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Original article

Reduced antioxidant capacity and increased subclinical inflammation markers in prepubescent obese children and their relationship with nutritional markers and metabolic parameters

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Objective: There are associations between some inflammatory and oxidative markers and obesity in adults, but whether prepubescent children of different weights also have such markers has not been studied. We investigated multiple inflammatory markers and levels of erythrocyte oxidant/antioxidant enzymes in prepubescent children of different weights.

Methods: Children aged 2–11 years were divided into three groups: 80 were underweight, 90 were obese but otherwise healthy, and 80 were healthy age- and sex-matched children of normal-weight. We analyzed inflammatory markers and the total oxidant status, total antioxidant status (TAS), and total thiol level were also determined, and the oxidative stress index was calculated as an indicator of the degree of oxidative stress.

Results: The obese group exhibited higher levels of fasting glucose, insulin, total cholesterol, triglycerides, the homeostatic model assessment of insulin resistance (HOMA-IR), and the homeostatic model assessment of β-cell function (HOMA-β), C-reactive protein (CRP), neutrophils, and neutrophil/lymphocyte ratio (NLR), as well as lower TAS and total thiol levels than the other two groups (all $P < 0.001$). Moreover, TAS and total thiols were negatively correlated with age in the obese group ($r = -0.212$, $P = 0.001$; $r = -0.231$, $P < 0.001$, respectively). CRP levels in plasma were positively correlated with the body mass index (BMI), insulin and glucose levels, HOMA-IR, HOMA-β, WBC and neutrophil counts, and the NLR, and were negatively correlated with TAS and total thiol levels in the overall studied population.

Discussion: The coexistence of increased obesity-related subclinical inflammation and decreased antioxidant capacity can be observed even in prepubescence, and may eventually increase the risk of long-term vascular damage.

Keywords: Inflammation markers, Oxidative/antioxidative status, Prepubescent children

Introduction

Obesity is becoming a global epidemic in both children and adults, and chronic multifactorial disorders result from the interaction between genetic, psychological, behavioral, environmental, socioeconomic, metabolic, and molecular factors.[1] Recent evidence suggest that obesity is characterized by increased oxidative stress and exacerbated inflammatory outcomes accompanying infiltration of immune cells in adipocytes.[2,3] Additionally, obesity has been linked to a low-grade proinflammatory state, in which impairments in the oxidative stress and antioxidant mechanism could be involved.[4] Oxidative stress is generally described as an imbalance in net levels of reactive oxygen species (ROS) relative to the body's antioxidant capacity, resulting in the accumulation of oxidative products.[5] ROS occur under physiological conditions and in many diseases, and cause direct or indirect damage to different organs.[6] It has been reported that there are several possible mechanisms by which obesity causes oxidative stress. The peroxisomal and

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mitochondrial oxidation of fatty acids can occur in oxidation reactions.[7] Obesity increases mechanical load, which in turn increases myocardial metabolism and oxygen consumption. The over-consumption of oxygen, which generates free radicals (superoxide, hydroxyl radical, hydrogen peroxide) in the mitochondrial respiratory chain, is coupled with oxidative phosphorylation in mitochondria.[8]

Recent studies demonstrated that excessive fat accumulation can cause cellular damage due to pressure from fat cells. This in turn leads to a high production of cytokines such as TNF-α, IL-1, and IL-6, which generates ROS in the tissues, increasing the lipid peroxidation rate. The formation of lipid hydroxyperoxides occurs in enzymatic or non-enzymatic reactions involving ROS which are responsible for toxic effects in the body via various tissue damages.[9] Also these increased cytokines are potent stimulators for the production of reactive oxygen and nitrogen by macrophages and monocytes. The elevated concentration of cytokines could be responsible for increased oxidative stress.[6]

Oxidative stress occurs when the intracellular concentrations of free radicals increase over physiological values, which results in cell damage by lipid peroxidation, DNA and protein damage, enzyme oxidation, and stimulation of proinflammatory cytokine release.[10,11] Oxidant status can be assessed by determining the total oxidant status (TOS). The adaptation of the cell to an increase in radical production consists of an increase in enzymatic antioxidant activity to prevent oxidative damage to cells. The antioxidant status of individuals depends on multiple factors. Enzymatic antioxidant (superoxide dismutase (SOD), catalase, glutathione peroxidase), the major non-enzymatic antioxidant (tripeptide glutathione), a subset of vitamins (α-tocopherol, ascorbic acid, carotenoids and flavonoids, zinc), and endogenous antioxidants (albumin, uric acid, and bilirubin) are the most widely used biomarkers of antioxidant status.[12] Another useful biomarker is the total antioxidant capacity which evaluates the integrated action of all antioxidants present in plasma.[13]

Oxidative stress might be a major mechanism underlying obesity-related co-morbidities, including atherosclerosis, insulin resistance, type 2 diabetes, and cancer.[14] Such metabolic and inflammatory disturbances in children often remain until adulthood and contribute to the development of various health problems.[15]

The enlargement of adipocytes by fat storage induces adipose tissue hypoxia and secretion of high levels of inflammatory cytokines and chemokines.[16] Increased secretion of these molecules by activated leukocytes, macrophages and other tissues has been demonstrated in obese patients.[17] The increased macrophage infiltration of adipose tissue may be an important component of the chronic inflammatory response in the development of insulin resistance.[18,19] Additionally, ROS are also released by activated neutrophils and other leukocytes; indeed, WBC secretion of oxidative and inflammatory surrounding the lipid droplets of the atheromatic core is a crucial biochemical step linking atherosclerotic plaques to thrombotic luminal occlusion.[20]

Many studies have shown a relationship between different oxidative stress and inflammatory markers in obese adults and older children/adolescents, but few studies have evaluated these together with oxidative/antioxidative status and inflammation markers in prepubescent children. Therefore, to investigate the potential relationships between multiple inflammatory and oxidative stress markers and the weight of children, we assessed white blood cell (WBC) count in serum, absolute neutrophil and lymphocyte counts, the neutrophil/lymphocyte ratio (NLR), C-reactive protein (CRP) levels, and oxidative/ antioxidative markers in three different groups (underweight, obese, and healthy children) aged 2–11 years. Our specific goal was to isolate effects according to age and gender, while eliminating the effects of puberty.

Method

This cross-sectional study was carried out at the Bezmialem Vakif University Hospital General Pediatrics Clinic in Istanbul. Randomly selected pediatric patients who attended the outpatient clinic for routine examinations between October 2014 and April 2015 were recruited. The study population consisted of 250 prepubescent children aged 2–11 years; The three study groups included 80 healthy anorexic children (with a body mass index [BMI] <the 18.5th percentile for each age and sex), 90 obese but otherwise healthy children (with a BMI ≥the 95th percentile), and 80 healthy children of normal-weight (with a BMI in the 18.5th to 95th percentile). The inclusion criteria consisted of a dietary history with adequate nutrient intake (in quality and quantity). Underweight patients were able to obtain food, but had poor appetites. The exclusion criteria included the presence of endocrine disease (Cushing's syndrome or hypothyroidism), acute or chronic inflammatory disease, malabsorption syndromes such as celiac disease or cystic fibrosis, infections or systemic illnesses, and the use of prescription medications, vitamins, or mineral supplements for any reason. Each children underwent a detailed physical examination (including evaluation for systemic and endocrine diseases). To exclude gluten enteropathy, we examined total IgA, tissue transglutaminase antibody IgA (anti tTG-IgA) in both underweight and normal-weight

children. We recruited four children who were diagnosed with celiac disease during the follow up. Consequently, children without any health problems except for anorexia were included in this study as underweight group. They had no dietary history of inadequate nutrient intake in means of quality or quantity. The children had the opportunity to obtain food, but they had only poor appetites. Subjects were classified as underweight, normal, or obese according to Cole's recently published BMI cut-offs for underweight/overweight/obese according to sex and age (for children aged 2–18 years).[21]

After 12 hours of fasting, venous blood samples were collected between 8:00 a.m. and 9:00 a.m. The samples were separated by centrifugation (10 minutes at $3082 \times g$, 4°C) and stored at –80°C for subsequent use. The complete blood count, and levels of iron and total iron-binding capacity in serum, ferritin, thyroid stimulating hormone (TSH), T4, vitamin B12, folic acid, glucose, albumin, insulin, total cholesterol, triglycerides, and CRP were assessed in all subjects. Total cholesterol and triglycerides were measured using the homogeneous colorimetric enzyme technique (Roche Cobas 8000 modular analyzer; Roche Diagnostics, Mannheim, Germany). Glucose was measured using the glucose oxidase technique (Advia 1800; Siemens Healthcare Diagnostics, Tarrytown, NY, USA); insulin levels were analyzed using the direct chemiluminescence technique (Advia Centaur, Siemens). IR was estimated from plasma measurements after fasting using the homeostatic model assessment of insulin resistance (HOMA-IR, calculated as insulin [μU/l] × glucose [mmol/l]/22.5). The criterion for IR in prepubescent children was an HOMA-IR of >2.5. The homeostatic model assessment of β-cell function (HOMA-β, calculated as $20 \times$ insulin [μU/l]/glucose [mmol/l] – 3.5) was estimated using the formulae developed by Wallace *et al.*[22]

The number of leukocytes and leukocyte subtypes were computed with an autoanalyzer (Sysmex XT-2000i Kobe, Japan). The NLR was defined as neutrophil count/lymphocyte count. CRP levels in serum were measured with a particle-enhanced immunoturbidimetric assay using the Roche Cobas 8000 modular analyzer. The cut-off value for CRP was assigned as 5.0 mg/l. The maximum intra-assay and inter-assay coefficients of variation were 2.38% and 4%, respectively.

Total oxidant and antioxidant status and oxidative stress index (OSI)

The total antioxidant status (TAS) and TOS of serum were determined using an automated measurement method with an automated analyzer (Chromate Manager 4300, Palm City, USA), as described in a previous study.[23] In the measurement of TAS, the absorbance of the colored dianisidyl radicals was monitored to determine the rate of Fenton's reaction, which initiates free-radical reactions with the production of a hydroxyl radical. Then, the antioxidative effect of the sample against potent free-radical reactions was measured in terms of mmol equiv/l Trolox (Rel Assay Diagnostics, Gaziantep, Turkey).

In the measurement of TOS, the intensity of the colored complex produced by the reaction of ferric ions, which were oxidized from a ferrous iono-dianisidine complex due to the presence of oxidants, with xylenol orange in an acidic medium was used to determine the total amount of oxidant molecules in the sample. The calibration of the assay was performed with hydrogen peroxide. TOS values are expressed in terms of micromolar hydrogen peroxide equivalents per liter (μmol H_2O_2 equiv/l) (Rel Assay Diagnostics, Gaziantep, Turkey).[24]

The OSI is the ratio of the TOS level to the TAS level. For the resulting micromolar unit, TAS was converted into millimoles per liter, and the OSI value was calculated according to the following formula: OSI (arbitrary unit) = TOS/TAS × 100.

The measurement of total free sulfhydryl groups (–SH) in serum samples was assayed according to Ellman's method, as modified by Hu and Chou.[25] Briefly, 1 ml of a buffer containing 0.1 M Tris, 10 mM EDTA (pH 8.2), and 50 μl serum was added to cuvettes, followed by 50 μl 10 mM DTNB in methanol. Blanks were run for each sample as a test, but without the DTNB. Following incubation for 15 minutes at room temperature, the sample absorbance was read at 412 nm on a Cecil 3000 spectrophotometer (Cecil Instruments, Cambridge, UK). Sample and reagent blanks made up the substrate. The concentration of sulfhydryl groups was calculated using reduced glutathione as the free sulfhydryl group standard, and the results are expressed as mmol/l. The CV to measure the serum –SH level was 3.9%.

Statistical analysis

Analyses were conducted using the IBM SPSS for Windows software package (ver. 20.0; IBM Corp., Armonk, NY, USA). The results are presented as means ± SD, with categorical variables presented as frequencies and percentages. The comparison of the groups was performed using a one-way analysis of variance (ANOVA) for parametric tests with Tukey's HSD *post hoc* test applied for multiple comparisons of the oxidant/antioxidant/inflammatory markers between the BMI groups. Obese children with IR were compared to obese children without IR using a *t*-test. A Pearson's correlation was used to determine the relationships between variables. A *P*-value of <0.05 was taken to indicate statistical significance.

Information concerning the aim of the study was provided to the children's parents at the time of enrollment; written informed consent was also obtained. Ethical approval was granted by the Bezmialem Vakif University Local Research Ethics Committee.

Results

Of the 250 children, 121 were male and 129 were female. The anthropometric and metabolic characteristics of the groups are summarized in Tables 1–3. The age and sex distribution did not differ among the three groups ($P = 0.840$ and 0.993, respectively). The underweight group had a significantly lower mean weight, weight *z*-score, mean height, height *z*-score, BMI, BMI *z*-score, and BMI percentage (all $P < 0.001$) than the control and obese groups.

There were no significant group differences in the levels of hemoglobin, hematocrit (hct), albumin, vitamin B12, or ferritin ($P = 0.790$, 0.055, 0.880, 0.060, 0.606, respectively) (Table 2). In addition, there were no significant group differences in WBC and lymphocyte counts, TOS levels, or OSI ($P = 0.055$, 0.187, 0.289, 0.075, respectively) (Table 3).

No significant differences were found between the underweight and normal-weight children in terms of fasting glucose, insulin, total cholesterol, triglycerides, HOMA-IR, HOMA-β, neutrophil counts, NLR, CRP, TAS, or total thiol level ($P = 0.940$, 0.427, 0.900, 0.740, 0.999, 0.524, 0.125, 0.540, 0.605, 0.933, 0.529, 0.778, respectively).

The obese group had higher fasting glucose, insulin, total cholesterol, triglyceride, HOMA-IR, HOMA-β, CRP, neutrophil count, and NLR but lower TAS and thiol levels than the other two groups (all $P < 0.001$). Moreover, TAS and total thiol level were negatively correlated with age in the obese group ($r = -0.212$, $P = 0.001$; $r = -0.231$, $P < 0.001$, respectively). However, WBC counts, neutrophils, NLR, and CRP levels all showed significant and positive correlations with BMI ($r = 0.176$, $P = 0.007$; $r = 0.176$, $P < 0.001$; $r = 0.205$, $P = 0.002$; $r = 0.471$, $P < 0.001$) (Fig. 1). TAS and total thiol levels were

Table 1 Comparison of demographic and anthropometric characteristics in underweight, normal-weight and obese children

Characteristics	Underweight children mean ± SD (range)	Healthy children mean ± SD (range)	Obese children mean ± SD (range)	*P** Value
Children (*n*)	80	80	90	
Gender				
Female	39 (48.75)	41 (51.25)	49 (54.5)	
Male	41 (51.25)	39 (48.75)	41 (45.5)	0.993
Age (years)	7.0 ± 2.6 (2–11)	7.2 ± 2.7 (3–11)	7.4 ± 2.7 (2–11)	0.840
Weight, kg	20.8 ± 7.4 (9.5–36.9)	25.8 ± 10.0 (12.5–59)	52.1 ± 16.5 (19.1–86)	<0.001
Weight, *z*-score	−1.47 ± 0.9 (−3.9 : 0.48)	0.17 ± 0.68 (−1.2:1.9)	2.15 ± 0.78 (−0.04:4.13)	<0.001
Height, cm	120.6 ± 19.1 (82–160)	122.0 ± 17.1 (90–159)	141.8 ± 16.6 (92.7–170)	<0.001
Height, *z*-score	−0.41 ± 1.0 (−2.9:2.3)	0.01 ± 0.84 (−1.5:2.0)	0.90 ± 0.9 (−1.6:2.7)	<0.001
BMI, kg/m^2	13.8 ± 1 (11.7–17.1)	16.75 ± 1.90 (14.2–21.8)	25.78 ± 4.2 (19.6–43.6)	<0.001
BMI-*z* score	−1.91 ± 0.8 (−4.9:−1.1)	0.23 ± 0.7 (−1.5:1.7)	2.32 ± 0.6 (0.9:4.5)	<0.001
BMI per.	6.3 ± 9.7 (0–24.3)	54.9 ± 22.50 (13.3–91.6)	98.1 ± 1.8 (95–100)	<0.001

BMI: body mass index, The data are expressed as number, mean values ± standard deviation (range), One-way ANOVA test was used for correlation analysis, $P < 0.05$.

Table 2 Comparison of laboratory characteristics of subjects according to BMI stage group

Parameters	Underweight children mean ± SD (range)	Healthy children mean ± SD (range)	Obese children mean ± SD (range)	*P** Value
Hb (g/dl)	12.46 ± 0.9 (9.6–14.8)	12.31 ± 0.9 (10.4–14)	12.65 ± 0.9 (10.4–14.7)	0.790
Hct	36.9 ± 2.55 (60–89.4)	36.6 ± 2.6 (65–87.6)	37.8 ± 3.5 (62.2–90.6)	0.055
fT4 (pmol/l)	15.27 ± 1.9 (10.6–19.9)	14.02 ± 2.13 (6.3–18.6)	13.63 ± 2.26 (2.57–18.9)	<0.001
TSH (IU/ml)	2.27 ± 0.91 (0.9–4.57)	3.04 ± 1.44 (0.7–6.7)	3.22 ± 2.26 (1.08–14.8)	<0.001
Albumin (g/dl)	4.43 ± 0.24 (4–5.2)	4.39 ± 0.22 (4–4.9)	4.44 ± 0.22 (4.1–5.2)	0.880
Vitamin B12 (pg/ml)	402.6 ± 190.2 (101–1582)	459.7 ± 178.8 (173–970)	399.5 ± 144.3 (104–721)	0.060
Ferritin (ng/ml)	32.02 ± 22.69 (4.1–107.7)	29.1 ± 21.1 (3.2–107.7)	29.38 ± 15.0 (5.4 ± 80.3)	0.606
Glucose (mg/dl)	87.6 ± 8.1 (59–99)	87.98 ± 6.3 (73–98)	92.81 ± 5.4 (79–103)	<0.001
Insulin (IU/ml)	8.1 ± 5.2 (1.03–26.5)	10.11 ± 6.1 (1.50–33.1)	19.36 ± 13.8 (3.9–82.5)	<0.001
HOMA-IR	1.78 ± 1.2 (0.19–6.3)	2.18 ± 1.35 (0.3–6.7)	4.44 ± 3.29 (0.9–19.79)	<0.001
HOMA-β	115.1 ± 139 (−854:414)	160.7 ± 107 (25–621)	241.7 ± 136.4 (55.5–873)	<0.001
Triglyceride (mg/dl)	65.7 ± 21.0 (36–135)	62.9 ± 17.3 (32–113)	92.2 ± 28.5 (32–148)	<0.001
Total cholesterol (mg/dl)	153 ± 17.2 (109–188)	154.6 ± 20 (108–223)	166 ± 28.7 (105–233)	<0.001

Hb: hemoglobin, fT4: Free thyroxin, TSH: thyroid stimulating hormone, HOMA-IR: Homeostatic model of assistance of insulin resistance, HOMA-β: Homeostatic model assessment of β-cell function.
The data are expressed as number, mean values ± standard deviation (range), One-way ANOVA test was used for correlation analysis, $P < 0.05$.

Table 3 Comparison of proinflammatory indicators of subjects according to BMI stage group

Parameters	Underweight children mean ± SD (range)	Healthy children mean ± SD (range)	Obese children mean ± SD (range)	*P** Value
WBC, $\times10^3/\mu l$	7.84 ± 2.14 (4.23–12.77)	7.75 ± 1.95 (4.58–12.30)	8.72 ± 1.99 (3.86–13.05)	0.035
NC, $\times10^3/\mu l$	3.82 ± 1.78 (1.20–9.6)	3.52 ± 1.47 (1.23–9.45)	4.58 ± 1.84 (1.00–10.73)	<0.001
LC, $\times10^3/\mu l$	3.13 ± 1.00 (1.40–6.13)	3.30 ± 1.10 (1.16–8.48)	3.00 ± 0.90 (1.17–6.19)	0.187
NLR	1.35 ± 0.80 (0.29–4.58)	1.20 ± 0.77 (0.29–4.68)	1.73 ± 1.18 (0.26–8.72)	<0.001
CRP, mg/l	1.50 ± 1.39 (0.0–9.0)	1.64 ± 1.22 (0.0–9.0)	4.11 ± 3.10 (0.0–15)	<0.001
TAS	1.33 ± 0.23 (1.01–1.98)	1.29 ± 0.21 (0.9–1.99)	1.19 ± 0.25 (0.9–1.99)	<0.001
TOS	11.27 ± 4.8 (6.78–32.1)	10.27 ± 3.34 (6.14–32.22)	11.33 ± 5.12 (6.78–32.12)	0.289
OSI	0.86 ± 0.39 (0.46–2.72)	0.81 ± 0.35 (0.31–3.29)	0.96 ± 0.48 (0.10–3.15)	0.075
Total thiol	0.36 ± 0.055 (0.14–0.48)	0.36 ± 0.04 (0.28–0.52)	0.33 ± 0.03 (0.28–0.46)	<0.001

WBC: White blood cell, NC: neutrophil count, LC: lymphocyte count, NLR: neutrophil/lymphocyte ratio, CRP: C-reactive protein, TAS: Total antioxidant status (mmol equiv/l Trolox), TOS: Total oxidant status (μmol H_2O_2 equiv/l), OSI: Oxidative stress index (arbitrary units), Total thiol (–SH free sulfhydryl groups) (mmol/l).
The data are expressed as number, mean values ± standard deviation (range), One-way ANOVA test was used for correlation analysis, $P < 0.05$.

negatively correlated with BMI ($r = -0.191$, $P = 0.004$; $r = -0.231$, $P < 0.001$) (Fig. 2). In addition, analysis showed a significant negative correlation between TAS and HOMA-IR, HOMA-β, CRP, WBC counts, neutrophils, NLR, and OSI, and a significantly positive correlation between TAS and total thiol levels. The Pearson's correlation for multiple comparisons of the oxidant/antioxidant/inflammatory markers between the BMI groups are summarized in Tables 4 and 5

Discussion

In the present study, we evaluated the pattern of oxidative stress and the different inflammatory markers in three different weight groups of prepubescent children. Although associations between these parameters

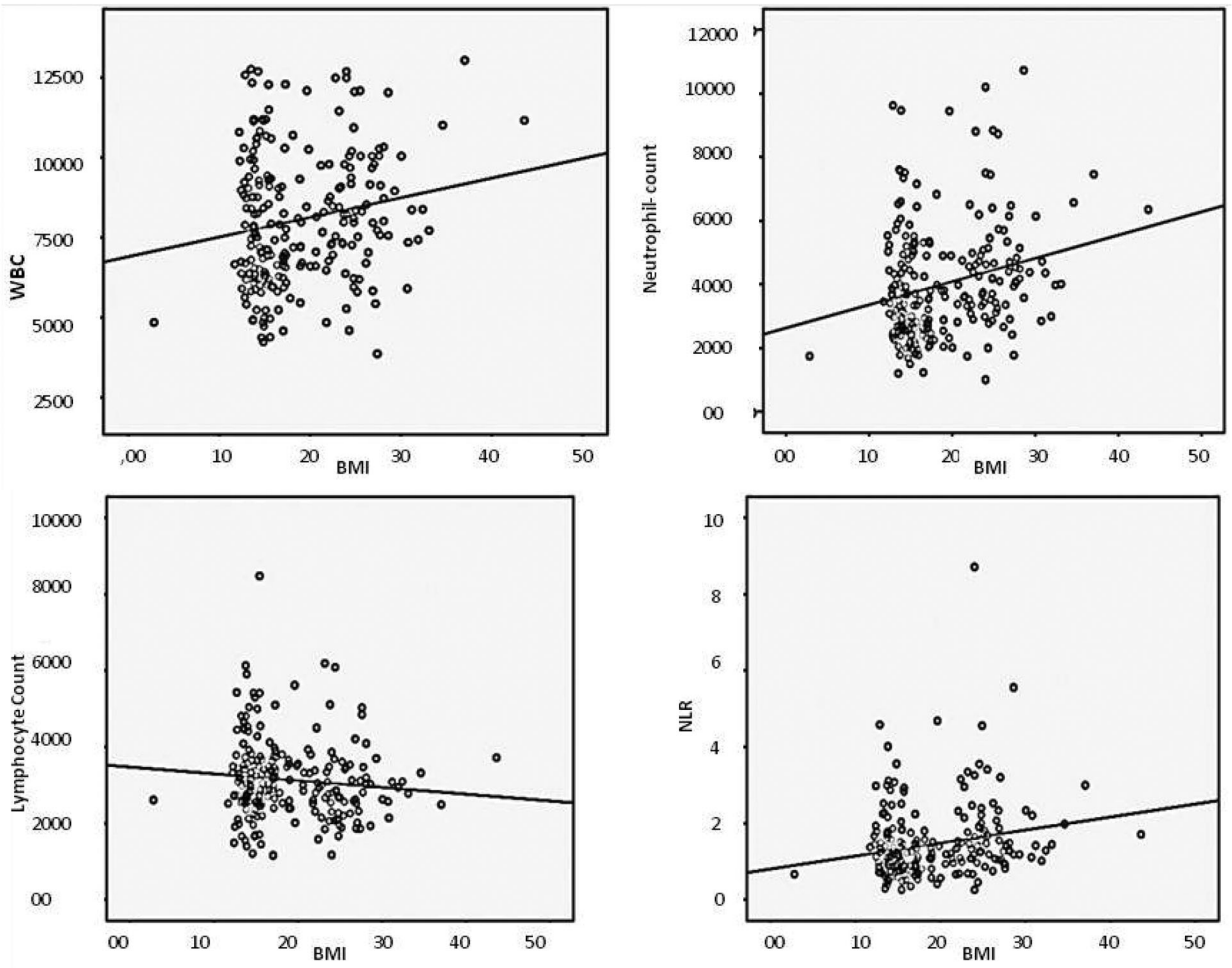

Figure 1 Scatter plot figures for Pearson's correlation of WBC, WBC subtypes, and NLR with BMI in overall population. WBC: White blood cell, BMI: Body mass index, NLR: Neutrophil–lymphocyte ratio, blood cell is expressed as $\times10^3/\mu l$.

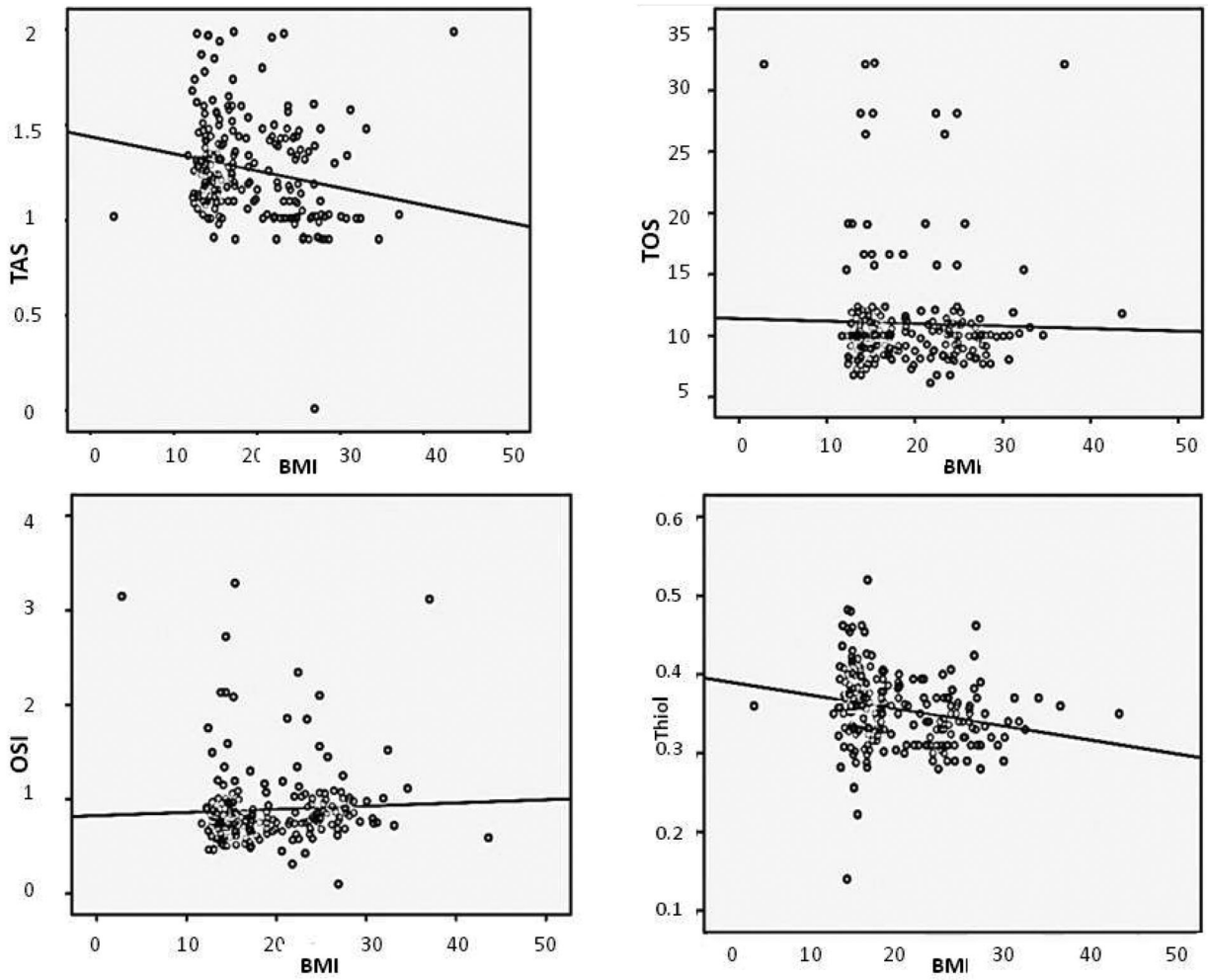

Figure 2 Pearson's correlation of TAS, TOS, OSI and Total thiol with BMI. TAS: Total antioxidant status (mmol equiv/l Trolox), TOS: Total oxidant status (μmol H_2O_2 equiv/l), OSI: Oxidative stress index (arbitrary units), Total thiol (mmol/l).

and obesity have been found to be greater for older children and adolescents,[26,27] it was unknown whether this relationship existed from an early age. We observed significantly higher levels of fasting glucose, insulin, total cholesterol, triglycerides, HOMA-IR, HOMA-β, CRP, neutrophil count, and NLR in obese prepubescent children compared to underweight and normal-weight children. However, antioxidant status and total thiol level were markedly lower in obese children compared to the other groups, and these parameters are negative correlated with age in obese group. Correlation analysis revealed a significant negative correlation between TAS and BMI, insulin, HOMA-IR, and HOMA-β. These observations indicated that at an early age, obese prepubescent children had concurrently elevated inflammatory and decreased antioxidant status compared to underweight/normal-weight and gender matched controls.

Previously many studies have evaluated antioxidant status in obese subjects and diverse results have been found. Kobayasi *et al.*[28] demonstrated that the production of superoxide anions and Cu/Zn SOD activity increased in obese mice. Sfar *et al.*[29] showed that the activity of antioxidant enzymes was markedly higher in obese children than in normal-weight ones. Similarly, Amaya-Villalva *et al.*[30] found that in adults, obese individuals had a higher antioxidant capacity, and antioxidant status was correlated with obesity-related indicators. However, other studies have determined that low antioxidant status and reduced antioxidant capacity are common in obese children and adolescents compared with normal children similar to our study results. For example, Beydoun *et al.*,[31] reported that obese U.S. adolescents with metabolic syndrome had lower serum carotenoid concentrations compared with their counterparts without MetS in recent national survey. Karamouzis *et al.*[32] recently 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. Another study, Olusi[33] found that erythrocyte Cu/Zn SOD activity was significantly lower in obese subjects than in controls. Similarly, Molnár *et al.*[34] showed that the TAS

Table 4 The results of Pearson's correlation between proinflammatory markers and other anthropometric and metabolic parameters

	r	*P*		*r*	*P*
WBC correlations with			NLR correlations with		
CRP	0.312**	<0.001	HOMA-IR	0.223**	0.001
Neutrophil	0.822**	<0.001	HOMA-β	0.174**	0.008
Lymphocyte	0.399**	<0.001	Insulin	0.219**	0.001
NLR	0.453**	<0.001	Glucose	0.150*	0.023
TAS	−0.145*	0.028	CRP	0.323**	<0.001
			WBC	0.453**	<0.001
Neutrophil correlations with			Neutrophil	0.825**	<0.001
HOMA-IR	0.188**	0.004	Lymphocyte	−0.533**	<0.001
HOMA-β	0.181**	0.006	TAS	−0.158*	0.016
Insulin	0.188**	0.004	TOS	0.140*	0.034
CRP	0.389**	<0.001	OSI	0.189**	0.004
WBC	0.822**	<0.001	CRP correlations with		
Lymphocyte	−0.155*	0.019	BMI	0.471**	<0.001
NLR	0.825**	<0.001	HOMA-IR	0.385**	<0.001
TAS	−0.179**	0.006	HOMA-β	0.311**	<0.001
OSI	0.151*	0.022	Insulin	0.383**	<0.001
Lymphocyte correlations with			Glucose	0.231**	<0.001
Insulin	−0.136*	0.039	WBC	0.312**	<0.001
WBC	0.399**	<0.001	Neutrophil	0.389**	<0.001
Neutrophil	−0.155*	0.019	NLR	0.323**	<0.001
NLR	−0.533**	<0.001	TAS	−0.142*	0.032
TOS	−0.157*	0.018	Total thiol	−0.157*	0.017
OSI	−0.170	0.01			

WBC: White blood cell, NC: neutrophil count, LC: lymphocyte count, NLR: neutrophil/lymphocyte ratio, CRP: C-reactive protein, TAS: Total antioxidant status, TOS: Total oxidant status, OSI: Oxidative stress index.
BMI per: body mass index percentile, HOMA-IR: Homeostatic model of assistance of insulin resistance, HOMA-β: Homeostatic model assessment of β-cell function. Statistical analysis by Pearson's correlation. $P < 0.05$.
*Correlation is significant at the 0.05 level (2 tailed).
**Correlation is significant at the 0.01 level (2 tailed).

in plasma and α-tocopherol levels were reduced in obese children with metabolic syndrome. Interestingly, Brown *et al.*[35] showed that there were no significant differences in TAS and a reduction in glutathione among normal-weight, overweight, and obese adults. These many diverse results can be explained by the age properties of study populations. We suggest that the long duration of oxidative stress in adults resulted in elevation of antioxidants for compensation. In studies with adolescents and

Table 5 The results of Pearson's correlation between oxidant/antioxidant status and other anthropometric and metabolic parameters

	r	*P*		*r*	*P*
TAS correlations with			TOS correlations with		
HOMA-IR	−0.210**	0.001	Lymphocyte	−0.157**	0.018
HOMA-β	−0.169**	0.010	NLR	0.140*	0.034
Insulin	−0.215**	0.001	OSI	0.917**	<0.001
			OSI correlations with		
CRP	−0.142*	0.032	HOMA-IR	0.157*	0.017
WBC	−0.145*	0.028	Insulin	0.154*	0.020
Neutrophil	−0.179**	0.006	Neutrophil	0.151*	0.022
NLR	−0.158*	0.016	Lymphocyte	−0.170*	0.010
Total thiol	0.163*	0.014	NLR	0.189*	0.004
OSI	−0.352**	<0.001	TAS	−0.352*	<0.001
Total thiol correlations with			TOS	0.917**	<0.001
CRP	−0.157*	0.017	Total thiol	−0.136*	0.040
TAS	0.163*	0.014			
OSI	−0.136*	0.040			

WBC: White blood cell, NC: neutrophil count, LC: lymphocyte count, NLR: neutrophil/lymphocyte ratio, CRP: C-reactive protein, TAS: Total antioxidant status, TOS: Total oxidant status, OSI: Oxidative stress index.
BMI per: body mass index percentile, HOMA-IR: Homeostatic model of assistance of insulin resistance. HOMA-β: Homeostatic model assessment of β-cell function. Statistical analysis by Pearson's correlation. $P < 0.05$
*Correlation is significant at the 0.05 level (2 tailed).
**Correlation is significant at the 0.01 level (2 tailed).

prepubescent studies the duration of obesity may be not enough for the increase of antioxidants.

An inadequacy of antioxidant defenses probably depends on different factors: obese individuals may have a lower intake of antioxidant- and phytochemical-rich food, such as fruits and vegetables.[36] Recent studies suggest that despite excessive dietary consumption, obese individuals have high rates of micronutrient deficiencies. In these studies it was demonstrated that deficiency in minerals and vitamins can also contribute to impairment of antioxidant defenses.[37] Also low levels of carotenoids, vitamin C and E, despite increased BMI, have been demonstrated in obese children. Furthermore, low concentrations of zinc, vitamins A and E in children who were overweight and obese were associated with lipids, inflammation and insulin resistance.[38] Otherwise, the consumption of antioxidants is normal, but obese individuals may have an increased utilization of these molecules, and inadequate physical activity may account for a decreased antioxidant state.[39]

In the recent studies, the role of specific nutrients has been investigated, and suggested that obese children should be urged to consume products containing greater levels of antioxidants. Codoñer-Franch *et al.* demonstrated that obese children on a hypocaloric diet supplemented with 100% mandarin juice, a product naturally rich in antioxidant such as vitamin C, carotenoids, and flavonoids, displayed a decrease in parameters of oxidative stress and an increase in antioxidant defense.[40] Murer *et al.*,[41] conducted to examine the effect of antioxidant supplementation on biomarkers of oxidative stress and inflammation in obese adolescents, and they found that combined antioxidant supplementation for 4 months significantly improve antioxidant status. Another study showed that fruit and vegetable intake was associated with lower concentrations of systemic oxidative stress and inflammation in early adolescents.[42]

Our study showed that TAS and the total thiol level was reduced in prepubescent obese children, suggesting that the balance between the oxidant and antioxidant system is disrupted due to a reduced TAS. Thiols are organic compounds that contain a sulphydryl group. Thiols constitute the greatest portion of total antioxidants and play a significant role in defense against ROS.[43] In addition, they play a significant role in detoxification, signal transduction, apoptosis, and various other functions at the molecular level. Decreased levels of thiols have been noted in various medical disorders including chronic renal failure and cardiovascular disorders.[44] In a similar study, Yokota *et al.*[45] demonstrated that total thiol levels in serum were decreased in patients with metabolic syndrome compared to healthy subjects.

Obesity is considered a state of chronic low-grade inflammation, and several studies in obese adults and older children have linked inflammation and oxidative stress.[46] Inflammation of adipose tissue causes the major events of the immune response, such as the early participation of neutrophils, the following procurement of diverse lymphocyte types, and the final procurement of both macrophage and mast cell polarization.[47] The adipose tissue macrophages plays a key role in systemic IR, glucose tolerance, and the development of metabolic syndromes and type 2 diabetes.[48] Wu *et al.*[49] showed that plasma CRP levels are positively related to anthropometric variables, such as body weight and BMI, insulin levels, and the IR index, and negatively related to high-density lipoprotein cholesterol (HDL-C) in Taiwanese children. The study including the youngest population about obesity and inflammation was done by Skinner *et al.*[50] They demonstrated that an increased risk of an abnormal neutrophil count and the CRP level are positively associated with higher weight in children, and that this relationship begins as early as the age of 3. Our study showed similarities to the recent literature, in that the obese group had a higher CRP, neutrophil count, and NLR. In addition, we found that plasma CRP levels, neutrophil counts, and NLR were positively correlated with BMI, insulin and glucose levels, HOMA-IR, and HOMA-β, and that these parameters were positively correlated with each other. Our results suggest that healthy young subjects with more body fat or a higher BMI have higher concentrations of inflammatory markers than their leaner peers, supporting the idea that obesity should be considered a state of chronic low-grade inflammation. Also Ryder *et al.*[51] demonstrated a positive correlation between leukocytes and the lymphocyte count and BMI and HOMA-IR in non-diabetic, obese adults without clinical and biochemical changes. Their results suggest that obese individuals without a metabolic syndrome could represent an initial stage of inflammation during obesity. Even in the absence of IR, inflammatory markers were increased. Ferrari *et al.*[52] demonstrated that the adiposity of European adolescents was sufficient to cause chronic inflammation, and the mean CRP values were significantly higher in overweight/obese adolescents than in thin/normal-weight adolescents. The causes of obesity-induced chronic inflammation have focused on monocyte/macrophage infiltration and activation in the adipose tissue.[53] Previous studies in adults have shown that CRP has now emerged as one of the most powerful predictors of inflammatory and lipid markers for the evidence of cardiovascular events in adults.[54] There are currently no guidelines associating the CRP level and cardiovascular risk in children, but adults with a CRP >3 mg/l are considered to have a 1.5- to 2-fold increased risk of cardiovascular disease.[55] In our study, obese children had markedly

elevated mean CRP values (4.11 ± 3.10 mg/l) compared to underweight/normal-weight children. These results suggest that the obese children in our study population had a high risk of future cardiovascular disease according to the adult cut-off point. However, there were no significant differences in the CRP level between underweight and normal-weight children ($P = 0.933$).

Childhood obesity as a consequence of high fat deposition has been demonstrated to be related to high levels of oxidative stress and redox imbalance. Warolin *et al.*[56] showed that oxidative stress was positively associated with the percentage of body fat and truncal fat in youth (8–17-years-old). In literature there are a few studies performed in obese children related to biomarkers of inflammation and oxidative stress. Oliver *et al.*[57] demonstrated that obese children had elevated biomarkers of inflammation (elevated total WBC counts and neutrophils) and oxidative stress, independent of gender ($n = 113$, 12.9 ± 0.3 years). Similarly in another study, Giannini *et al.*[58] demonstrated that the oxidative status were higher in pre-pubertal lean and obese subjects compared with controls whereas no differences were documented between lean and obese children. Although the general consensus is that obesity increases oxidative stress even during childhood, we did not find significant differences among the different weight groups. This could be explained by the fact that our study population consisted of prepubescent children with a mean age of 7.0–7.4 years.

Our work had some limitations. The study sample size was small for the proper investigation of the subgroups of obesity such as IR, and also the relationship between variables, thus providing a lack of statistical significance in terms of the TOS and OSI values. A prospective study with a large sample size may provide more reliable data in the future.

In conclusion, we found that obese children have higher inflammatory markers and lower TAS values than underweight and normal-weight children even in prepubescence, which indicates a risk of future cardiovascular events. But we could not demonstrate an increased burden of inflammation or decreased antioxidant status in underweight children. Further studies to determine the cause-and-effect relationship between inflammation and oxidation in children are warranted.

Disclaimer statements

Contributors All authors contributed equally.

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