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Oxidative Stress in Obesity and Metabolic Syndrome in Children and Adolescents

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Key Words
Oxidative stress · Obesity · Metabolic syndrome

Abstract
Background/Aims: The aim of this study was to investigate the alterations in the oxidant/antioxidant status in obese children with and without metabolic syndrome (MetS). ***Methods:*** We recruited 25 Caucasian obese children with MetS, 30 Caucasian children with simple obesity and a control group of 30 Caucasian children. We performed diacron-reactive oxygen metabolites (d-ROMs) test and biological antioxidant potential (BAP) test in order to evaluate the oxidant-antioxidant status in recruited patients. ***Results:*** d-ROM level was significantly higher in obese children with and without MetS ($p = 0.005$). The total antioxidant capacity (BAP level) was reduced in MetS and noMetS children compared to controls ($p = 0.009$). The subjects without MetS had higher d-ROMs test and lower BAP/d-ROMs ratio than subjects with MetS (although not significant). The ratio BAP/d-ROMs was higher in controls than noMetS and MetS children ($p < 0.0001$). d-ROM level was higher in prepubertal subjects with MetS than pubertal ones ($p = 0.03$). A direct correlation was found between d-ROM levels and BMI SDS ($p = 0.0005$), while an inverse correlation was found between BAP and BMI SDS ($p = 0.004$) and BAP/d-ROMs and BMI SDS ($p = 0.0001$). ***Conclusions:*** This result confirms that fat accumulation plays a key role in the pathogenesis of systemic oxidative stress already during pediatric age.

Introduction

Oxidative stress, which occurs as a consequence of an imbalance between the formation of reactive oxygen species (ROS) and their inactivation by antioxidant defense system [1], plays an important role in the pathogenesis of inflammation, endothelial dysfunction and atherosclerotic vascular disease [2–4]. Adipose tissue is the major source of ROS production, and fat accumulation is closely related to increased oxidative stress via NADPH oxidase activation [5, 6]. The increase in ROS in accumulated fat leads to the deregulated production of adipocytokines, as well as tumor necrosis factor-alpha, interleukin-6, plasminogen activator inhibitor, adiponectin, and to an increase of systemic oxidative stress with the involvement of other organs including the liver, skeletal muscle and the aorta [6, 7]. The increased ROS secretion into peripheral blood is also involved in the induction of

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insulin resistance (IR), through the impairment of both pancreatic β-cell insulin secretion and glucose transport in skeletal muscle and adipose tissue [6, 8]. More recently, it has been demonstrated that free fatty acids, which are elevated in obese subjects, serve as signaling molecules activating protein kinases such as protein kinase C, jun kinase and the inhibitor of nuclear factor-kb kinase-β. These kinases can then impair insulin signaling by increasing the inhibitory serine phosphorylation of insulin receptor substrates, the key mediators of insulin receptor signaling [9].

The decreased insulin sensitivity represents one of the main pathogenic mechanism underlying metabolic syndrome (MetS), a multifactorial condition characterized by several risk factors for atherosclerosis, including hyperglycemia, dyslipidemia, hypertension and abdominal obesity [10, 11]. Oxidative stress has been demonstrated to be an early instigator of the obesity-associated development of MetS in obese mice [6]. Moreover, MetS has also been independently associated with elevated systemic oxidative stress because of the impairment of the antioxidant activity of small-, high-density lipoprotein (HDL) subfractions, closely related to the presence of hypertriglyceridemia, hyperinsulinemia, and IR in subjects with MetS phenotype [12]. A synergistic effect of obesity and MetS on biomarkers of oxidative stress and inflammation has been demonstrated in adult subjects [13]. Moreover, the risk of cardiovascular heart disease (CHD) is markedly greater in obese middle-aged men with MetS than in those without MetS [14]. However, little is known about the relationship between oxidative stress and MetS phenotype in obese children.

The aim of this study was to investigate the alterations in the oxidant/antioxidant status in prepubertal and pubertal obese children with and without MetS, and to evaluate the influence of obesity and MetS phenotype on biomarkers of oxidative stress during pediatric age.

Materials, Subjects and Methods

Subjects

We recruited 55 consecutive Caucasian obese children who had been referred to the Department of Pediatrics of the University of Bari, Italy, between March 2009 and January 2010. All the subjects were affected by severe obesity (BMI >2 SD for the mean for age and sex). Twenty-five of them had MetS (12 males; 11 prepubertal; mean age 11.9 ± 3.2 years), and the remaining 30 had simple obesity (noMetS; 14 males; 15 prepubertal; mean age 10.9 ± 3.2 years). Exclusion criteria were endocrinological disorders or genetic syndromes associated with obesity, acute infection or presence of diseases that could have an influence on oxidative stress (i.e. asthma). Also excluded from the study were subjects who were under some form of medication (i.e. antioxidant vitamins such as ascorbate, tocopherols, alphacarotene, or polyphenol-containing nutraceuticals) during or a week before the blood samples were taken.

All recruited children had the same consumption of fruits, fruit or vegetable juices, tea, coffee or other dietary antioxidants as they had received dietary and behavioral recommendations and they had monitoring visits every 3 months.

The children with MetS were diagnosed according to the up-to-date definitions by Cook et al. [15], in the presence of three of the following criteria: elevated triglycerides, low HDL cholesterol, elevated blood pressure, abdominal obesity measured as waist circumference, and hyperglycemia. Twenty out of the 25 MetS subjects (80%) had abdominal adiposity, 10 out of 25 (40%) hypertension, 5 out of 25 (20%) HDL cholesterol <5th percentile, 5 out of 25 (20%) hyperglycemia (fasting glucose ≥110 mg/ dl), and 9 out of 25 (36%) elevated triglyceride levels.

The children with noMetS had normal lipid profiles, aminotransferases, fasting glucose levels and insulinemia.

As control group, 30 Caucasian normal-weight children (15 males; 16 prepubertal; mean age 10.4 ± 3.9 years; BMI <1.7 SD), who had been admitted to our Department of Pediatrics for minor diseases, were recruited. Exclusion criteria were hypertension, dyslipidemia and acute infections.

Informed consent was obtained from the parents and the children. All the procedures used were in accordance with the guidelines of the Helsinki Declaration on Human Experimentation.

Anthropometric Measurements

All the children underwent a medical visit to assess general health status, including anthropometric parameters. Standing height (H) was measured by a wall-mounted Harpenden Stadiometer and weight (W) with patient in underwear was measured by an electronic scale with digital readings accurate to 0.1 kg. Waist circumference was measured midway between the lower rib margin and the iliac crest in the standing position and at the end of a gentle expiration. BMI was calculated by dividing W in kilograms by the square of H in meters. Obesity was defined in presence of BMI greater than 95th percentile for age and sex, in accordance with Italian growth charts [16]. In addition, BMI SDS was derived from population standards [16]. Pubertal development was assessed according to the criteria of Tanner [17]. Blood pressure was measured three times while the subjects were seated by using a standard Riva-Rocci sphygmomanometer with an appropriate size cuff and a stethoscope, and the two last measurements were averaged for the analysis according to the American Heart Association guidelines [18]. Hypertension was defined as mean systolic blood pressure (SBP) and mean diastolic blood pressure (DBP) higher than the 95th percentile for age after adjustment for height [18].

Laboratory Procedures

Biochemical Analysis

After an overnight fast, blood samples were taken from the study population and the controls to evaluate lipid profile, fasting insulin and glucose. Total cholesterol, HDL cholesterol, low-density lipoprotein (LDL) cholesterol, triglycerides and glucose concentrations were measured by using an automated analyzer (Roche Diagnostics, Mannheim, Germany). Serum insulin was

Table 1. Baseline clinical characteristics and anthropometric measurements of the study population

	MetS (n = 25)	noMetS (n = 30)	Controls (n = 30)	p value
Age, years	11.9 ± 3.2	10.9 ± 3.2	10.4 ± 3.9	NS
Males/females	12/13	14/16	17/13	NS
Pubertal stage	14 pubertal (6/8)	15 pubertal (6/9)	14 pubertal (8/6)	NS
Tanner I/II/III/IV	0/0/12/2	0/3/9/3	0/4/5/5	
Weight SDS	2.02 ± 0.7*	1.98 ± 0.65*	–0.10 ± 0.8	<0.0001
Height SDS	0.43 ± 1.14	0.27 ± 1.26	–0.09 ± 1.09	NS
BMI SDS	2.14 ± 0.46*	2.3 ± 0.35*	0.37 ± 0.46	<0.0001
Waist circumference, cm	96 ± 13.0*	92 ± 11*	71 ± 5.3	<0.0001
SBP SDS	1.16 ± 1.2*, †	0.23 ± 0.8*	–0.04 ± 0.94	<0.0001
DBP SDS	0.27 ± 0.94	–0.08 ± 0.83	0.07 ± 0.81	NS

Figures in parentheses indicate number of males/females. * $p < 0.05$ vs. controls; † $p < 0.05$ vs. noMetS subjects.

measured by a chemiluminescent assay (DPC, Los Angeles, USA). In order to ascertain IR status, the homeostasis model assessment of IR (HOMA-IR) was calculated according to the following formula: [fasting insulin (μU/ml) × fasting glucose (mmol/l)]/22.5 [19]. As marker of inflammation, C-reactive protein (CRP) levels were measured by an ultrasensitive immunoturbidimetric assay (CRP Latex HS, Roche Diagnostics).

Oxidant-Antioxidant Status

Systemic oxidative stress was studied by measuring serum hydroperoxide concentration by means of a commercially available spectrophotometric method (diacron-reactive oxygen metabolites, d-ROMs, test, Diacron International S.a.S., Grosseto, Italy) [20]. Hydroperoxides are the products of dehydrogenation and peroxidation of several cellular components, including proteins, peptides, amino acids, lipids and fatty acids. When a biological sample is dissolved in an acidic buffer, the hydroperoxides react with the transition metal irons liberated from the proteins in acidic medium, and are converted to alkoxyl (R-O•) and peroxyl (R-OO•) radicals, according to Fenton's reaction. Such radicals, in turn, are able to oxidize an alkyl-substituted aromatic amine (A-NH2, which is solubilized in a chromogenic mixture) and thus to transform it in a pink-colored derivative. Finally, this colored derivative is spectrophotometrically quantified (absorption at 505 nm). The intensity of developed colour is directly proportional to the concentration of ROMs, according to Lambert-Beer's law. The serum levels of the d-ROMs range from 250 to 300 Carratelli Units (U.CARR), where 1 U.CARR corresponds to 0.8 mg/l H_2O_2/dl. Values higher than 300 U.CARR indicate increasing levels of oxidative stress [21].

The biological antioxidant potential (BAP test) was measured by a spectrophotometric method (Diacron International), which determines the ability of plasma barrier components (e.g. proteins, bilirubin, uric acid, cholesterol, etc.) to give 'reducing equivalents' (i.e. electrons or hydrogen atoms) to reactive species, which thus would avoid dangerous radical chains [22]. Therefore, in the BAP test, plasma sample is dissolved in a colored solution that has been previously obtained by mixing a source of ferric ions ($FeCl_3$, ferric chloride) with a chromogenic substrate. By spectophotometrically assessing the intensity of discoloration, the amount of reduced ferric ions can be calculated and the antioxidant power of blood plasma tested can be measured. The reference values of BAP, which are expressed in terms of iron-reducing activity by using C vitamin as antioxidant, are >2,200 μmol/l for an optimum status, <1,600 μmol/l for a deficient status and <1,400 μmol/l for a high deficiency status, as determined by BAP method [22].

In order to highlight the differences between antioxidant and pro-oxidant potential, for the first time we have studied the ratio between BAP and d-ROMs.

Statistical Analysis

Data are presented as mean ± standard deviation (SD). The χ^2 test or Fisher's exact test was used, as appropriate, to compare percentages and nominal variables. For comparison of continuous variables, we used the Student's t test. The Kruskal-Wallis test was used for inter-group comparisons. Sperman's rho correlation test was used to investigate the relationship between continuous variables. A probability (p) value <0.05 was considered significant. Statistical analyses were performed by using SPSS 13.

Results

Baseline Characteristics

Baseline clinical characteristics and anthropometric measurements of all the subjects are reported in table 1. The three groups (Mets, noMetS and controls) were homogeneous for gender, age and pubertal stage. SBP measurement was significantly different between the three groups ($p < 0.0001$), and increased SBP values were found in the MetS subjects as compared to the noMetS ones ($p < 0.0001$), while DBP was not different in the population of the obese children as compared to the controls.

Table 2. Biochemical characteristics of the study population

	MetS (n = 25)	noMetS (n = 30)	Controls (n = 30)	p value
Fasting glucose, mg/dl	90.8 ± 10.4*, †	84.6 ± 9.2*	81.1 ± 8.1	0.0008
Fasting insulin, μU/ml	23.2 ± 8.6*, †	14 ± 11.1*	9.7 ± 4.5	<0.0001
HOMA-IR	4.2 ± 2.2*, †	1.6 ± 0.80*	1.3 ± 0.33	<0.0001
Total cholesterol, mg/dl	158 ± 23.7	150 ± 23.1	144 ± 18.3	NS
LDL cholesterol, mg/dl	89.3 ± 17.9	89.3 ± 30.3	78.1 ± 21	NS
HDL cholesterol, mg/dl	45.5 ± 12.2*	51.9 ± 9.2*	58.6 ± 9.3	0.0002
Triglycerides, mg/dl	112.9 ± 61*	79.2 ± 58.6*	58 ± 17.8	0.0001
CRP, mg/l	3.8 ± 4.1*, †	1.9 ± 2.1*	0.7 ± 0.3	0.0002

* $p < 0.05$ vs. controls; † $p < 0.05$ vs. noMetS subjects.

Table 3. Oxidant/antioxidant status of the study population

	MetS (n = 25)	noMetS (n = 30)	Controls (n = 30)	p value
d-ROMs test, U.CARR	416.4 ± 88.7*	446.1 ± 98.9*	357 ± 78.5	0.005
BAP test, μmol/l	2,067 ± 530*	2,168 ± 473.5*	2,438 ± 466.7	0.009
BAP/d-ROMs ratio	5.14 ± 1.5*	4.98 ± 1.2*	6.85 ± 1.6	<0.0001

* $p < 0.05$ vs. controls.

Laboratory Investigation

The biochemical characteristics of the study population are reported in table 2.

As expected, all the biochemical parameters were significantly higher in the obese as compared to the controls, except for total cholesterol and LDL cholesterol.

The children with MetS have significantly higher levels of fasting glucose ($p = 0.03$), insulin ($p = 0.009$), HOMA-IR ($p = 0.004$), triglycerides ($p = 0.009$) and CRP ($p = 0.03$) than the noMetS ones.

Oxidant-Antioxidant Status

The oxidant-antioxidant status of the study population is reported in table 3.

d-ROM level was significantly higher in the MetS and noMetS obese children (416.4 ± 88.7 and 446.1 ± 98.9 U.CARR, respectively) as compared to the controls (357.8 ± 78.5 U.CARR; $p = 0.005$). The total antioxidant capacity (BAP level) was reduced in the obese children with and without MetS (2,067 ± 530 and 2,168 ± 473 μmol/l, respectively) compared to the controls (2,438 ± 466.7 μmol/l; $p = 0.009$). No significant differences were found in d-ROM and BAP levels between the MetS and noMetS subjects. In addition, as reported in table 3, subjects without MetS had higher d-ROMs test and lower BAP/d-ROMs ratio than subjects with MetS (although not significant). The BAP/d-ROMs ratio was significantly higher in the controls as compared to the noMetS and MetS children (6.85 ± 1.67 vs. 4.98 ± 1.20 and vs. 5.14 ± 1.56; $p < 0.0001$).

Oxidant-Antioxidant Status in Pre-Pubertal and Pubertal Subjects

d-ROM level was higher in the prepubertal subjects with MetS as compared to the pubertal ones ($p = 0.03$; fig. 1). No differences were found in relation to pubertal stage in the noMetS children. Moreover, no differences were found in relation to the gender in the study population.

Correlation between Oxidant-Antioxidant Status and Clinical and Biochemical Parameters

A direct correlation was found between d-ROM level and BMI SDS ($p = 0.0005$; $r^2 = 0.14$), while an inverse correlation was found between BAP and BMI SDS ($p = 0.004$; $r^2 = 0.04$) and BAP/d-ROMs and BMI SDS ($p = 0.0001$;

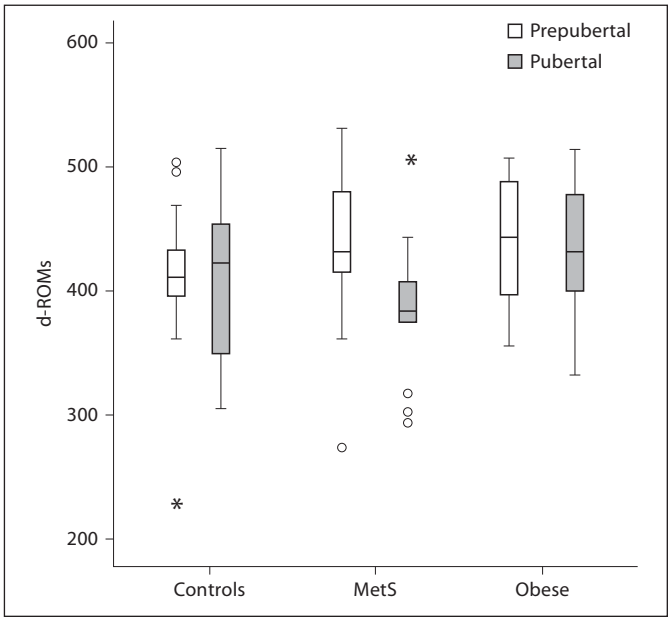

Fig. 1. d-ROM level in prepubertal and pubertal MetS subjects.

$r^2 = 0.2$; fig. 2). Moreover, we found an inverse correlation between BAP/d-ROMs ratio and waist circumference ($r^2 = 0.1$), total cholesterol ($r^2 = 0.06$), triglycerides ($r^2 = 0.04$) and CRP ($r^2 = 0.03$).

Discussion

In our study, a significant impaired oxidant/antioxidant status has been documented in obese children with and without MetS. This result confirms that fat accumulation plays a key role in the pathogenesis of systemic oxidative stress, already during pediatric age. In addition, our data have shown that oxidative stress correlates with BMI SDS; therefore, a greater accumulation of fat may cause a greater impairment of oxidant/antioxidant status. These findings suggest that obesity can act as a strong risk factor for subsequent pathologic conditions.

Oxidative stress has been associated with obesity, hyperglycemia, hypertension and atherosclerosis [23–26]. Previous studies demonstrated that the earliest signs of CHD, such as coronary artery fatty streaks, are already present in childhood and rapidly increase during adolescence, particularly in those with elevated BMI [27, 28]. MetS is a multifactorial condition leading to accelerated atherosclerosis and increased risk of diabetes [29]. Importantly, the risk for CHD is markedly greater in adult obese with MetS than

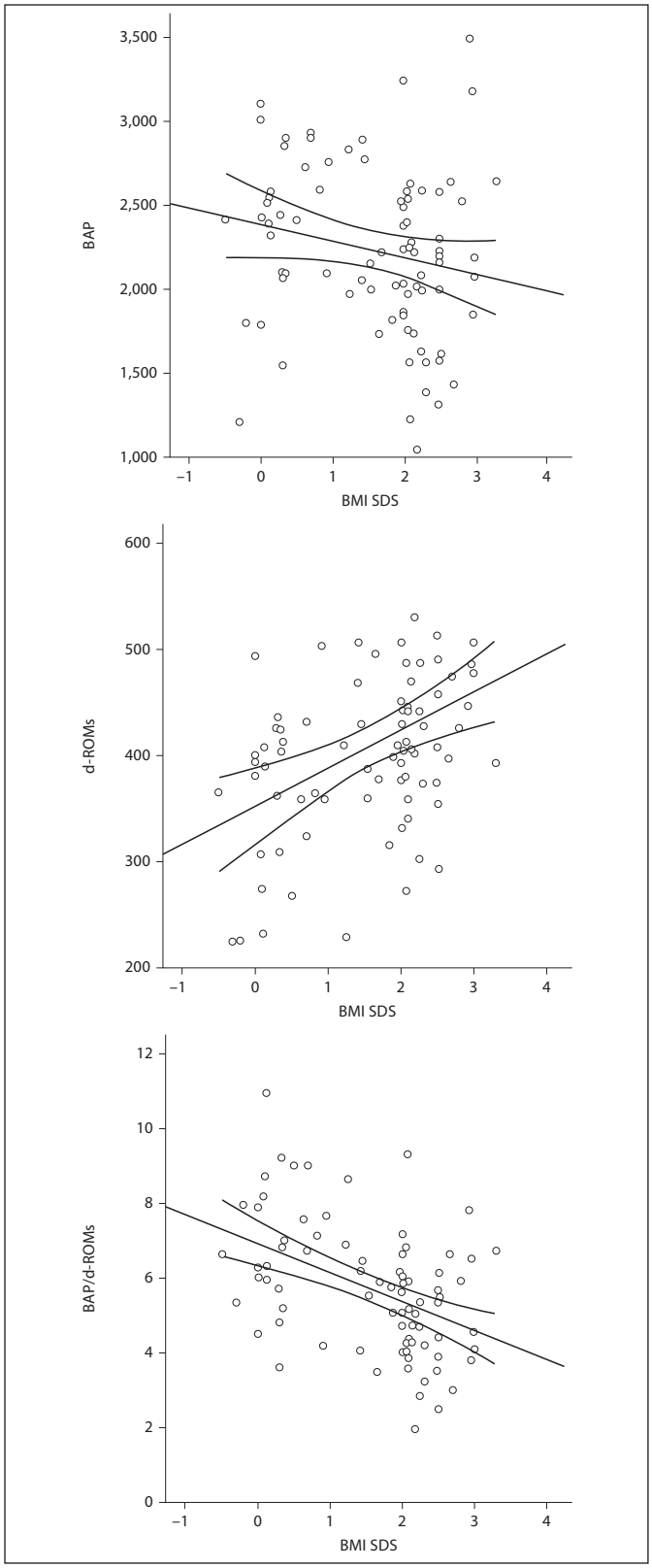

Fig. 2. Correlation between BAP and d-ROM levels and BAP/d-ROMs ratio and BMI SDS in all (obese and nonobese) children.

those without it [14]. Although the main pathogenic mechanism relies on IR, several lines of evidence demonstrated a close link between MetS, a state of chronic low-level inflammation, and oxidative stress [30, 31]. In particular, the adult obese with MetS show a decreased antioxidant protection and increased lipid peroxidation, and the enhanced oxidative stress in these patients seems to be associated with visceral adiposity and liver steatosis [32]. It has been recently demonstrated that excessive visceral fat accumulation and decreased adiponectin in obese children might increase oxidative stress, and this event is associated with the development of metabolic complications [33].

In our study, no significant differences were found in d-ROM and BAP levels between the obese children with MetS and those without MetS; in addition, as reported in table 3, subjects without MetS had higher d-ROMs test and lower BAP/d-ROMs ratio than subjects with MetS (although not significant). Furthermore, for the first time we calculated the BAP/d-ROMs ratio, and we found that it was higher in the controls than in the obese with and without MetS, which confirmed that severe childhood obesity is associated with the alterations of antioxidant biological barrier components. Moreover, we found an inverse correlation between BAP/d-ROMs ratio and waist circumference, which suggests the important role of visceral adiposity in the alteration of antioxidant status.

Our data show that an accelerated formation of ROS and a reduction of the antioxidant protection occur earlier to the emergence of other comorbidities, because our simply obese patients did not present with dyslipidemia, hyperinsulinemia or hypertension. These results are in agreement with previous data and demonstrate that obesity is an independent risk factor for plasma lipid peroxidation in children [34–36], but they are in contrast to other studies, which, however, evaluated only the alterations of antioxidant status [37]. In our study, we evaluated oxidant/antioxidant status by using two tests which have been reported as clinically significant markers in various diseases [38, 39]. In addition, our data clearly demonstrate that d-ROM level was higher in the pre-pubertal obese subjects with MetS than the pubertal ones. The finding of higher d-ROM levels in prepubertal obese subjects with MetS than pubertal ones despite the physiological IR of puberty is very interesting and confirms previous data that demonstrated an enhanced oxidative stress combined with reduced antioxidant capacity in obese prepubertal and adolescent girls with full or partial MetS, suggesting that the presence of metabolic alteration could worsen the imbalance between oxidative and antioxidative systems already during prepubertal age [40].

In our study population, we found an inverse correlation between BAP/d-ROMs ratio and total cholesterol, triglycerides and CRP, which suggests that the alterations of lipid profile and the inflammation, which are associated with the MetS phenotype, could represent other factors influencing the oxidative stress in children. A strong correlation between oxidative stress and IR has been reported in the adults [41]. Although this association was also demonstrated in obese children, it seems to depend on the degree of severity of IR, so that with the increase in severity of IR the production of ROS and consequently the development of comorbidities would increase [42]. In this study, the obese subjects without MetS were not insulin resistant, and only MetS children with higher values of HOMA-IR presented increased levels of d-ROMs; this would explain the lack of correlation between oxidative stress and IR in our population. Instead, other factors such as visceral adiposity seem to have a significant role in the alteration of antioxidant/oxidant status in pediatric age.

In conclusion, our findings clearly demonstrate the role of the obesity in the alteration of oxidant/antioxidant status in children, and underline the importance of careful follow-up of obese children already during prepubertal age to detect the development of metabolic abnormalities which can lead to MetS. Simple interventions, such as dietary restriction and weight loss, should be highly encouraged and maintained over time to reduce the increased risk of cardiovascular disease.

Disclosure Statement

The authors have nothing to disclose.

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