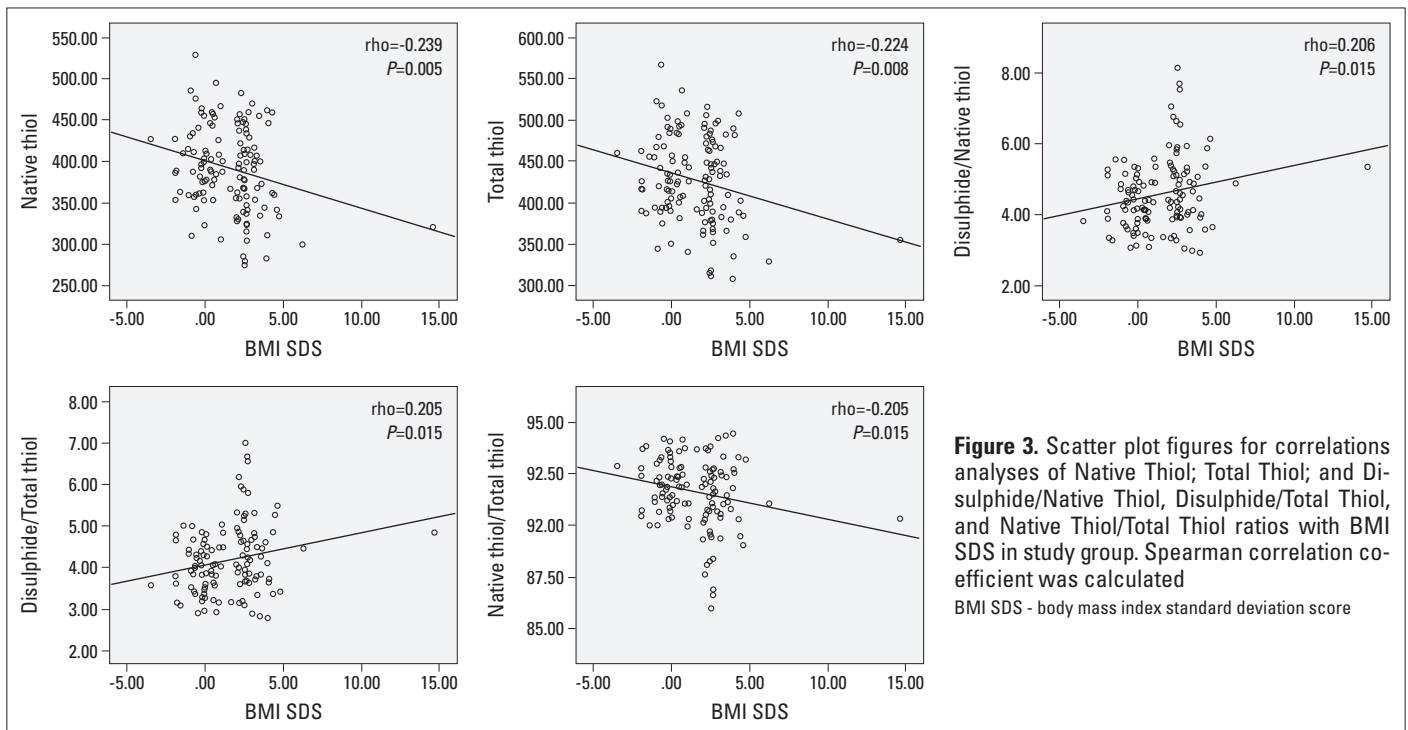
native thiol (r=0.206, p=0.015) and disulphide/total thiol (r=0.205, p=0.015) ratios in the obese group (Fig. 3). However, there was no significant correlation between BMI SDS and disulphide level (r=0.053, p=0.533). Furthermore, there was a negative correlation of LVMI with native thiol (r=−0.174, p=0.041) and total thiol (r=−0.172, p=0.043) in the obese group (Fig. 4).

ROC curve evaluating thiol/disulphide homeostasis parameter thresholds is summarized in Figure 5. Specificities of disulphide/native thiol and disulphide/total thiol ratios determined using the optimum cut-off values, 4.973 for the former and 4.582 for the latter, were higher in obesity group; sensitivities of both disulphide/native thiol and disulphide/total thiol ratios deter-

mined using the same optimum cut-off value were low. The sensitivity and specificity of native and total thiol, disulphide values, and native thiol/total thiol ratios were low.

Discussion

In our study, the thiol/disulphide homeostasis antioxidant parameters were lower in the obese children, whereas the oxidant parameters were higher in them. Thus, thiol/disulphide homeostasis was found to shift toward disulphide formation. In addition, there was an increase in the values of cardiovascular markers such as SBP, EATT, and LVMI with elevation in levels of inflam-

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Figure 3. Scatter plot figures for correlations analyses of Native Thiol; Total Thiol; and Disulphide/Native Thiol, Disulphide/Total Thiol, and Native Thiol/Total Thiol ratios with BMI SDS in study group. Spearman correlation coefficient was calculated

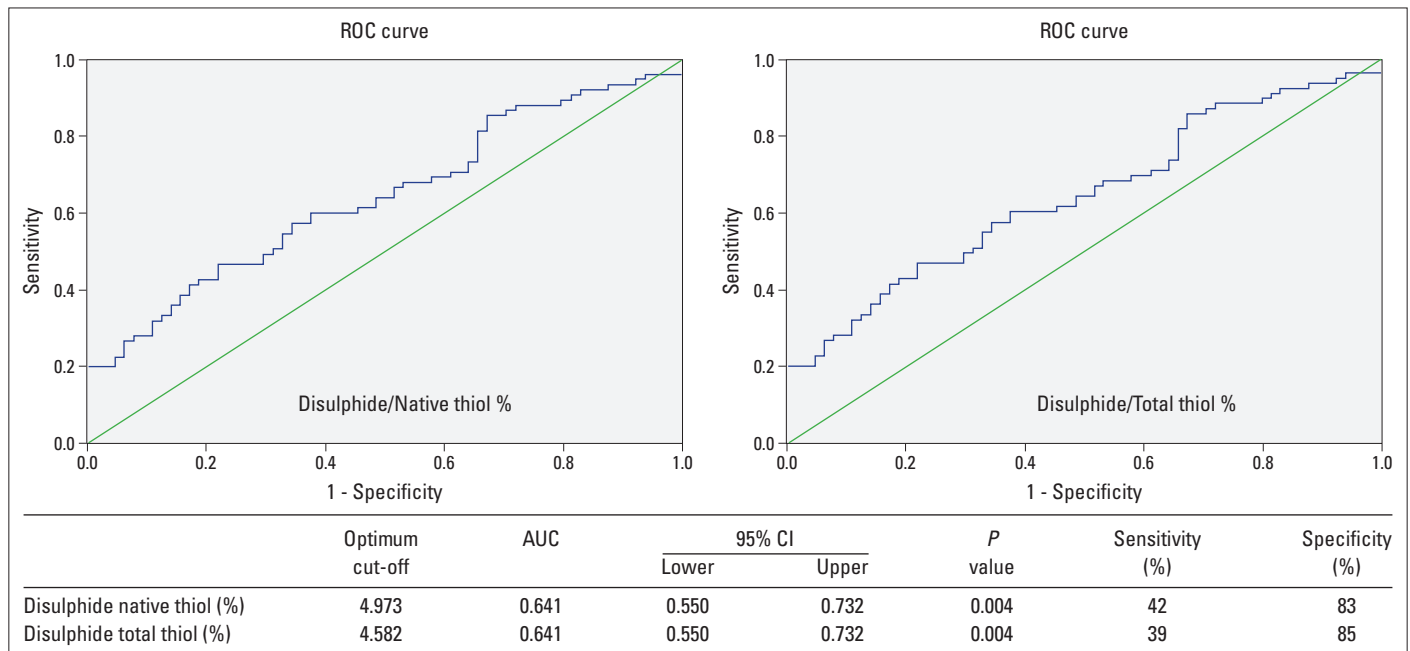BMI SDS - body mass index standard deviation score
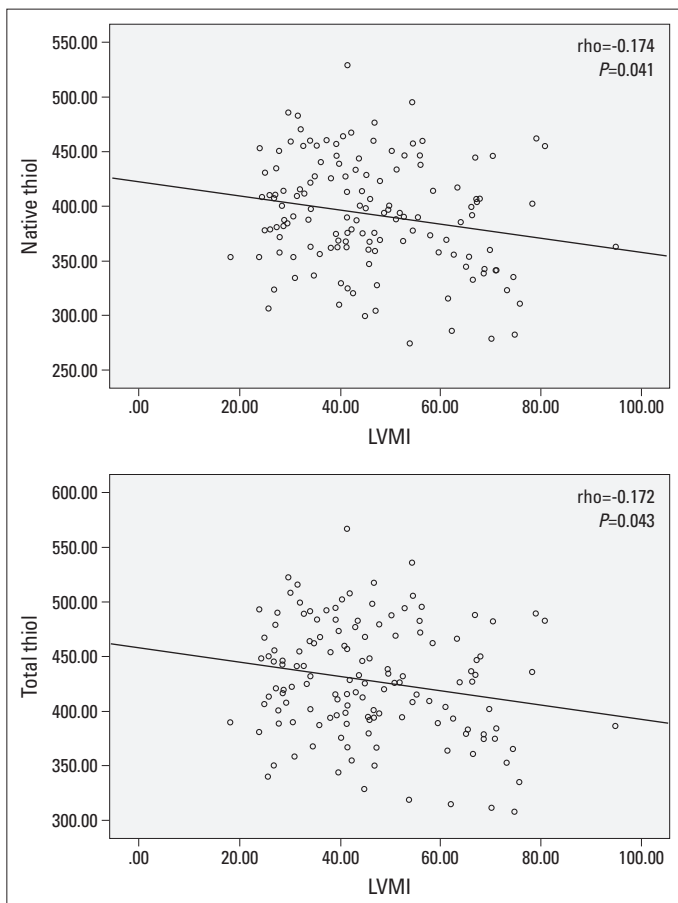
Figure 4. Scatter plot figures for correlations analyses of LVMI with native and total thiol in obese group. Spearman correlation coefficient was calculated

LVMI - left ventricular mass index

matory markers such as WBC, NLR, L, MCV, PLT, and hs-CRP in obese children. Furthermore, there was a negative correlation between thiol/disulphide homeostasis antioxidant parameters and BMI SDS, some inflammatory and cardiovascular markers.

Although different mechanisms have been postulated in the pathogenesis of obesity-related complications, the most widely accepted hypothesis is that adipose tissue inflammation plays a critical role and an oxidant status emerges in obese individuals (30). Oxidative stress is a product of imbalance between reactive oxygen species (ROS) and antioxidant molecules. ROS are involved in many aspects of atherogenesis, including oxidized LDL formation, endothelial activation, monocyte-derived macrophage recruitment, vascular smooth muscle cell proliferation, and matrix remodeling. Physiologically, ROS perform important signaling functions as intracellular messengers, and one of their key targets are protein thiols (31). Thiols are mercaptans that contain sulfhydryl residues. They are the main molecules coordinating antioxidant protective mechanisms (1). Thiols form disulphide bonds by engaging in oxidation reaction with ROS. Disulphide bonds are reversible bonds that are formed between protein thiol groups and low molecular weight thiols via oxidation of cystine residues due to oxidative stress. The disulphide bonds can be reduced to thiol groups; thus, dynamic thiol/disulphide homeostasis is sustained (12, 13). Total thiols comprise free forms, reduced to glutathione forms, and proteins-bonded forms in intracellular and extracellular spaces. Native thiol comprises only reduced thiols, whereas total thiol comprises both reduced and oxidized thiols (14).

Thiol/disulphide homeostasis was first described as a newly developed method by Erel et al. (29). They defined this method

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	Optimum cut-off	AUC	95% CI		P value	Sensitivity (%)	Specificity (%)
			Lower	Upper			
Disulphide native thiol (%)	4.973	0.641	0.550	0.732	0.004	42	83
Disulphide total thiol (%)	4.582	0.641	0.550	0.732	0.004	39	85

Figure 5. ROC curve for values of Disulphide/Native Thiol and Disulphide/Total Thiol ratios of study groups
AUC - area under curve; CI - confidence interval

as that an easy, inexpensive, practical, fully automated, and optionally manual spectrophotometric assay can be utilized to determine plasma dynamic thiol/disulphide homeostasis, which consists of thiol–disulphide exchanges. This method can be easily performed using lower quantities of serum samples, which is a substantial advantage for studies conducted in children (29). Furthermore, its usefulness as a novel and sensitive oxidative stress mediator has been investigated in various diseases (16, 17, 21–24). However, the relationship between thiol/disulphide homeostasis and inflammatory and cardiovascular markers in obese children has not been reported in the literature.

Until now, many studies have evaluated oxidant–antioxidant status, and diverse results have been reported in obese children. Vehapoğlu et al. (32) found significantly lower total antioxidant status and total thiol levels in obese children aged 2–11 years. Karamouzis et al. (33) demonstrated that the loss of the normal homeostatic balance between the oxidant–antioxidant state leads to enhanced oxidative stress combined with a reduced antioxidant capacity in obese prepubescent and adolescent girls. Molnár et al. (34) showed that TAS levels in plasma and α-tocopherol levels were reduced in obese children with metabolic syndrome. Özler et al. (35) reported an impaired thiol/disulphide homeostasis in obese adolescents with polycystic ovary syndrome. However, some studies have reported that high antioxidant status and increased antioxidant capacity are common in obese children compared with those in normal children, different from our study results. Sfar et al. (36) showed that the activity of antioxidant enzymes was markedly higher in obese children than in normal-weight ones. Different from these results, Brown et al. (37) found that there were no significant differences in TAS and glutathione levels between normal weight,

overweight, and obese adults. In our study, the thiol/disulphide homeostasis antioxidant parameter levels were low in the obese children, whereas the oxidant parameter levels were higher in these subjects. BMI SDS was positively correlated with thiol/disulphide oxidant parameters and negatively correlated with antioxidant parameters. This result showed that thiol/disulphide homeostasis was impaired in obese children, triggering oxidative stress. It also showed that impaired thiol/disulphide homeostasis in obese children was associated with BMI SDS. Specificities of disulphide/native thiol and disulphide/total thiol ratios were high, while sensitivities of these ratios were low in obese group. Sensitivities and specificities of native and total thiol, disulphide levels, and native thiol/total thiol ratio were low. The results of our study were consistent with those reported by studies on obesity and excessive oxidative stress. We believe that thiol/disulphide homeostasis may be a reliable indicator of oxidant–antioxidant status in obese children.

Many studies have reported that oxidative stress was triggered by an increase in ROS production due to an increase in the lymphocyte count (5, 38, 39). Positive correlations were observed between lymphocyte count and thiol/disulphide oxidant parameters in our study, whereas negative correlations were observed between lymphocyte count and antioxidant parameters. These results showed an increase in inflammatory markers associated with thiol/disulphide homeostasis in obese children. The presence of low MCV and high WBC, L, and PLT counts and high hs-CRP levels also support the presence of chronic inflammation in obese children.

In our study, values of cardiovascular markers such as SBP, EATT, and LVMI were higher in obese group than in control group. There was a positive correlation between cardiovascular mar-

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kers and BMI SDS. Furthermore, there was a negative correlation of thiol/disulphide homeostasis antioxidant parameters with BMI SDS and LVMI. Our results suggest that as BMI SDS values increase, the cardiovascular markers deteriorate, and this may be related to impairment of thiol/disulphide homeostasis. Eventually, the thiol/disulphide homeostasis may be a new biomarker that mediates the cardiovascular effects of chronic inflammation in obese children.

Study limitations

The major limitation of this case-control study was the relatively small number of participants. Another limitation of our study was the lack of evaluation of other inflammatory markers and cardiovascular risk factors. Furthermore, thiol/disulphide parameters were not compared with other enzymatic and non-enzymatic oxidative stress parameters.

Conclusion

In this study, we demonstrated that thiol/disulphide homeostasis, which is one of the important parameters of oxidative stress, was impaired in obese children. This impairment was strongly associated with increased inflammation in obesity. In addition, cardiovascular involvement may have contributed to the impairment of this homeostasis. Our study provided insight into these issues, but further in-depth studies are still required.

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