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Evaluation of oxidative stress and inflammation in obese adults with metabolic syndrome

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Abstract

Background: Obesity and metabolic syndrome increase the risk of cardiovascular morbidity and mortality. Oxidative stress seems to be involved in the pathophysiology of diabetes and cardiovascular complications of metabolic syndrome. The aim of our study was to evaluate the level of oxidative stress and inflammation in obese adults with and without metabolic syndrome.
Methods: Oxidative stress and inflammation markers (total amount of free radicals, malondialdehyde, allantoin, α_1-antiproteinase, oxidized/reduced glutathione ratio, high-sensitive C-reactive protein, fibrinogen), total antioxidant capacity and lipid standardized α-tocopherol were determined in obese subjects fulfilling at least three criteria of metabolic syndrome according to the National Cholesterol Education Program-Adult Treatment Panel III guidelines (n=20 patients), in obese subjects without metabolic syndrome (n=20 patients) and in 48 healthy controls.
Results: Oxidative stress and inflammation markers were significantly elevated in the obese subjects, especially in those exhibiting metabolic syndrome. According to multidimensional statistical analysis, oxidative stress was independently related to triacylglyceride concentration, abdominal fat, low high-density lipoprotein cholesterol and low lipid standardized α-tocopherol in the patients with metabolic syndrome.
Conclusions: High levels of free radicals together with low antioxidant capacity detected in obese adults indicate elevated oxidative stress, which is – together with systemic inflammation – further potentiated in the case of obese patients with metabolic syndrome. This imbalance in oxidative/antioxidative status and subclinical inflammatory state leads to higher risk of atherosclerotic and diabetic complications.
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Keywords: inflammation; metabolic syndrome; oxidative stress.

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Introduction

Obesity, which nowadays represents a disease with rising prevalence, is the main factor leading to metabolic syndrome (MetS), which is characterized by a constellation of insulin resistance and cardiovascular risk factors, including atherogenic dyslipidemia, abnormal glucose tolerance, hypertension and visceral obesity. In addition, various other abnormalities of inflammation, hemostasis and fibrinolysis are often considered a part of this syndrome (1). Accumulation of these symptoms was reported to unequivocally increase the risk of cardiovascular morbidity and mortality (2–5). In particular, MetS leads to a higher risk of coronary events, myocardial infarction, heart failure (6), stroke and diabetes (7). MetS can also result in other pathologic states, such as non-alcoholic fatty liver steatosis and elevated occurrence of various types of carcinomas, e.g., colon cancer. The underlying mechanisms of the pathophysiology of MetS complications are common inflammatory stress conditions and an increase in the formation of free radicals (reactive oxygen and nitrogen species; RONS). It is the oxidative stress which may play a considerable role in the pathological development of diabetes and cardiovascular complications.

The aim of our study was to assess the impact of oxidative stress in obese subjects with and without MetS. In addition to the parameters generally used for assessing oxidative stress [malondialdehyde, ratio of oxidized and reduced glutathione (GSSG/GSH), total antioxidant status, α-tocopherol, allantoin, ceruloplasmin), we decided to determine the level of other parameters: $\alpha_1$antiproteinase and free radicals. α_1-Antiproteinase, also known as α_1-antitrypsin or α_1-proteinase inhibitor, is a predominant component of α_1 fraction of human plasma. It is a member of the serine proteinase inhibitor superfamily responsible for the inhibition of trypsin-, chymotrypsin-, or elas-

tase-proteolytic capacity. RONS can inactivate α_1-antiproteinase by oxidizing essential methionine of the active site loop to methionine sulfoxide. As α_1-antiproteinase is very sensitive to oxidants, it may be a good biomarker to evaluate the oxidative damage in biological systems. Free radical concentration was determined by a novel method based on the determination of electron acceptance by chlorophyllin, which was developed recently by Votruba et al. (8). This method is suitable for measurement of the total amount of all types of free radicals, both reactive oxygen and reactive nitrogen species (9). The method was validated by comparison with the basic method of electron paramagnetic resonance (8) with excellent correlation ($r=0.997$).

Subjects and methods

Subjects

This study included 40 obese adults characterized by waist circumference, body mass index (BMI) and age. The basic group (BG) of obese subjects was divided into two subgroups. The first subgroup comprised patients fulfilling at least three criteria of MetS (A, $n=20$), the second subgroup was without expressed MetS symptoms (B, $n=20$). These subgroups did not differ significantly in waist circumference, BMI and age. The control group consisted of 48 healthy normal-weight blood donors (C) (for the group characteristics, see Table 1).

All the obese adults were recruited from trainees of the Healthy Lifestyle Courses arranged by the Health Institute, which provided guided energy intake reduction under medical supervision.

All the subjects recruited for the study were <70 years of age and were either abstainers or moderate alcohol consumers (≤ 20 g/day). The patients who had any serious health complications were excluded. None of the studied subjects exhibited renal, hepatic, gastrointestinal, pulmonary, endocrine or oncological diseases. Written informed consent was obtained from all the participants before starting the protocol, and the study was approved by the Hospital Ethical Committee on Human Research.

The biochemical parameters related to MetS and to oxidative stress were followed in all the subjects. The occurrence of MetS was defined according to the National Cholesterol Education Program-Adult Treatment Panel III guidelines (NCEP/ATPIII) (1) including the presence of at least three criteria among the following: waist circumference >102 cm in men and >88 cm in women, hypertriacylglyceridemia ≥ 150 mg/dL (1.695 mmol/L); low levels of high-density lipoprotein cholesterol (HDL-C) <40 mg/dL (1.036 mmol/L) in men and <50 mg/dL (1.295 mmol/L) in women; high blood pressure $\geq 130/85$ mm Hg; high fasting blood glucose ≥ 110 mg/dL (6.1 mmol/L).

Methods

Blood samples Venous blood was obtained under standard conditions, from 7.00 to 8.00 am after fasting for at least 12 h. The serum and plasma samples were kept frozen at –70°C until assayed. Glutathione analysis was performed from whole blood. The samples, deproteinized with phosphoric acid, were centrifuged and the supernatant was frozen at –70°C.

Oxidative stress parameters The free radical concentration was determined by a direct spectrophotometric method based on determination of electron acceptance by chlorophyllin (Free Radicals kit, Sevapharma, Prague, Czech Republic). The total antioxidant capacity was quantified using a Total Antioxidant Status kit (Randox, Crumlin, UK). GSSG and GSH in whole blood were analyzed by means of reverse-phase HPLC (Shimadzu, Kyoto, Japan) with fluorescence detection (excitation $\lambda=350$ nm and emission $\lambda=420$ nm) (10). The concentration of α-tocopherol was measured in serum by means of reverse-phase HPLC (Shimadzu) using isocratic gradient and UV/VIS detection at 292 nm (11). Malondialdehyde serum level was monitored by means of reverse-phase HPLC (Shimadzu) with UV/VIS detection at 532 nm (12). Allantoin was determined by means of reverse-phase HPLC (Shimadzu) with UV/VIS detection at 360 nm (12); α_1-antiproteinase was measured using a Bioxytech α_1AP-410 Assay kit (OxisResearch, Foster City, CA, USA).

MetS parameters The plasma levels of glucose, total cholesterol, triacylglycerides, HDL-C, low-density lipoprotein cholesterol (LDL-C), fibrinogen, high-sensitive C-reactive protein (hsCRP), albumin, transferrin, ceruloplasmin and uric acid were determined by standard procedures using an automatic biochemistry analyzer (Dimension, Dade-Behring, Deerfield, IL, USA).

Clinical examination Blood pressure and physical data (weight, height, waist circumference) were determined during a complete clinical examination. A detailed questionnaire concerning dietary habits and lifestyle was filled out by each of the subjects.

Statistical analysis

Between-group differences in continuous variables were analyzed with the use of the Hotelling T^2-test for independent groups using the software NCSS2000 (Dr. Jerry L. Hintze, Kaysville, UT, USA). Principal component analysis and anal-

Table 1 Group characteristics.

	Obese people with MetS (A, n=20)	p-Value (A vs. B)	Obese people without MetS (B, n=20)	p-Value (B vs. C)	Control group (C, n=48)	p-Value (A vs. C)
Age, years	51.30±7.52	–	48.71±13.68	–	52.12±5.43	–
Male/female, n	11/9	–	9/11	–	26/22	–
Blood pressure systolic, mm Hg	133.00±9.80	–	123.35±16.87	–	128.75±12.50	–
Blood pressure diastolic, mm Hg	88.00±6.21	–	82.25±8.10	–	85.02±7.64	–
BMI, kg/m^2	37.03±6.23	–	33.57±4.70	***	21.86±4.15	***
Waist circumference, cm	113.53±12.48	–	101.72±13.82	***	79.45±9.30	***

Data are expressed as means±SD; statistical significance: ***$p<0.001$.

ysis of correlation matrix were carried out using the software STATISTICA (Statsoft, Tulsa, OK, USA). All the results are expressed as means with standard deviation in parentheses.

Results

Comparison of obese subjects with healthy controls

When classical biochemical parameters were compared, dyslipidemia was confirmed in the BG of obese patients (both with and without MetS) with a significant elevation of triacylglyceride concentration [BG: 1.67 (0.89) mmol/L vs. C: 1.09 (0.58) mmol/L, $p<0.01$] and a significant decrease in HDL-C in comparison with healthy controls [BG: 1.27 (0.34) mmol/L vs. C: 1.60 (0.38) mmol/L, $p<0.01$]. A significantly higher level of systemic inflammation in obese patients was assessed by plasma hsCRP levels [BG: 4.21 (2.89) mg/L vs. C: 1.72 (1.19) mg/L, $p<0.01$]. Increased coagulability in obese subjects was confirmed by higher plasma fibrinogen levels [BG: 4.01 (0.89) g/L vs. C: 3.75 (0.83) g/L, $p>0.05$). Obese subjects exhibited higher plasma concentration of uric acid [BG: 311.85 (68.99) μmol/L vs. C: 243.75 (62.09) μmol/L, $p<0.001$].

Higher oxidative stress in obese patients was confirmed by significantly elevated levels in all of the following markers: free radicals : 7.83 (5.20) mmol/L vs. C: 4.58 (0.82) mmol/L, $p<0.01$, malondialdehyde : 2.56 (0.47) μmol/L vs. C: 1.18 (0.19) μmol/L, $p<0.001$, oxidized form of α_1-antiproteinase [BG: 36.50 (12.34) μmol/L vs. C: 26.59 (9.60) μmol/L, $p<0.001$] and the GSSG/GSH ratio [BG: 13.49 (5.68) vs. C: 6.53 (2.65), $p<0.01$].

Higher levels of oxidative stress markers in obese subjects also coincided with the lower levels of HDL-C and ceruloplasmin [BG: 0.23 (0.06) g/L vs. C: 0.38 (0.11) g/L, $p>0.001$]. Low antioxidant capacity [BG: 0.81 (0.14) mmol/L vs. C: 0.81 (0.09) mmol/L, $p>0.05$] as well as low lipid standardized α-tocopherol [BG: 3.44 (0.57) μmol/L vs. C: 3.36 (0.55) μmol/L, $p>0.05$] were found in both groups.

Comparison of obese subjects suffering from MetS with those without MetS

When the obese subjects were divided on the basis of the presence of MetS, all the 20 patients exhibiting MetS according to the NCEP/ATPIII definition (subgroup A) suffered from higher blood pressure. The subjects in this subgroup had significantly higher levels of fasting blood glucose, as well as significantly lower levels of HDL-C, than the obese subjects without MetS (Table 2).

The maximum level of free radicals found in Mets patients correlated with the highest level of malondialdehyde, product of lipoperoxidation, as well as the highest GSSG/GSH ratio, an important marker of intracellular oxidative stress, or the highest level of the oxidized form of α_1-antiproteinase. The elevation of all the markers followed seems to prove the opinion that MetS is accompained by increased oxidative stress (Table 3).

When the levels of individual antioxidants were compared, the concentration of lipid standardized α-tocopherol was low in all the groups. The total antioxidant capacity was low in both groups of obese subjects, but significantly higher in patients with MetS than in those without MetS. As the presence of MetS coincided with elevated level of uric acid (which exhibits an antioxidative capacity), the monitored difference in total antioxidant status between the tested groups of obese subjects may be given by the monitored differences in uric acid levels. Furthermore, a lower level of ceruloplasmin was determined in the MetS group (Table 3).

Based on the principal component analysis, the plot of component weights (Figures 1 and 2) proves the interesting correlations between individual parameters. The same conclusions were made from an analysis of the correlation matrix. The following significant correlations were found: (a) in the MetS group, as illustrated in Figure 1, there was a positive correlation between malondialdehyde and waist circumference, as well as with BMI. There was also a positive correlation between the free radical amount and triacylglyceride concentration, a negative correlation between free radicals and HDL-C, as well as between free radicals and lipid standardized α-tocopherol. The lipid standardized α-tocopherol was inversely associated with hypertriacylglyceridemia and positively correlated with ceruloplasmin concentration. Furthermore, a positive correlation was found between hsCRP and BMI or fasting blood glucose.

Table 2 Basic biochemical parameters of the study groups.

	Obese people with MetS (A, n=20)	p-Value (A vs. B)	Obese people without MetS (B, n=20)	p-Value (B vs. C)	Control group (C, n=48)	p-Value (A vs. C)
Glucose, mmol/L	6.63±2.14	*	5.24±0.52	–	5.12±0.70	**
Total cholesterol, mmol/L	4.88±0.8	–	5.17±0.89	–	5.21±0.60	–
HDL-C, mmol/L	1.04±0.23	***	1.47±0.23	–	1.60±0.38	***
LDL-C, mmol/L	3.13±0.90	–	3.09±0.85	–	2.92±0.44	*
Triacylglycerides, mmol/L	1.67±0.89	*	1.23±0.90	–	1.09±0.58	***
Index athero (Klimov)	3.87±1.16	*	2.68±0.93	–	2.48±0.93	**
Fibrinogen, g/L	4.14±0.85	–	3.90±0.93	–	3.75±0.83	*
High-senstive CRP, mg/L	4.25±2.37	–	4.06±3.30	**	1.72±1.19	***

Data are expressed as means±SD. Statistical significance: *$p<0.05$, **$p<0.01$, ***$p<0.001$. Index athero $= \frac{\text{total cholesterol}-\text{HDL-C}}{\text{HDL-C}}$.

Table 3 Oxidative stress status parameters in the study groups.

	Obese people with MetS (A, n=20)	p-Value (A vs. B)	Obese people without MetS (B, n=20)	p-Value (B vs. C)	Control group (C, n=48)	p-Value (A vs. C)
Free radicals, mmol/L	8.68±3.70	*	6.34±1.80	*	4.71±0.77	**
Total antioxidant capacity, mmol/L	0.91±0.12	*	0.73±0.10	–	0.81±0.09	–
Malondialdehyde, μmol/L	2.67±0.43	–	2.45±0.49	***	1.18±0.19	***
α_1-Antiproteinase, μmol/L	34.96±12.65	–	39.23±11.26	***	26.59±9.60	***
GSSG/GSH, %	15.60±6.51	*	11.78±4.40	***	6.53±2.65	***
Allantoin, μmol/L	4.14±2.10	–	3.52±1.10	–	4.01±1.03	–
Vitamin E, μmol/L	23.51±4.85	–	22.38±5.66	–	23.22±4.80	–
Vitamin E/cholesterol+triacylglycerides	3.19±0.55	*	3.53±0.55	*	3.26±0.55	–
Albumin, g/L	41.47±2.50	–	40.61±3.31	–	41.15±3.11	–
Transferrin, g/L	2.43±0.30	–	2.44±0.30	*	2.32±0.58	–
Ceruloplasmin, g/L	0.20±0.03	*	0.24±0.08	***	0.38±0.11	***
Uric acid, μmol/L	354.20±62.20	***	276.56±53.88	**	243.75±62.09	***

Data are expressed as means±SD; statistical significance: *p<0.05, **p<0.01, ***p<0.001.

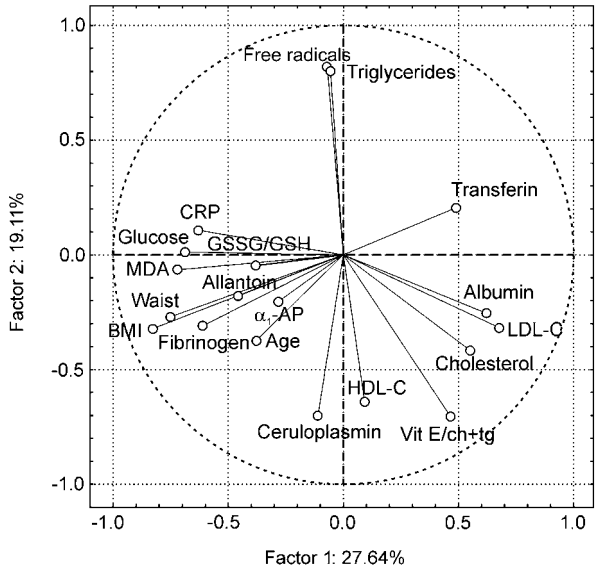

Figure 1 Component weight plot in the group of obese patients with MetS. MDA, malondialdehyde; α_1-AP, α_1-antiproteinase; Vit E/ch+tg, vitamin E/cholesterol+triglycerides.

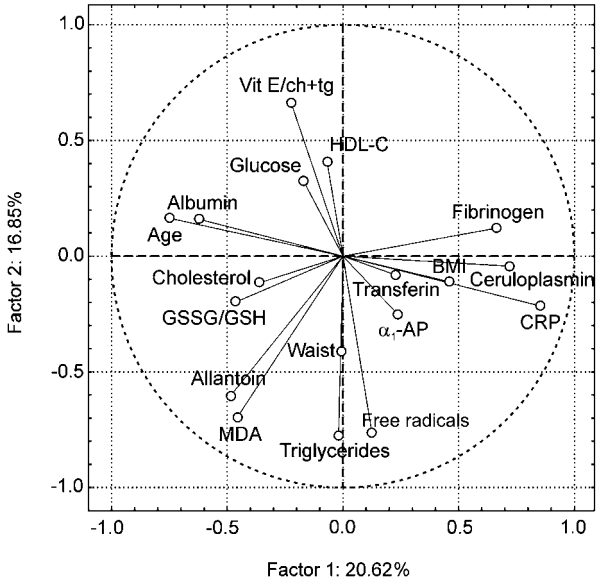

Figure 2 Component weight plot in the group of obese patients without MetS. MDA, malondialdehyde; α_1-AP, α_1-antiproteinase; Vit E/ch+tg, vitamin E/cholesterol+triglycerides.

(b) In the obese group without MetS, as illustrated in Figure 2, a positive correlation was found between waist circumference and malondialdehyde and between free radical amount and triacylglyceride concentration. A negative correlation was obtained between free radicals and lipid standardized α-tocopherol.

Discussion

The results obtained show that systemic oxidative stress is significantly elevated in obese subjects, especially in those exhibiting a MetS phenotype, which according to its definition also involves (apart from central obesity) atherogenic dyslipidemia (hypertriacylglyceridemia and subnormal HDL-C levels), hyperglycemia and hypertension. Increased oxidative stress was associated not only with hypertriacylglyceridemia and deficient HDL antioxidative capacity, but also with a chronic inflammatory state as indicated by an elevation of CRP levels (in all obese subjects, higher in MetS group). The correlation analysis revealed that the increased level of free radicals in the MetS group was also intimately related to the low levels of lipid-standardized vitamin E, thereby suggesting that abnormalities in both lipid metabolism and antioxidative substances underlie the increased oxidative stress in the MetS group. The mechanisms involved in elevation of oxidative stress and inflammatory burden may include increased production of superoxide anion via the nicotinamide adenosine diphosphate oxidase pathway (13–15) observed during hypertriacylglyceridemia, hypertension and obesity. The occurrence of small dense LDL particles, which are more susceptible to oxidation than large buoyant LDL (16) is proportional to the degree of hypertriacylglyceridemia. A low HDL-C level

contributes to oxidative stress, apart from other mechanisms, due to diminished antioxidative capacity (17). Several mechanisms have been suggested to explain the link between hyperglycemia and lipid peroxidation. Hyperglycemia leads to elevated formation of RONS as a consequence of non-enzymatic protein glycation and glucose autoxidation (18, 19). Glucose may combine directly with LDL phospholipids or apolipoprotein B lysine group to form advanced glycosylation end-products that facilitate lipid peroxidation (20). Hyperglycemia also induces the enzymatic production of superoxide through activation of NAD(P)H oxidase in vascular cells (21).

Our results are in accordance with the above-mentioned data and indicate that hypertriacylglyceridemia, a low HDL-C level and lack of antioxidant vitamin E are important factors associated with the oxidative stress in patients with MetS. A low level of lipid-standardized α-tocopherol in patients with MetS was also found by Ford et al. (22), who suggested that high levels of oxidative stress deplete endogenous and exogenous pools of antioxidants. Block et al. (23) and Dietrich et al. (24) found a link between BMI and plasma isoprostanes, as well as with other markers of oxidative stress, such as reduced erythrocyte glutathione and glutathione peroxidase (25) or association of high waist circumference with high serum concentration of CRP and oxidized LDL (26–30). Besides elevated markers of oxidative stress, we also found increased levels of fibrinogen in the MetS group, which indicates a higher coagulability in these patients. Moreover, an increased level of the inflammatory marker CRP indicates a link between MetS and higher level of systemic inflammation. This link between oxidative stress and low-grade systemic inflammation in MetS has also been suggested by Das and Van Guilder et al. (29, 30).

As inflammation pathways that generate the mediators of inflammation, such as adhesion molecules and interleukins, are all induced by oxidative stress (31), a higher oxidative stress correlates with the presence of inflammation. It has been suggested that the sub-clinical pro-inflammatory state often observed in atherosclerosis, cancer and aging is caused by a mitochondrial over-generation of free radicals (32). It is difficult to pinpoint the primary stimulus, as oxidative stress could be induced by a low-grade systemic inflammation. Several authors have suggested that a low degree of inflammation in obese individuals is caused by a high secretion of pro-inflammatory cytokines, such as tumor necrosis factor-α (33, 34). This, in turn, induces the production of interleukin-6. Interleukin-6 is a prime regulator of CRP synthesis in the liver, which leads to a low-grade inflammatory state that induces the production of free radicals and leads to increased lipid peroxidation (35). Weinbrenner et al. (26) showed that elevated CRP concentrations were related to increased abdominal fat, which is in accordance with our findings.

The mechanisms by which abdominal adiposity per se could induce elevation of oxidative stress were suggested. Furukawa et al. (36) found a correlation between fat accumulation and systemic oxidative stress in humans and mice. They observed that production of RONS increased selectively in adipose tissue of obese individuals and it was accompanied by augmented expression of NADPH oxidase and decreased expression of antioxidant enzymes. In cultured adipocytes, elevated levels of fatty acids increased oxidative stress, which caused dysregulated production of adipocytokines, including adiponectin, plasminogen activator inhibitor-1, interleukin-6 and monocyte chemotactic protein-1 (36, 37). Furthermore, overnutrition and decreased physical activity lead to increased glucose and free fatty acid loads in cells. Their transformation into energy is accompanied by an increased generation of free radicals (38). The impaired glucose tolerance is characterized by increased postprandial hyperglycemia, which induces oxidative stress. The persistence of such a condition produces an exhaustion of β cells, leading to overt diabetes mellitus. However, as all the obese patients in our study (both with and without MetS) had a similar anthropometric profile (no differences in BMI or waist circumference), oxidative stress seems to be enhanced by a combination of risk factors associated with MetS rather than by obesity per se.

In their study of subjects from the offspring cohort of the Framingham Heart Study, Keaney et al. (39) found that oxidative stress in an almost healthy population is related to smoking, diabetes and obesity. They did not demonstrate any strong positive association between oxidative stress and total cholesterol, blood pressure or age. There were only a very small number of smokers in our study ($n=2$ in each group); therefore, the effect of smoking could not be evaluated. As all the patients with MetS and approximately one-half of our obese subjects without MetS were receiving antihypertensive therapy, we may have lost the discriminatory value of blood pressure as a correlate of oxidative stress. In accordance with Holvoet et al. (40) and Ford et al. (22), we did not find any association of MetS with a higher concentration of LDL-C.

Summarizing our data by multidimensional statistical analysis, we can draw the conclusion that oxidative stress is independently related to triacylglyceride concentration, abdominal fat, low HDL-C and low lipid standardized α-tocopherol in obese patients with MetS. High levels of free radicals together with the low total antioxidant capacity detected in the case of obese patients indicate elevation of oxidative stress, which is potentiated especially in the case of obese patients with MetS. This imbalance in oxidative/antioxidative status and chronic low-grade inflammation may result in a higher risk of atherosclerotic and diabetic complications in obese adults. Future studies are needed to elucidate the precise mode of action of MetS factors.

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