Systemic Oxidative Alterations Are Associated with Visceral Adiposity and Liver Steatosis in Patients with Metabolic Syndrome

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Abstract

Although evidence suggests the link between chronic inflammation and oxidative stress as the main mechanism responsible for endothelial dysfunction and cardiovascular complications in patients with metabolic syndrome, little is known about the determining role of each metabolic syndrome component in such alterations. This study investigated the relation between systemic oxidative alterations and metabolic syndrome features in 41 patients. Compared with control subjects, serum vitamin C and α-tocopherol concentrations were lower and those of lipid peroxides [thiobarbituric acid reactive substances (TBARs)] were higher in metabolic syndrome patients (P < 0.001). A linear relation was observed between visceral fat thickness and serum TBARs:cholesterol ratio (r = 0.541, P < 0.001), whereas negative correlations were found between α-tocopherol and BMI (r = −0.212, P < 0.05) and the grade of liver steatosis (r = −0.262, P < 0.02). Patients with metabolic syndrome and liver steatosis had higher serum hyaluronate (HA) concentrations (P < 0.001). Serum HA was positively correlated with serum alanine amino transferase (r = 0.715, P < 0.001) and the homeostasis monitoring assessment index (r = 0.248, P < 0.03). The presence of metabolic syndrome was predicted from a linear combination of visceral fat and all oxidative variables. In metabolic syndrome patients, serum nitrosothiols and vitamin C concentrations, which were lower (P < 0.001) than in control subjects, were inversely related to the grade of hypertension (r = −0.645, P < 0.001 and r = −0.415, P < 0.007, respectively). In conclusion, metabolic syndrome patients exhibited decreased antioxidant protection and increased lipid peroxidation. Our results indicate a strong association between increased abdominal fat storage, liver steatosis, and systemic oxidative alterations in metabolic syndrome patients and diminished nitrosothiols and vitamin C concentrations as important factors associated with hypertension in these patients. J. Nutr. 136: 3022–3026, 2006.

Introduction

The metabolic syndrome, a multifactorial condition leading to accelerated atherosclerosis and increased risk of diabetes, is associated with high mortality rate and major cardiovascular events (1,2). Although the main pathogenic mechanism relies on insulin resistance, an abundance of evidence has emerged demonstrating a close link among the metabolic syndrome, a state of chronic low-level inflammation, and oxidative stress (3). Oxidative stress plays an important role in the pathogenesis of vascular alterations by either triggering or exacerbating the atherosclerotic process accompanying the metabolic syndrome (4). In particular, experimental and clinical observations indicate oxidative stress as an important pathogenic mechanism in obesity-associated metabolic syndrome and in the development of diabetes and its complications (5,6).

In the metabolic syndrome, excessive fat accumulation induces free radical generation and favors oxidative damages in adipose tissue (5). In patients with metabolic syndrome, oxidative stress may be amplified by a concomitant antioxidant deficiency that may favor the propagation of the oxidative alterations to the extracellular space, thus promoting endothelial dysfunction and cardiovascular damage (7,8). Similarly, a unifying hypothesis based on excessive superoxide production explains the appearance of insulin resistance, β-cell dysfunction, impaired glucose metabolism, and macro- and microvascular complications in type 2 diabetes (9).

The complex equilibrium between antioxidants and pro-oxidant insults, as well as between reduced and oxidized antioxidants status, determines outcomes in studies of tissue degeneration and inflammation (10). Recent evidence suggests that, beyond their specific antioxidant properties, vitamins E and C exert protective effects against atherogenesis by countering LDL oxidation, endothelial damage, inflammatory cytokine synthesis, and by favoring nitric oxide (NO)2 production and

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2 Abbreviations used: ALT, alanine amino transferase; HA, hyaluronic acid; HOMA, homeostasis monitoring assessment; NAFLD, nonalcoholic fatty liver disease; NO, nitric oxide; RSNO, nitrosothiol; TBAR, thiobarbituric acid reactive substance.
arterial dilatation (11,12). Concerning this latter point, paradoxically, hyperglycemic status results simultaneously in both increased NO production and decreased NO diffusion, with a final low NO availability (13,14). Changes in NO metabolism involving its derivatives, peroxynitrite and nitrosothiols, may have important implications in the appearance of diabetes and metabolic syndrome associated endothelial dysfunction (15).

Despite all these observations linking oxidative stress and the metabolic syndrome, little is known about the determining role of each single component of the metabolic syndrome in the circulating oxidative stress markers. Therefore, this study aimed to characterize oxidative stress marker abnormality in patients with metabolic syndrome and to gain more insight into the relations between the major constituent factors.

Patients and Methods

This study was conducted on 41 consecutive patients with metabolic syndrome (demographic and clinical characteristics reported in Table 1) admitted at our day hospital department. Twenty-three age- and sex-matched healthy individuals served as control subjects. Diagnosis of metabolic syndrome was made according to clinical and biochemical parameters; modified criteria of the Third Report of the National Cholesterol Education Program Adult Treatment Panel III were adopted (16). Patients with diabetes complications, cardiovascular or cerebrovascular disease, chronic liver or renal disease, or tobacco or alcohol abuse were excluded. This was the patients’ first diagnosis and they were not previously reported (17) and accordingly divided into grades (I-III). Serum albumin excretion were measured using routine automated assay methods after an overnight fast. Insulin resistance was calculated by the homeostasis monitoring assessment (HOMA) formula. Written consent was obtained from all participants. The study was approved by the Ethical Committee of the Medical Faculty at Bari University.

| TABLE 1 | Demographic and ultrasonographic characteristics of patients with metabolic syndrome and of healthy controls¹ |

<table>
<thead>
<tr>
<th>Metabolic syndrome</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects, n</td>
<td>41</td>
</tr>
<tr>
<td>Age, y</td>
<td>53 ± 6</td>
</tr>
<tr>
<td>Females, n</td>
<td>22</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>33 ± 6</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>114 ± 14*</td>
</tr>
<tr>
<td>Arterial hypertension, %</td>
<td>83</td>
</tr>
<tr>
<td>Fasting hyperglycemia, %</td>
<td>71</td>
</tr>
<tr>
<td>Diabetes, %</td>
<td>32</td>
</tr>
<tr>
<td>Serum triglycerides, mmol/L</td>
<td>1.85 ± 0.96*</td>
</tr>
<tr>
<td>Serum total cholesterol, mmol/L</td>
<td>5.1 ± 1.0*</td>
</tr>
<tr>
<td>Serum HDL cholesterol, mmol/L</td>
<td>1.0 ± 0.3*</td>
</tr>
<tr>
<td>Serum insulin, pmoL/L</td>
<td>194 ± 97</td>
</tr>
<tr>
<td>Urine albumin, mg/L</td>
<td>232 ± 108*</td>
</tr>
<tr>
<td>Serum C-reactive protein, mg/L</td>
<td>1.5 ± 0.9*</td>
</tr>
<tr>
<td>Serum ALT, IU/L</td>
<td>54 ± 32*</td>
</tr>
<tr>
<td>Liver steatosis, %</td>
<td>95</td>
</tr>
<tr>
<td>Preperitoneal fat, mm</td>
<td>14 ± 5*</td>
</tr>
<tr>
<td>Visceral fat, mm</td>
<td>72 ± 41*</td>
</tr>
<tr>
<td>Subcutaneous fat, mm</td>
<td>20 ± 9*</td>
</tr>
</tbody>
</table>

¹ Values are means ± SD or %. *Different from controls, P < 0.02. ² Measured in fasting subjects.

Abdominal ultrasonography. Liver ultrasonography scanning was performed using a Hitachi apparatus equipped with a convex 3.5-MHz probe. Liver steatosis was diagnosed as previously described (18) and graded semiquantitatively according to a previously reported scale (19): 0 = absent, 1 = mild, 2 = moderate, and 3 = severe steatosis. Subcutaneous, preperitoneal, and visceral fat thickness was measured, as previously described (20), using a linear-array probe (7.5 MHz). All examinations were performed by the same operator who was unaware of the experimental design. The common carotid intima-media thickness was measured using ultrasonography and standard techniques (21).

We collected blood from subjects who fasted overnight and the blood was immediately centrifuged at 3000 × g; 5 min to obtain serum. For assessing vitamin C, serum was precipitated with 30 g/L metaphosphoric acid and centrifuged. The supernatant was adjusted to pH 3.5 with 0.44 mol/L citrate buffer and mixed with dichlorophenolindophenol (22). Absorbance was read spectrophotometrically at 510 nm. Serum α-tocopherol was determined according to the method of Hatam and Kayden (23); samples were saponified in saturated KOH in a water bath, extracted with hexane, nitrogen dried, and resuspended in methanol/ascorbate before injection onto the chromatograph. Serum levels of thiobarbituric acid reactive substances (TBAR) were determined with the spectrophotometric method described by Slater and Sawyer (24). Serum nitrosothiol (RSNO) levels were measured following the method described by Cook et al. (25), which uses a mixture of sulfanilamide/ N-(1-Naphthyl)ethylenediamine dihydrochloride as reagents. Standards were prepared by reacting equal molar-reduced glutathione and nitrite in water. Serum hyaluronate (HA) level is a widely used indirect index of liver fibrosis. HA levels were determined by a standardized ELISA kit (Corgenix). Serum HA normal range was defined to be between 0 and 700 nmol/L. All the reagents were purchased from Sigma-Aldrich or as otherwise stated.

Statistical analysis. Descriptive statistics, including means, SD, ranges and percentages, were used to characterize the study subjects. Because of lacking normal distribution, the Mann-Whitney rank sum test was used to make comparisons. Differences between proportions were tested by chi-square. To address the relation between variables, Spearman rank correlations and forward stepwise multiple regression analysis were performed. The effect of anthropometric, metabolic, and ultrasonographic variables on oxidative markers, determined in all participants (n = 64), were calculated as independent factors. Calculations were performed with the NCSS 2004 statistical software.

Results

All the enrolled patients fulfilled the definition criteria for the metabolic syndrome. Patients with metabolic syndrome differed significantly from healthy controls in serum concentrations of triglycerides, total and HDL-cholesterol, fasting insulin, and liver transaminases (Table 1). Based on BMI, 68% of subjects were classified as obese (BMI ≥ 30 kg/m²), 29% overweight (25 kg/m² ≤ BMI ≤ 29.9 kg/m²), and only 1 had normal anthropometric values. The incidences of the characteristics of metabolic syndrome in our cohort of patients reflected those reported in larger studies (16): 85% of the patients had abdominal adiposity, 85% hypertension, 76% low HDL cholesterol, 71% fasting hyperglycemia, 32% diabetes, and 46% elevated triglyceride levels. Overall, 51% of the patients had 3 major diagnostic criteria for metabolic syndrome, 37% exhibited 4 major criteria, and 12% of them had all 5 characteristics.

At abdominal ultrasonography, most of the patient images indicated liver steatosis (Table 1): 44% had grade I, 36% grade II, and 18% grade III. Fat distribution in patients with metabolic syndrome was prevalent at the visceral level, and at all 3 sites measured, patients had significantly more fat than control subjects (Table 1). There was no correlation between the presence and grade of hepatic steatosis and/or mesenteric adiposity.
with any combinations of major diagnostic criteria of the metabolic syndrome. Among anthropometric variables, although there was a positive relation between BMI and waist circumference ($r = 0.648, P < 0.001$), the amount of visceral fat correlated only with BMI ($r = 0.529, P < 0.001$).

The analysis of the serum antioxidant vitamin concentrations, together with the concentrations of the oxidative metabolic variables, allowed us to distinguish patients with metabolic syndrome from control subjects. In particular, the concentrations of vitamin C and α-tocopherol were lower ($P < 0.001$) and serum TBARS higher ($P < 0.001$) in subjects with metabolic syndrome (Table 2). We found a negative correlation between BMI and α-tocopherol levels ($r = -0.212, P < 0.05$). When the concentrations of α-tocopherol and TBARS were related to the serum levels of total cholesterol, the resulting ratios (α-tocopherol:cholesterol and TBARS:cholesterol) better represented the oxidative metabolic alterations (Table 2). As a consequence, the ratio α-tocopherol:TBARS was significantly lower in subjects with metabolic syndrome ($6.7 ± 4.6$ vs. $16.6 ± 8.1 \mu mol/nmol, P < 0.02$).

Serum cholesterol-adjusted α-tocopherol concentrations and the grade of liver steatosis were inversely related ($r = -0.263, P < 0.02$). In addition, compared with control subjects, patients with metabolic syndrome and liver steatosis had higher HA concentrations ($843 ± 571$ vs. $229 ± 100 \mu mol/L, P < 0.001$) that were correlated with serum ALT ($r = 0.715, P < 0.001$) and the HOMA index ($r = 0.248, P < 0.03$). The amount of visceral fat and the serum TBARS:cholesterol ratio (Fig. 1) were correlated ($r = 0.541, P < 0.001$), with the regression analysis indicating that the presence of excess visceral fat was an important and independent factor resulting in the observed differences in the serum TBARS:cholesterol ratio ($r^2 = 0.315, P < 0.001$). By forward stepwise multiple regression analysis considering all the study subjects ($n = 64$), the presence of metabolic syndrome was predicted from a linear combination of independent variables (visceral fat, liver steatosis, serum α-tocopherol:cholesterol, and TBARS:cholesterol ratios).

Compared with controls, subjects with metabolic syndrome had lower circulating RSNO levels ($423 ± 93$ vs. $538 ± 70 \mu mol/L, P < 0.001$). Serum vitamin C and RSNO concentrations were lower in hypertensive patients with metabolic syndrome (Fig. 2) than in control subjects and both were inversely related to the grade of hypertension ($r = -0.415, P < 0.007$ and $r = -0.645, P < 0.001$, respectively). Oxidative variables were not related with the levels of C-reactive protein, homocysteine, urate, urine albumin, and with the carotid intima-media thickness.

**Discussion**

Several human conditions have been closely related to excess free radicals or to inadequate antioxidant defense (26), and oxidative stress has been associated with obesity, hypertension, and hyperglycemia (3,5,27). Although some of the constituent characteristics of the metabolic syndrome share common pathogenic mechanisms, however, the impact of each in determining inflammatory process-triggered oxidation is still unclear.

This study shows that subjects with metabolic syndrome exhibited an unbalanced serum redox balance with a decreased lipid antioxidant capacity and increased lipid peroxidation. These findings are consistent with a purported incapacity of the body to correct excess oxidation. Also, by combining the serum levels of antioxidant vitamins with those of oxidative variables, our findings allowed us to distinguish patients with metabolic syndrome from healthy control subjects and better define the unbalanced redox status by giving a more accurate evaluation of antioxidant vitamin changes.

**TABLE 2** Serum redox variables in patients with metabolic syndrome and in healthy controls

<table>
<thead>
<tr>
<th></th>
<th>Metabolic syndrome</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin C, µmol/L</td>
<td>94 ± 42*</td>
<td>144 ± 34</td>
</tr>
<tr>
<td>α-Tocopherol, µmol/L</td>
<td>16.0 ± 4.8*</td>
<td>23.4 ± 6.8</td>
</tr>
<tr>
<td>TBARS, nmol/L</td>
<td>3.0 ± 1.3*</td>
<td>1.6 ± 0.4</td>
</tr>
<tr>
<td>α-Tocopherol:cholesterol, nmol/nmol</td>
<td>0.0308 ± 0.0111*</td>
<td>0.0527 ± 0.0151</td>
</tr>
<tr>
<td>TBARS:cholesterol, nmol/nmol</td>
<td>0.059 ± 0.018*</td>
<td>0.035 ± 0.009</td>
</tr>
</tbody>
</table>

1 Values are means ± SD. *Different from controls, P < 0.001.

**Figure 1** The positive relation between serum thiobarbituric reactive substances (TBARS):total cholesterol ratio and visceral fat in healthy individuals ($n = 23$) and in patients with metabolic syndrome ($n = 41$).

**Figure 2** Serum concentrations of vitamin C (Panel A) and nitrosothiols (Panel B) in healthy controls ($n = 23$) and in patients with metabolic syndrome ($n = 41$) divided according to the grade of blood hypertension (I-III). Values are means ± SD. Asterisks indicate different from controls: *P < 0.02, **P < 0.001.
Serum tocopherols, in fact, tend to partition into lipids, act as membrane stabilizers by protecting lipids from oxidation, and also regulate inflammatory reactions and metabolic pathways, including platelet aggregation (11). In combination with tocopherols, vitamin C counters free radicals and regulates vitamin E metabolism by recycling oxidized tocopherols. The synergic action of these 2 vitamins is also modulated by the intervention of reduced glutathione, which maintains vitamin C in the reduced form (28). Both vitamins E and C appear to be important for the prevention of cardiovascular events. In fact, vitamin E consumption has been associated with a lower risk of coronary heart disease (29) and with reduced low-density lipoprotein oxidation (30). Recently, vitamin E supplementation prevented the onset of type 2 diabetes (31) and improved nonalcoholic fatty liver disease (NAFLD) in obese children (32). However, controversial results exist and evidence also suggested that high-dose vitamin E may not be totally innocuous and may be associated with increased risk of death and heart failure (33). Unfortunately, most of these studies did not evaluate pre- or posttreatment vitamin E levels or vitamin E/lipid ratios.

In this study, the determination of the vitamin E/cholesterol ratio was used to better define the actual vitamin E status and the potential requirement in subjects who are likely to raise LDL cholesterol oxidation. By expressing the concentrations of α-tocopherol and TBARS in relation to cholesterol level, we demonstrated the existence of an inverse relation between the serum cholesterol-adjusted α-tocopherol concentrations and the grade of liver steatosis and a linear relation between the extent of visceral fat and the TBARS:cholesterol ratio, thus indicating a close association with systemic lipid peroxidation enhancement. These observations support the hypothesis that the enhanced systemic oxidative stress in patients with metabolic syndrome is associated with visceral adiposity and liver steatosis (7,8,34). Accumulation of fat in the abdominal region and in the liver induces increases in lipid peroxidation and damage through excess of free fatty acids, lipoprotein-bound lipids, cytokines, and vasoactive peptides. The measurement of its thickness is a reliable index for assessing the amount of visceral adiposity and has been found to be an independent determinant of all components of the metabolic syndrome (35). In our study, the relation between ultrasound-estimated visceral fat and systemic metabolic disorders was more accurate than the measurement of waist circumference and was in line with previous investigations showing an independent association among ultrasonographic/measured mesenteric fat, metabolic risk factors, and BMI (36).

In conditions of altered lipid metabolism, the liver is damaged (37) and represents a contributory source for systemic oxidative alterations (34). Concerning this, our results confirmed previous observations of a close relation between metabolic syndrome and NAFLD (38) and add information on the risk of fibrosis in these subjects. In fact, the findings of increased HA levels in subjects with metabolic syndrome and ultrasonographic features of fatty liver correlated with serum ALT levels and HOMA values, known factors associated with increased risk of disease evolution (39).

Other major findings that emerged from this study were the low RSNO and vitamin C levels in patients with metabolic syndrome and higher grade of hypertension. This observation points to a pathogenic role of decreased NO derivatives and vitamin C in the development of hypertension and vascular complications in patients with metabolic syndrome. This argumentation is supported by some considerations. NO, an odd member of the free radical family, exerts a modulatory activity on cell membrane function and its derivative RSNO, an unstable thioester, exhibits different functions within the cell (40) and in the extracellular compartment through the nitrosylation of proteins and enzymes (41). Circulating RSNO acts as free NO donor (42) for the vascular tone modulation (15) and also as a mediator of the immune response (43), both functions being altered in atherosclerosis. By representing a storage form of thiols and glutathione in particular, extracellular RSNO exerts antioxidant functions and favors removal of toxic products (44), contributing to the role of extracellular microenvironment in regulating redox status and function of cell surface proteins (45).

Vitamin C is known to improve selectively elastic artery (12) by either contrasting endothelial cell oxidation or by regulating collagen synthesis (46) through the stimulation of both endothelium-dependent and -independent arterial vasodilation (47). Vitamin C administration was able to restore the endothelium-dependent vasodilation in patients with hyperglycemia (48). Consistent with these observations, our findings suggest that hyperglycemia and hypertension may impair the vascular function in patients with MS through oxidative stress events. This mechanism may have particular importance when hypertension and metabolic syndrome are associated with abdominal obesity and NAFLD.

In conclusion, subjects with metabolic syndrome exhibited decreased antioxidant protection and increased lipid peroxidation. Our findings emphasize the role of oxidative stress in the pathophysiologic interactions among the constituent factors of the metabolic syndrome, lipid oxidation, and the onset of cardiovascular damage, contributing to define the metabolic risk profile of the subjects (49). The close association with abdominal obesity and liver steatosis suggests a strong interrelation between these forms of fat accumulation and systemic oxidative alterations. The assessment of oxidative stress indices may allow the design of tailored therapeutic approaches for patients with metabolic syndrome.

### Literature Cited