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Original Article

Dynamic thiol / disulfide homeostasis in metabolically healthy obese adolescents

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Abstract

Aim: This study aim to discuss the possible role of oxidant-antioxidant balance in providing metabolic health in obese adolescents by determining the oxidant-antioxidant status.

Material and Methods: 98 metabolically healthy obese and 75 normal-weight adolescents participated in the study. Obese individuals were grouped according to the severity of obesity. Biochemical parameters including thiol/ disulfide homeostasis were analyzed. Serum native thiol (SH), total thiol (SH+SS) and disulfide (SS) concentrations were determined using a novel automated measurement method. Disulfide to native thiol ratio (SS/SH), disulfide to total thiol ratio(SS/(SH+SS)), and native thiol to total thiol ratio (SH/(SH + SS)) were calculated and were presented as percentage.

Results: Native and total thiol levels-as an antioxidative parameter-was significantly higher in obese adolescents compared to healthy-weight adolescents ($p<0.001$). Native thiol/total thiol ratio was slightly higher in the obese group, but not statistically significant ($p=0.4$). Disulfide levels, disulfide/native thiol and disulfide/total thiol ratios were not different between the groups. We found that the levels of the antioxidant parameters, native thiol, and total thiol increased significantly along with the severity of the obesity, while other parameters remained comparable. We also found a significant increase in antioxidant parameters correlated with the severity of obesity ($p<0.001$).

Conclusion: This study concluded that the increased antioxidant status and activity in obese adolescents may be related to the metabolic health.

Keywords: Thiol/Disulfide; obesity; adolescent; oxidative stress

INTRODUCTION

Obesity defined as an abnormal or excessive fat accumulation that presents a risk to health (1) dyslipidemia, hepatic dysfunctions, cardiovascular diseases, renal function disorders, cancer, asthma, sleep disorders, and endocrine disorders (2). Despite being obesity, some individuals do not exist any of the deleterious metabolic effects of obesity or might be at substantially lower risk than expected for their degree of obesity. This subgroup has been described as having metabolically healthy obesity (MHO) (3-4). Many questions have been raised regarding the biological basis, transitory nature, and predictors of MHO.

Potential mechanisms involved in the genesis of MHO are not yet known. A robust detoxification mechanism that can cope with the oxidant environment created by increased adipose tissue may be one of the main mechanisms to preserve the metabolic well-being. Enhanced free radical production and /or depleted cellular antioxidant defense system may contribute to the metabolic effects of obesity.

Thiols are a major antioxidant and play an important role in the eradication of the reactive oxygen radicals through non-enzymatic paths (5-6). Dynamic thiol/disulfide homeostasis, which is novel indicator of the oxidative stress status, plays a key role in antioxidant protection, detoxification, signal transduction, apoptosis, regulation of enzymatic activity, the function of some transcription factors and some cellular signaling mechanisms (7-8).

The aim of present work is to evaluate prooxidant/ antioxidant status in MHO adolescents and to discuss the possible role of antioxidant activity response to provide metabolic health.

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MATERIAL and METHODS

Adolescents, who were diagnosed with obesity following the anthropometric evaluation, were included in this prospective, cross-sectional study. The anthropometric parameters were determined as the mean value of at least two measurements performed by the same investigator. Weight was obtained with subjects wearing light clothing (shorts or dress and teeshirt, without shoes) to the nearest 0.1 kg with an electronic weighing device. Height was measured without shoes to the nearest 0.1 cm by a wall-mounted stadiometer (Harpenden).

Obesity was defined according to sex- and age-specific body mass index (BMI) cut-off points [9]. The body-mass index was calculated with the following formula: BMI=weight (kg)/height2 (m^2). The weight categories according to age-specific and sex-specific BMI percentiles that we used in the present study were as follows: Class 1 obesity (≥95th percentile to <120% of the 95th percentile) ; Class 2 obesity (≥120% to <140% of the 95th percentile, or BMI ≥35 whichever was lower); Class 3 obesity (≥140% of the 95th percentile, or BMI ≥40, whichever was lower) (9-10).

Metabolically healthy obesity (MHO) defined as an obese person according to BMI does not have obesity-related metabolic abnormalities such as dyslipidemia, insulin resistance, hypertension and an unfavorable inflammatory profile (11).

Blood serum samples were obtained after 10-12 hours fasting period. Fasting plasma glucose and insulin levels, total cholesterol (TC), triglyceride (TG), low and high-density lipoprotein choles-terol (LDL-C and HDL-C), alanine aminotransferase (ALT) levels were analyzed. Serum glucose and lipid profile measurements were performed using the Roche modular system/Integra 800 device and kit (Mannheim, Germany). Serum TC >200mg/dL, TG>150mg/dL, LDL-C >130mg/dL or HDL-C <40mg/dL were accepted as dyslipidemia (12). For ALT, we used the reference intervals defined by our hospital laboratory. The upper limit of ALT was 41U/L. Insulin resistance was calculated using the Homeostatic Model Assessment of fasting IR (HOMA-IR)with the following for-mula was: fasting plasma insulin (μU/mL) x fasting plasma glucose (mg/dL)/405 (13). The accepted HOMA-IR cut-off value was ≥3.16 for both genders (14).

Patients with monogenic, syndromic obesity or chronic systemic diseases, had an infection with or without fever within the last 15 days, were smokers, had hypertension, dyslipidemia or metabolic dysfunctions like impaired glucose tolerance were not included in the study.

The study was approved by the Ethics Committee of the Medical Faculty at the Yıldırım Beyazıt University (11.01.2016; 2016/01/16).

Measurement of Thiol/Disulfide Homeostasis Parameters

The thiol/disulfide parameters were determined by a new spectrophotometric method using an automatic clinical chemical analyzer (Roche, Cobas 501, Mannheim, Germany) as previously de-scribed by Erel and Neşelioğlu (15). In summary, reducible disulfide bonds were reduced for the formation of the free functional thiol groups. Formaldehyde was used for the removal of the not-used and consumed sodium borohydride. All native and reduced thiol groups measured after the reaction with DTNB (5,5′-dithiobis-(2-nitrobenzoic acid)). Half of the difference between total and native thiols provided the dynamic disulfide amount. Native-thiol (SH), total thiol(SH + SS), and disulfide(SS) levels were measured as μmol/L. Disulfide to native thiol ratio (SS/SH), disulfide to total thiol ratio (SS/(SH+SS)), and native thiol to total thiol ratio (SH/(SH + SS)) were calculated and were presented as percentage.

Statistical Analysis

The statistical analysis was done with the SPSS (SPSS for Windows v.20.0, SPCC Inc, USA) software package. Quantitative variables were given in numbers and percentages. The mean values of normally distributed variables were compared with the Student's t-test and non-normally distributed variables were compared with the Mann-Whitney U test. Proportional parameters were evaluated with the Chi-square test. The accepted limit of statistical significance was $p<0.05$.

RESULTS

This study included 173 adolescents, of whom 98 were obese and there were 75 healthy, normal-weight adolescents. Within the obese group, 35 (35.8%) were moderately obese (class 2 obesity group), 63 (64.2%) were severely obese (class 3 obesity group). The mean duration of obesity in our patients was 5.04 ± 3.12 years.

HOMA-IR, plasma glucose, plasma insulin, TC, LDL and HDL cholesterol, TG levels were similar between obese patients and healthy control subjects, also between moderately and severely obese patients. The demographic, clinical and laboratory characteristics of the participants are displayed in Table 1.

Obese patients had higher native thiol (SH) and total thiol (SH+SS) levels than control subjects ($p<0.001$ for both parameters). Although the native thiol/total thiol (SH/SH+SS) ratio was slightly high in the obese group, there was no statistically significant difference between the groups ($p=0.4$). In addition disulfide (SS) levels, disulfide/native thiol (SS/SH) and disulfide/total thiol (SS/SH+SS) ratios were not different between groups (Table-2).

Native thiol (SH) and total thiol (SH+SS) levels were statistically higher in severely obese patients (class 3) than moderately obese patients (class 2). Disulfide (SS) levels, disulfide/native thiol (SS/SH) and disulfide/total thiol (SS/SH+SS) ratios were not different among moderately and severe obese patients. (Table-2)

Table 1. Demographic, clinical and laboratory parameters of class 2 obesity group, class 3 obesity group and healthy control groups

	Control group(n=75)	Class 2 obesity group (n=35)	Class 3 obesity group (n=63)	p value*	p value[a]	p value[b]	p value[c]
Age (years), Mean ±SD	13.34 ± 1.5	13.04 ± 1.19	13.62 ± 1.77	0.201	0.86	0.63	0.97
Gender (Female) (n,%)	54 ± 72	35 ± 55.5	25 ± 71.4	0.095			
BMI (kg/m2) Mean ±SD	21.1 ± 1.2	25.7 ± 21.7	35.1 ± 26.9	0.001	0.049	<0.001	0.04
SBP (mmHg) Mean ±SD	106 ± 10.9	110.16 ± 16.4	115.3 ± 15.7	0.001	0.08	<0.001	0.93
DBP (mmHg) Mean ±SD	71.4 ± 7.5	73.8 ±12.91	74.6 ± 11.09	0.164	0.15	0.054	0.62
FBG (mg/dL) Mean ±SD	87.4 ± 6.7	89.35 ±10.26	90.6 ± 7.83	0.061	0.158	0.006	0.39
Fasting insulin (µU/mL) Mean ±SD	17.5 ± 8.5	19.5 ± 10.4	21.4 ±15.56	0.16	0.16	0.07	0.23
HOMA-IR Mean ±SD	3.02 ±1.36	2.93 ± 1.26	3.12 ± 1.15	0.77	0.63	0.32	0.76
ALT (U/L) Mean ±SD	20.8 ± 7.5	23.73 ± 37.9	25.5 ± 22.5	0.46	0.32	0.058	0.59
HDL (mg/dL) Mean ±SD	43.3 ± 10	44.3 ± 10.5	41.6 ± 9.6	0.39	0.31	0.84	0.10
LDL (mg/dL) Mean ±SD	103.9 ± 26.9	104.5 ± 24.3	106.8 ± 26.1	0.80	0.45	0.26	0.66
TG (mg/dL) Mean ±SD	110.2 ± 85.5	122.2 ± 85.5	130.7 ± 60.1	0.29	0.24	0.051	0.30
Cholesterol (mg/dL) Mean ±SD	179.3 ± 37.5	177.3 ± 35.7	183.8 ± 35	0.64	0.60	0.23	0.80

SD: standard deviation, BMI: Body mass index , SBP: Systolic blood pressure, DBP: Diastolic blood pressure, FBG: Fasting blood glucose, ALT: Alanine aminotransferase, HDL:High-density lipoprotein, LDL: Low-density lipoprotein cholesterol,TG: Triglyceride HOMA-IR: Homeostasis model assessment of insulin resistance
p value* Significance in analysis of variance (among three groups)
p value[a] Significance between control group and class 2 group
p value[b] Significance between control group and class 3 group
p value[c] Significance between class 2 and class 3 groups

Table 2. Thiol / disulfide homeostasis parameters of class 2 obesity group, class 3 obesity group and healthy control groups

	Control group(n=75)		Class 2 obesity (n=35)		Class 3 obesity (n=63)		p value*	p value[a]	p value[b]	p value[c]
	Mean ±SD	Median (IQR)	Mean ±SD	Median (IQR)	Mean ±SD	Median (IQR)				
Native thiol (µmol/L)	361.89±49.72	368.65 (73.4)	390.8±47.6	392.1 (48.9)	419.14±44.2	421.5 (56)	< 0.001	0.001	<0.001	0.005
Total thiol (µmol/L)	393.86±49.33	403.6(72.1)	423.8±52.03	430.6 (60.4)	456±48.2	48.2 (45.8)	< 0.001	0.001	<0.001	0.003
Disulfide (µmol/L)	18.67±8.06	16.8 (9.3)	18.49±6.56	18.9 (5.7)	19.02±6.2	17.8 (8.05)	0.93	0.89	0.82	0.69
Disulfide/Native thiol, %	5.25±2.34	4.8 (2.51)	4.77±1.83	4.73 (1.55)	4.59±1.72	4.05 (1.6)	0.23	0.20	0.14	0.63
Disulfide/Total thiol, %	4.76±2	4.41 (2.1)	4.37±1.62	4.32 (1.34)	4.19±1.42	3.75 (1.41)	0.24	0.23	0.14	0.57
Native thiol / Total thiol, %	91.78±3.20	92.2 (3.77)	92.2±2.38	91.9 (2.9)	91.9±2.36	92.4 (2.83)	0.59	0.33	0.79	0.51

p value*. Significance in analysis of variance (among three groups)
p value[a] Significance between control group and class 2 group
p value[b] Significance between control group and class 3 group
p value[c] Significance between class 2 and class 3 groups

DISCUSSION

With this study, significant increased antioxidant parameters has been documented in MHO compared to the normal-weight ones. Considering the increase in antioxidant parameters as biomarker reflecting antioxidant activity, our study results may indicate the importance of antioxidant defense in providing metabolic health.

Obesity is a chronic low-level inflammatory process characterized by the increase of the body fat mass. The energy balance, which is disrupted by the excessive energy intake, leads to hypertrophy and hyperplasia in the adipocytes resulting in fat accumulation. This causes a hypoxic and inflammatory environment in the accumulated fat tissue, stimulates the release of inflammatory cytokines, chemokines (16,17). These inflammatory changes induce systemic oxidative stress through multiple biochemical mechanisms as well as local oxidative effects of obesity so that act synergistically in metabolic abnormalities associated with obesity. Therefore, evaluation of oxidative status has been reported to aid in identification of patients with high risk of complications.

Until now, many studies evaluating the oxidant-antioxidant status in obese children and adolescents have been reported. Many of them have found increased prooxidant status in obese individuals, contrary to our study (18-21). In addition, some researches in the literature have drawn attention to the presence of increased antioxidant enzyme activity in obese individuals (22-24). nevertheless, the increased prooxidant status was prevailed in these individuals. These findings were also confirmed that obesity itself is associated with increased free radical production resulting in an increased antioxidant response and prooxidant stress. Unlike these studies, Brown et al (18) reported that there was no difference between healthy-weight, overweight and obese adults for the total antioxidant enzyme activity, while different studies stated that total antioxidant enzyme activity was even lower in the obese individuals compared to healthy controls (25-26). These conflicting results may be explained by the age, obesity duration and severity, physical activity differences, eating behavior among obese patients. However, none of these studies, similarly our study, aimed to evaluate these factors that may cause oxidant / antioxidant activity changes.

The mechanisms responsible for altered antioxidant activity in obesity are unclear. Knowing these is important for providing and maintaining metabolic health in obesity. Therefore, it may be essential to know what the change in oxidant/antioxidant balance is in obese patients who do not have the metabolic disorder associated with obesity. In this context, our study is one of the first studies that investigated oxidant/antioxidant status in MHO. First time, Mengen et al (27) found that prooxidant parameters were significantly high in obese children. When they divided obese patients into two groups as metabolically healthy and unhealthy, they demonstrated that oxidant/antioxidant parameters did not differ among groups. Similarly, it was not different between MHO and normal-weight controls. In our study, there was significantly increased in antioxidant parameters in MHO. This finding may be significant for understanding the possible effect of antioxidant activity on providing metabolic health in obesity.

Besides these, previous studies have been reported that decreased antioxidant activity and antioxidant levels in the presence of long-term obesity. Similarly, the lower antioxidant levels was observed in individuals with severe obesity (25-28). Long-term obesity and severe obesity leads to prooxidant status causing a loss of antioxidants due to increased demand for antioxidants to combat free radicals. These results can speculated by the study of Dobrian et al. (29). They showed that antioxidant activity and antioxidant status was increased in the early stages of obesity. Furukawa et al (30) showed a decrease in antioxidant levels in proportion to the severity of obesity. As a result, they stated that their patients had a prooxidant status obesity-associated complications. Considering the characteristics of our obese patient group, we can evaluate the effect of these two factors on oxidant / antioxidant activity. our patients' obesity duration were long-term and degrees of obesity were moderately and severity. Nevertheless, most of them had antioxidant status and there were no metabolic disorders associated with obesity. Furthermore, there was a significant increase in antioxidant levels in the severely obese than moderately obese. Our result can be considered as a reflection of increased antioxidant activity to maintain metabolic health against increased oxidative stress.

Recent studies' findings indicate that an increase in physical activity in combination with a reduction of energy intake provides in reducing oxidative stress by increasing sensitivity to oxidative stress and antioxidant enzyme activity (31-32). Most probably, not to evaluate the impact of these factors, is one of the most important limitations of our study. Other limitations are that we have not compared metabolically unhealthy obese and the low number of patients.

CONCLUSION

In conclusion, our data show that the increased antioxidant status and activity in obese children may be related to the metabolic health. Maintaining and increasing antioxidant activity to prevent complications related to obesity may be an important question for future studies. For this, change in antioxidant activity may assess by the different method, for example physical activity, calorie restriction, adequate and appropriate vitamin and mineral intake.

Competing interests: The authors declare that they have no competing interest.

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Ethical approval: The study was approved by the Ethics Committee of the Medical Faculty at the Yıldırım Beyazıt University (11.01.2016; 2016/01/16).

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