



Original Article

Behavior of the total antioxidant status in a group of subjects with metabolic syndrome



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ABSTRACT

Aims: Our purpose was to examine the total antioxidant status (TAS) in subjects with metabolic syndrome (MS) subdivided according to the presence or not of diabetes mellitus.

Methods: We enrolled 106 subjects (45 women, 61 men) with MS subsequently subdivided in diabetics (14 women, 29 men) and nondiabetics (31 women, 29 men). TAS was obtained using an Assay kit which relies on the ability of plasma antioxidant substances to inhibit the oxidation of 2,2'-azino-bis(3-ethylbenzthiazoline sulfonic acid) to the radical ABTS*+.

Results: In the group of MS subjects a significant decrease in TAS ($p < 0.05$) in comparison with normal controls was evident. This difference was present between normal subjects and nondiabetic subjects with MS ($p < 0.001$) but not between normal and diabetic subjects with MS. Examining the linear regression among TAS, age, anthropometric profile, blood pressure values and glycometabolic pattern, conflicting data were found.

Conclusions: Although we know that TAS includes several enzymatic and non enzymatic antioxidants, we retain that the difference observed in the two subgroups of subjects with MS must be looked in particular into two pathophysiological aspects regarding bilirubin and uric acid.

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1. Introduction

Up to now several antioxidants, including retinol, retinyl esters, carotenoids (β -carotene, α -carotene, β -cryptoxanthin, lycopene and zeaxanthin/lutein), vitamin E and vitamin C, have been examined in patients with metabolic syndrome (MS) [1,2] while previously observational studies have regarded the levels of serum carotenoids in MS [3] and in type 2 diabetes mellitus (DM) [4]. Conversely, many research have looked at the effects of long-term antioxidant supplementation in adults with risk of MS [5] and in type 2 diabetic subjects [6] with contrasting results.

The antioxidant defence includes the hydrosoluble antioxidants such as uric acid, vitamin C, total proteins and albumin, the liposoluble antioxidants, as bilirubin and vitamin E, and the enzymatic antioxidants such as superoxide dismutase, catalase, glutathione peroxidase. In the clinical practice the antioxidant status may be altogether investigated as total antioxidant capacity (TAC), ferric reducing ability of plasma (FRAP), total antioxidant activity (TAA), total radical-trapping antioxidant parameter (TRAP) and total antioxidant status (TAS).

We know that MS represents a multifactorial status characterized by different combinations of three or more of the following clinical conditions: diabetes mellitus, arterial hypertension, dyslipidemia and visceral obesity, and in this syndrome as well as in its principal features the behavior of total antioxidant status has been examined. In many papers [7–12] that have regarded MS, this parameter has been found reduced. In the paper of Sebekova et al. [13] regarding the association of MS risk factors in health omnivores (traditional western mixed diet) and vegetarians, FRAP was different between the two groups, after the subdivision according to the risk factor. In fact, while in omnivores the trend of antioxidant status changed in relation to the number of risk factors, although not significantly, that did not happen in the vegetarian group.

There are more information about antioxidant status in the principal components of MS. In morbid obesity [14] and in overweight subjects without MS [12] no statistical difference was observed in total antioxidant status. In type 2 DM the antioxidant status resulted reduced in some papers [15–17] although Savu et al. [18] observed its increase in comparison with control subjects. These authors [18] in subjects with uncomplicated type 2 DM also found a significant increase in plasma concentrations of uric acid and copper and in ceruloplasmin activity. As it is known, copper

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is a cofactor of superoxide dismutase, which acts as an antioxidant enzyme, but at the same time it may exert pro-oxidative effects, as well as ceruloplasmin. The antioxidant status significantly improved in morbidly obese with type 2 DM undergoing bariatric surgery [19]. This parameter, according to some authors, clearly distinguished diabetic subjects with and without proteinuria even if others did not observe this distinction [20]. In type 2 diabetic subjects the plasma total antioxidant status was also related to the glycometabolic control and to vascular complications [15]. Lapolla et al. [21] noted significantly lower levels of TRAP in type 2 diabetics with peripheral artery disease (PAD) in comparison with type 2 diabetics without PAD. In arterial hypertension some authors have described a TAS decrease [22–24]; this finding has been noted also in subjects with prehypertension [22]. In hypertensives with metabolic syndrome TAS was reduced in comparison with hypertensives without MS [25]. The total antioxidant status was also negatively correlated with insulin resistance in hypercholesterolemic patients [26] while the data regarding this parameter after lipid-lowering therapy seem to be controversial; in fact, while bezafibrate reduces the plasma total antioxidant activity [27] and atorvastatin rises this parameter [28], simvastatin seems to act on this parameter with different effects [29,30].

Considering this preamble our aim was to examine the TAS in MS subjects according to the presence or not of DM.

2. Subjects and methods

We enrolled 106 consecutive subjects with MS (61 men and 45 women), defined following the International Diabetes Federation (IDF) criteria [31]. Subsequently MS subjects were subdivided in diabetics (29 men and 14 women) and in nondiabetics (32 men and 31 women).

In the entire group of MS subjects mean age was 53.5 ± 8.9 years, BMI was 32.21 ± 4.53 , waist circumference was 106.7 ± 11.2 cm, SBP and DBP were respectively 132.1 ± 16.3 and 81.2 ± 9.9 mmHg, fasting blood glucose was 114.3 ± 44.3 mg/dl, total cholesterol was 213.9 ± 53.0 mg/dl, HDL-cholesterol was 40.4 ± 10.8 mg/dl, LDL-cholesterol was 133.2 ± 46.5 mg/dl and triglycerides were 220.2 ± 147.8 mg/dl. In the subgroup of diabetic MS subjects mean age was 58.9 ± 6.0 years, BMI was 33.2 ± 5.0 , waist circumference was 114.4 ± 11.7 cm, SBP and DBP were respectively 136.0 ± 20.5 and 79.6 ± 11.4 mmHg, fasting blood glucose was 147.5 ± 54.2 mg/dl, total cholesterol was 193.1 ± 53.1 mg/dl, HDL-cholesterol was 41.4 ± 12.8 mg/dl, LDL-cholesterol was 112.8 ± 39.7 mg/dl and triglycerides were 204.2 ± 150.9 mg/dl. In the subgroup of nondiabetic MS subjects mean age was 49.7 ± 8.6 years, BMI was 31.5 ± 4.2 , waist circumference was 102.8 ± 8.8 cm, SBP and DBP were respectively 130.0 ± 13.3 and 82.2 ± 8.9 mmHg, fasting blood glucose was 92.2 ± 10.3 mg/dl, total cholesterol was 228.0 ± 48.3 mg/dl, HDL-cholesterol was 39.7 ± 9.3 mg/dl, LDL-cholesterol was 147.9 ± 45.8 mg/dl and triglycerides were 231.0 ± 145.9 mg/dl.

The study was approved by Ethical Committee and each subject gave informed consent.

Blood samples were collected by venous puncture from the antecubital vein of each subject and immediately transferred to glass tube anticoagulated with EDTA-K3 to evaluate TAS. Total antioxidant status was obtained using an Assay kit (Calbiochem, La Jolla, USA) which relies on the ability of plasma antioxidant substances to inhibit the oxidation of 2,2'-azino-bis(3-ethylbenzthiazoline sulfonic acid) (ABTS) to the radical cation ABTS^{•+} by a peroxidase [32]. The radical concentration was measured by spectrophotometry.

The same parameter was also examined in a group of 54 normal subjects (35 men and 19 women, mean age 41.3 ± 7.4 years) recruited from the hospital staff members.

The values were expressed as means \pm s.d.; the differences between subjects with metabolic syndrome, diabetics and nondia-

betics, and normal controls were evaluated using Student's *t* test for unpaired data; the correlations were performed employing the linear regression test. The null hypothesis was rejected for *p* values less than 0.05.

3. Results

The obtained data show that, in comparison with normal subjects, in the whole group of MS subjects was evident a decrease in TAS ($N = 0.986 \pm 0.238$; MS = 0.882 ± 0.223 , $p < 0.05$). This datum was also present between normal subjects and nondiabetic subjects with MS ($N = 0.986 \pm 0.238$; NDMS = 0.809 ± 0.211 , $p < 0.001$) but not between normal subjects and diabetic subjects with MS ($N = 0.986 \pm 0.238$; DMS = 0.987 ± 0.199). The total antioxidant status was also significantly different ($p < 0.001$) between diabetic and nondiabetic MS subjects. Examining the linear regression between TAS, age, anthropometric profile, blood pressure values and glycometabolic pattern, we found in the whole group of MS subjects a positive correlation between TAS and age ($r = 0.251$, $p < 0.01$), TAS and waist circumference ($r = 0.386$, $p < 0.001$), TAS and fasting glucose level ($r = 0.250$, $p < 0.05$), and also between TAS and triglycerides ($r = 0.238$, $p < 0.05$). In the subgroup of nondiabetic MS subjects we noted a positive correlation between TAS and waist circumference ($r = 0.310$, $p < 0.05$) and a negative correlation between TAS and HDL-cholesterol ($r = -0.290$, $p < 0.05$), while in the subgroup of diabetic subjects with MS we observed a positive correlation between TAS and diastolic blood pressure ($r = 0.418$, $p < 0.05$) and between TAS and total cholesterol ($r = 0.388$, $p < 0.05$), TAS and LDL-cholesterol ($r = 0.400$, $p < 0.05$) and also between TAS and triglycerides ($r = 0.408$, $p < 0.01$).

4. Discussion

The reduction of total antioxidant status observed in the entire group of MS subjects confirms almost quite what has been observed previously in MS and also in its principal components, although the datum that deserves to be underlined regards the different behavior observed in diabetic and nondiabetic subjects with MS. Even if in diabetes mellitus the observations regarding the TAS are not univocal [15–18], we think that this distinction needs specific considerations. We know that the evaluation of total antioxidant status includes enzymatic and non-enzymatic antioxidants and it is not possible to exclude that the difference observed in these two subgroups of MS subjects must be looked especially into two pathophysiological aspects concerning the levels of bilirubin and uric acid.

Some data, in fact, have underlined the protective role played by bilirubin in metabolic syndrome [33,34], but we do not dispose of its values in the two subgroups. As it is known, bilirubin acts as antioxidant irrespective of whether it is unconjugated, conjugated or albumin bound and at the same time we know that in diabetes mellitus is present an erythrocyte mechanical fragility, significantly correlated to fasting plasma glucose and to lipid peroxidation [35], that, inducing intravascular hemolysis, theoretically may induce a bilirubin increase.

The other pathophysiological aspect regards the role of uric acid in metabolic syndrome. A relationship between hyperuricemia and various MS components (hypertension, obesity, diabetes mellitus) has been demonstrated [36–39] and serum uric acid levels seem to be clearly associated with the prevalence of MS [36] and the incidence of DM [40]. In addition, in type 2 diabetic subjects hyperuricemia is correlated with the vascular complications, such as retinopathy, cerebrovascular and coronary artery diseases, and nephropathy [41].

In the last years a paradoxical role of uric acid has been hypothesized as it could act as an antioxidant in plasma and

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