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ANTIOXIDANTS, TRACE ELEMENTS AND METABOLIC SYNDROME IN ELDERLY SUBJECTS

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Abstract: *Objective:* To examine whether concentrations of several trace elements and activities of several antioxidant enzymes are modified in metabolic syndrome, and to evaluate their possible association with metabolic syndrome components. Additionally, concentration of CRP, as a marker of inflammation, was measured. *Design:* Cross-sectional study. *Participants:* The study group consisted of 100 subjects, aged 71-88 years. *Measurements:* Anthropometric measurements and biochemical analyses of fasting blood samples were performed by standardized methods. According to the International Diabetic Federation (IDF) criteria, metabolic syndrome was diagnosed in 64 subjects. Whole blood glutathione peroxidase (GPx), erythrocyte superoxide dismutase (SOD) and catalase (CAT), serum selenium (SSe), copper (SCu) and zinc (SZn), glucose, lipoprotein profile and C-reactive protein (CRP) were determined in all subjects. *Results:* No clear influence of metabolic syndrome on SSe, SZn and SCu concentration and SOD and CAT activity was found. However, significantly higher GPx was found in subjects with metabolic syndrome than in subjects without metabolic syndrome (p=0.029), as well as in subjects with hypertriglyceridemia than in control subjects (p=0.038). After adjusting for potentially confounding variables by multiple regression, significant positive relationship between SCu and CRP was found, indicating that elevated levels of Cu could have influence on inflammatory mechanisms. *Conclusion:* Our results suggest that GPx and CRP, as biomarkers of oxidative stress and chronic inflammation, respectively, have significant role in the pathogenesis of metabolic syndrome.

Key words: Trace elements, antioxidant enzymes, metabolic syndrome, obesity, elderly.

Introduction

Cardiovascular diseases are the leading cause of death in most European transitional countries, including Croatia. Factors associated with an increase in cardiovascular disease also have a strong relation to metabolic syndrome and include abdominal obesity, high blood glucose, elevated triglycerides, low level of high-density lipoprotein cholesterol (HDL), and high blood pressure. Many dietary and lifestyle factors may contribute to increasing risk of developing metabolic syndrome and cardiovascular disease (1), and oxidative stress and low antioxidant status may be associated with unfavourable metabolic pattern. Available data are inconsistent.

It has been suggested that subjects with low serum selenium (Se) have an increased risk of cardiovascular disease (2) and that diabetic patients may be deficient in Se relative to healthy subjects (3). However, the beneficial effect of Se supplement failed to be confirmed (4, 5), especially in a Se-replete population (such as the United States) in which long-term supplementation with Se may increase diabetes risk (6, 7). On the other hand, in diabetic patients with chronic complications or macrovascular diseases increased concentrations of plasma copper (Cu) were observed (8). Cu supplementation exerted beneficial effects in diabetic mice by reducing glucose levels (9).

As key component of many enzymes and proteins, Se, zinc (Zn) and Cu could play an important role in immunity, glucose and lipid metabolism, and cardiovascular function (10-12). Moreover, Se, Cu and Zn are essential components of antioxidant enzymes involved in antioxidant defence protecting cells against oxidative damage: Se-glutathione peroxidase (GPx), and Cu,Zn-superoxide dismutase (SOD). Inadequate Zn and/or Se status may reduce human life expectancy either by accelerating the aging process or by increasing susceptibility to various diseases (13-15). Oxidative stress has been reported to increase in elderly subjects, possibly arising from an uncontrolled production of free radicals by aging mitochondria and decreased antioxidant defences (16). Imbalance between oxidative stress and antioxidant capacity can play an important role in the development and progression of more than 100 disorders and diseases.

The aim of this study was to examine whether concentrations of several trace elements (SSe, SZn, and SCu) and activities of several antioxidant enzymes (GPx, SOD and CAT) are modified in metabolic syndrome, and to evaluate their possible association with metabolic syndrome components. Additionally, concentration of CRP, as a marker of inflammation was measured.

Subjects and methods

Subjects

This investigation was part of the big national multicentre study on epidemiology of respiratory diseases in Croatian

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population. The study started in 1969, with checkups in 1972, 1982 and in 2006. At the beginning (1969), the study was based on a random sample of Croatian residents from five communities (N=4223 subjects) and age of participants was in the range of 30 to 54 years. In the mean time, due to the withdrawal or deaths, number of participant was drastically decreased (N=325 subjects). Samples for trace element status and metabolic syndrome components determination were collected from the residents of two rural municipalities in the period of the latest follow-up.

The study group consisted of 100 mobile elderly subjects, predominantly from rural areas in Croatia: 20 women and 17 men from the coastal area (Adriatic island Vis) and 38 women and 25 men from the continental region (Virovitica-town and surroundings). Subjects aged 71-88 years, living in their own homes and consuming self-selected diets.

A standardized questionnaire including data on age, dietary habits, smoking, alcohol consumption, and medical and occupational history was completed by a physician for each subject. Smoking intensity was expressed as the number of cigarettes smoked per day. Alcohol consumption was expressed as the number of drinks per week, where one drink corresponds to 3 dL beer, 1 dL wine, or 0.3 dL brandy. There were 14 active smokers, 14 former smokers and 72 never-smokers, and 49 alcohol consumers, 18 only occasional alcohol consumers and 33 non-consumers of alcohol. Medical check-up and diagnoses of chronic diseases were standardized according to the 9th International Classification of Diseases and Causes of Death. Routine medical examination included weight, height (body mass index [BMI] was calculated as weight/[height]^2), waist circumference, and blood pressure. Blood pressure was measured by a standard mercury sphygmomanometer, regularly calibrated, using a standardized method. Two consecutive blood pressure readings were performed in a sitting position after a 10-minute rest, and the mean values were used for calculations.

The study was performed in accordance with the ethical principles of the Helsinki Declaration. Participants provided informed written consent, as approved by the local authorized Ethical Committee.

Criteria for metabolic syndrome

Metabolic syndrome was diagnosed on the basis of anthropometric measurements, biochemical parameters (serum concentrations for glucose, triglycerides and HDL-cholesterol) and arterial blood pressure. According to criteria of the International Diabetes Federation, metabolic syndrome was diagnosed if waist circumference was ≥94 cm for males, ≥80 cm for females, plus any two of the following: glucose concentration ≥5.6 mmol/L (or previously diagnosed diabetes mellitus and antidiabetic therapy), triglycerides >1.7 mmol/L, HDL-cholesterol <1.03 mmol/L (males), and <1.29 mmol/L (females), and blood pressure ≥130/85 mmHg). Patients who used drugs for hypertension, lipid abnormalities or type 2 diabetes were also categorized as meeting the related metabolic syndrome criteria.

Analyses of biological specimens

Serum Se (SSe) was measured by electrothermal AAS method with the Zeeman background correction (17). Serum copper (SCu) and zinc (SZn) concentrations were measured by flame AAS method (18). The accuracy for all three SSe, SCu, and SZn measurements was controlled by analyzing reference serum samples (Seronorm, Nycomed Pharma, Oslo, Norway and Second Generation, J. Versieck, Gent, Belgium). The accuracy was also controlled by the laboratory's regular participation in the Trace Elements External Quality Assessment Scheme (TEQAS, Guildford, UK), and our results were consistently categorized as being Acceptable (as opposed to Borderline or Unacceptable as the remaining two options).

The GPx (EC 1.11.1.9) activity in blood was determined using the method described by Belsten et al (19). The SOD (EC 1.15.1.1) activity in the erythrocytes was determined using Ransod commercial test (Randox, Crumlin, UK) according to the manufacturer's directions. The results were expressed per gram of haemoglobin (U/g Hb). Catalase (CAT, EC 1.11.1.6) activity in the erythrocytes was determined according to Aebi (20) at 25 °C, pH 7.0, at 240 nm. It was calculated using the molar extinction coefficient (43.6 mM^{-1} cm^{-1}). The results were expressed per gram of haemoglobin (U/g Hb). Haemoglobin (Hb) in whole blood and erythrocytes was measured at 540 nm by standard cyanmethaemoglobin method using Haemiglobincyanide standard (Mallinckrodt Baker B.V., Deventer, Holland). To verify the analytical procedure, three levels of Hb (Mallinckrodt Baker B.V., Deventer, Holland) were used.

Concentration of glucose and C-reactive protein (CRP) were determined by standardized methods on an Olympus AU400 selective autoanalyser (Olympus, Tokyo, Japan) using reagents from the same manufacturer (Olympus, Hamburg, Germany). The lipoprotein parameters were measured enzymatically by a Roche COBAS MIRA autoanalyser (Roche Diagnostics, GmbH, Mannheim, Germany).

Statistics

Statistical analysis was performed using statistical software Statistica 7.0 (StatSoft Inc., USA). Because of the skewed distribution of some of the measured parameters, results are expressed as median and range, and the significance of the difference between groups was calculated by using the Mann-Whitney U-test (z, P). Spearman's rank correlation (r, P) was calculated for associations between each of the measured parameters. In the forward stepwise multiple regression, concentrations of SSe, SCu, SZn, activity of GPx, SOD and CAT were each analysed as a dependent variable, and age, smoking, alcohol, sex, region, waist circumference, glucose, triglycerides, HDL, blood pressure, low-density lipoprotein cholesterol (LDL) and CRP as predictors that were simultaneously introduced in the model. In addition, multiple

logistic regression models were used to analyse factors associated with metabolic syndrome. The first model studied association between metabolic syndrome and age, smoking, alcohol, sex, region, SSe, SCu, SZn, GPx, SOD and CAT. In the second model, components of metabolic syndrome (waist circumference, glucose, triglycerides, HDL, systolic and diastolic blood pressure) were added. Chi-square test was used to evaluate gender difference in the prevalence of metabolic syndrome. Kruskal-Wallis test was used to evaluate difference in measured parameters between more than two subgroups. The statistical significance was defined as a $p < 0.05$.

Results

Median and range of SSe, SCu, and SZn concentration in the study population were 64.49 (33.15-123.26), 1340 (668-2051), and 896.5 (623-1195) µg/L, respectively. The prevalence of obesity among participants was 21% and over half were overweight (57%). There was no significant difference in measured trace elements and antioxidant enzymes between obese and non-obese subjects. Significant relationship between BMI and SSe (r=0.469, p=0.004), BMI and SZn (r=0.388, p=0.021) and BMI and GPx (r=0.376, p=0.026) was observed only in participants without metabolic syndrome. The percentage of hypertension (blood pressure ≥130/85 mmHg) in total study population was 81%. Even 22/36 (61%) subjects without metabolic syndrome was hypertensive.

Table 1 shows relevant data in 64 subjects with metabolic syndrome and 36 subjects without metabolic syndrome. Both study groups were similar with regard to age, smoking habits, and alcohol consumption. In addition, sex ratio in both groups is similar, i.e. rough 60% of subjects in both groups were female. Significant difference between the groups was found for BMI, waist circumference, fasting concentrations of glucose, triglycerides, HDL-, LDL- and total cholesterol, and CRP concentration. However, no significant difference between the groups was found for blood pressure. Subjects with metabolic syndrome had significantly higher GPx activity than subjects without metabolic syndrome (p=0.029), whereas there was no significant difference in SSe, SCu and SZn concentration and SOD and CAT activity between the groups. No significant difference in the prevalence of metabolic syndrome between women (67%) and men (62%) was found. Only one man and eight women met all five criteria for metabolic syndrome, whereas six men and six women met no criteria for metabolic syndrome.

Table 2 shows SSe, SCu and SZn concentrations, and GPx, SOD and CAT activities with regard to cut-off levels for components of metabolic syndrome. No significant difference in SSe, SCu and SZn, GPx, SOD and CAT was found among different BMI subgroups. Women with waist circumference ≥80 cm had significantly higher SSe and SZn than women with waist circumference <80 cm (p=0.017 and p=0.027, respectively). Subjects with triglycerides >1.7 mmol/L had significantly higher activity of GPx than control subjects (p=0.038).

Table 1

Median and range of anthropometric and laboratory variables in 64 subjects with metabolic syndrome and 36 subjects without metabolic syndrome, and the significance of the difference between the groups (p)

Parameter	With metabolic syndrome[a] (N=64)	Without metabolic syndrome[a] (N=36)	p
Age (years)	76 (72-88)	78 (71-87)	0.368
Sex ratio (M/F) (%)	28/36 (44/56)	14/22 (39/61)	
Smoking (cigarettes/day)	0 (0-20)	0 (0-15)	0.594
Alcohol (drinks*/week)	10 (0-122)	6.5 (0-95)	0.789
BMI (kg/m^2)	28.1 (22.4-34.6)	25.7 (18.3-37.1)	0.001
Waist circumference (cm)	104 (82-124)	94 (67-118)	0.000034
Systolic blood pressure (mm Hg)	150 (110-195)	150 (110-190)	0.695
Diastolic blood pressure (mm Hg)	90 (60-125)	90 (80-110)	0.774
Glucose (mmol/L)	6.2 (4.2-16.0)	4.85 (3.6-7.5)	0.0003
Triglycerides (mmol/L)	1.745 (0.59-8.06)	1.055 (0.55-1.93)	1.93×10^{-7}
HDL (mmol/L)	0.965 (0.63-1.84)	1.385 (0.80-2.59)	3.43×10^{-7}
LDL (mmol/L)	3.77 (3.00-6.49)	3.28 (1.12-5.77)	0.003
Total Cholesterol (mmol/L)	5.61 (3.01-9.25)	5.31 (3.21-7.70)	0.022
C - Reactive Protein (mg/L)	2.52 (0.32-49.95)	1.685 (0.32-32.75)	0.003
SSe (µg/L)	66.9 (33.1-123.3)	61.2 (36.9-100.1)	0.193
SCu (µg/L)	1350 (851-2051)	1272 (668-1672)	0.301
SZn (µg/L)	891 (623-1195)	908 (722-1106)	0.794
GPx (U/L)	17796 (8842-28103)	15945 (10320-30473)	0.029
SOD (U/g Hb)	1509 (1265-1308)	1543 (1228-1915)	0.513
CAT (U/g Hb)	38.4 (16.5-78.6)	35.0 (13.9-71.0)	0.544

a. Metabolic syndrome according to criteria of the International Diabetes Federation; BMI - body mass index, HDL –high-density lipoprotein cholesterol, LDL – low-density cholesterol, SSe - serum selenium, SCu - serum copper, SZn - serum zinc, GPx – whole blood glutathione peroxidase, SOD – erythrocyte superoxide dismutase, CAT – erythrocyte catalase

Spearman Rank correlations between antioxidant enzymes and trace elements with regard to metabolic syndrome components and CRP, performed in all participants, showed significant relationship between SSe and CRP (r=-0.202, p=0.045), SCu and glucose (r=-0.211, p=0.038), SCu and CRP (r=0.311, p=0.002), and between GPx and triglycerides (r=0.211, p=0.039).

After adjusting for potentially confounding variables by multiple regression, only positive relationship between SCu and CRP remained significant (Beta 0.331; Coeff. (B) 161.27; SE of B 49.79; P=0.002). Results of logistic regression showed no significant association between metabolic syndrome and SSe, SZn, SCu, GPx, SOD and CAT.

Discussion

No clear influence of metabolic syndrome on SSe, SZn and SCu concentration and SOD and CAT activity was found. However, significantly higher activity of GPx in subjects with hypertriglyceridemia than in control subjects, and in subjects with metabolic syndrome than subjects without metabolic syndrome confirmed that oxidative stress is involved in the pathogenesis of metabolic syndrome. Significant relationship

Table 2
Concentration of SSe, SCu and SZn, and activity of GPx, SOD and CAT with regard to cut-off levels for components of metabolic syndrome

Parameter	N	SSe (µg/L)	SCu (µg/L)	SZn (µg/L)	GPx (U/L)	SOD (U/g Hb)	CAT (U/g Hb)
Waist circumference (cm)							
<94 (male)	7	60.76 (44.60-96.36)	1434 (904-2051)	886 (737-914)	16160 (14024-19379)	1592 (1348-1814)	30.7 (16.5-60.2)
≥94	35	66.05 (37.84-123.26)	1259 (805-1653)	877 (690-1093)	17256 (9269-30473)	1473 (1228-2365)	36.5 (19.5-61.6)
<80 (female)	8	49.26 (36.93-63.32)	1339 (877-1489)	872 (813-915)	15266 (13254-17170)	1517 (1307-1666)	42.2 (22.5-71.0)
≥80	50	64.79 (33.15-118.70)[a]	1384 (668-1759)	943 (623-1195)[b]	16242 (8842-28103)	1524 (1271-1915)	38.5 (13.9-78.6)
Glucose (mmol/L)							
<5.6	64	61.90 (37.84-123.26)	1345 (805-2051)	891 (623-1195)	16268 (10320-30473)	1543 (1228-1915)	36.4 (13.9-78.6)
≥5.6	33	66.90 (33.15-100.08)	1352 (668-1934)	915 (787-1087)	17533 (8842-28103)	1505 (1271-2365)	38.8 (20.5-71.0)
HDL cholesterol (mmol/L)							
≥1.03 (male)	19	66.05 (37.84-123.26)	1313 (805-2051)	830 (718-1074)	16838 (11251-30473)	1497 (1228-1785)	33.7 (16.5-56.2)
<1.03	23	60.76 (40.48-91.02)	1254 (851-1653)	886 (690-1093)	17697 (9269-20915)	1482 (1264-2365)	36.8 (22.8-61.6)
≥1.29 (female)	20	60.37 (36.93-100.08)	1272 (668-1672)	935 (813-1083)	15789 (10320-22273)	1543 (1289-1748)	34.8 (15.9-71.0)
<1.29	38	65.79 (33.15-118.70)	1401 (877-1934)	934 (623-1195)	16263 (8842-28103)	1524 (1271-1915)	41.0 (13.9-78.6)
Triglycerides (mmol/L)							
≤1.7	66	63.34 (33.15-100.08)	1307 (668-2051)	890 (718-1106)	16146 (9269-30473)	1507 (1228-2365)	35.3 (13.9-71.0)
>1.7	34	65.70 (38.92-123.26)	1379 (851-1934)	916 (623-1195)	18238 (8842-28103)[c]	1521 (1264-1841)	38.6 (19.5-78.6)
Systolic pressure (mm Hg)							
<130	10	65.84 (33.15-100.08)	1223 (668-1464)	836 (788-1027)	17032 (9269-19913)	1524 (1350-2365)	44.2 (22.1-61.6)
≥130	90	64.49 (36.93-123.26)	1340 (805-2051)	908 (623-1195)	16284 (8842-30473)	1509 (1228-1915)	36.6 (13.9-78.6)
Diastolic pressure (mm Hg)							
<85	45	60.47 (33.15-118.70)	1384 (668-1759)	886 (623-1195)	16051 (9269-28103)	1548 (1228-2365)	38.9 (15.9-78.6)
≥85	55	64.81 (37.84-123.26)	1280 (805-2051)	914 (718-1106)	17132 (8842-30473)	1491 (1247-1915)	35.0 (13.9-60.6)

P-value was determined by Mann-Whitney U-test for the difference between the cut-off levels: a. P=0.017; b. P=0.027; c. P=0.038 compared to control

between SCu and CRP after adjusting for potentially confounding variables by multiple regression indicates a possible role of Cu in inflammatory mechanisms.

Trace element status

SZn and SSe concentrations in the present study were slightly lower, and SCu concentrations higher compared to previous studies performed in Croatian adults (21-24). This could be related to the older age of participants in the present study. Namely, the risk of Zn and Se deficiency seems to increase in relation to age (16, 25, 26), especially in elderly suffering from chronic diseases (27, 28). Concentration of Se in human organism varies widely between geographical areas, depending on its content in soil and plants, dietary Se intake, bioavailability and retention, nutrient interactions and other factors. Therefore, maintaining an adequate Se status in the elderly may be difficult, particularly in eastern European countries, where Se intakes are low compared to the recommended dietary daily intake for optimal GPx activity or immune functions.

Trace elements and antioxidant enzymes with regard to obesity

It has been reported that significantly lower serum Se and GPx were observed in obese compared to non-obese patients with suspect metabolic syndrome, although serum Se was higher in the two patient groups than in the control group (29). In addition, significant inverse association between serum Se and waist circumference was found (30). Serum Cu was significantly higher in obese than non-obese subjects, and a positive correlation was found between SCu and BMI (29). Our results showed no significant difference in measured trace elements and antioxidant enzymes between obese and non-obese subjects. However, significant positive relationship was found between BMI with each of SSe, SZn and GPx, but only in the group of subjects without metabolic syndrome. Concentrations of SZn and SSe were significantly higher in women with abdominal obesity (waist circumference ≥80 cm) than in control.

Trace elements and antioxidant enzymes with regard to glucose and lipid profile

Increased oxidative stress and impaired antioxidant defence have been suggested to contribute to initiation and progression of diabetes and glucose intolerance (31, 32). Moderate increase in glucose could affect oxidative status (32), and the balance between several components of the anti-oxidant defence appears to be sensitive to glucose levels. Only few papers have been published specifically on association between components of metabolic syndrome with regard to antioxidant enzymes and trace elements, and previous results are inconsistent. For example, several studies reported positive association of total, LDL and HDL cholesterol with plasma Se (7, 10, 33) and Cu (33), inverse association of HDL cholesterol with SSe (30), and total and HDL cholesterol with SCu (11). No effect of Se-containing supplementation on total cholesterol was observed after adjusting for baseline total cholesterol levels and lipid-lowering medications (4). No difference in SSe, SZn, and SCu levels between normo- and hyperlipidemic subjects was observed (34). Results of our study could not confirmed association between components of metabolic syndrome with regard to measured trace elements and antioxidant enzymes.

Namely, after adjusting for potentially confounding variables by multiple regression, significant inverse relationship between SCu and glucose, and positive relationships between GPx and triglycerides, observed in our participants, disappeared.

Trace elements and antioxidant enzymes with regard to metabolic syndrome

Significantly higher SCu was reported in patients with metabolic syndrome than in control subjects (35), whereas no significant difference in SSe between patients with and without metabolic syndrome was found (29, 30). Although no beneficial effects of antioxidant supplementation in a generally well-nourished population was found in France, baseline SZn were positively associated with the risk of developing metabolic syndrome (5). In the present study, participants with metabolic syndrome had significantly higher GPx than those without metabolic syndrome. In addition, activity of GPx was higher in subjects with hypertriglyceridemia (regardless of metabolic syndrome) than in control. It is possible that chronic oxidative stress, as in diabetes and other impairments of metabolic pattern, contribute to increase of GPx activity as a result of up-regulation of the enzyme, indicating an adaptive, compensatory phenomenon (36).

Trace elements, antioxidant enzymes and CRP

Our results of stepwise multiple regression confirmed significant positive relationship between CRP and SCu, and suggest a possible participation of Cu in inflammation. This is in accordance with previous results showing increased Cu concentrations in inflammation, infection, and various chronic diseases (11, 37, 38). Namely, it has been proposed that Cu-induced oxidative stress might determine an inflammatory response (11). Moreover, Bo et al. (11) hypothesized that inflammation might represent the results of Cu damage instead of the cause for the increase in Cu concentration.

Some inconsistencies between the present and previously published data may be explained by the difference in status of trace elements and their interactions among different populations. Relative high prevalence of obesity, overweight and hypertension observed among our participants, and presence of one or more components of metabolic syndrome even in subjects without metabolic syndrome could have impact on the interpretation of the data. It is also possible that the small number of participants in this study contributed to greater random variability of results. Although we adjusted the data for various potential confounders, the possibility of uncontrolled or unknown confounders cannot be ruled out. However, our participants are older than those in previous studies and therefore our results are comparable to other studies which comprised subjects of the similar age. In addition, other studies did not consider simultaneous association between age, smoking, alcohol, SSe, SCu, SZn, GPx, SOD and CAT with regard to metabolic syndrome and CRP in elderly.

In conclusion, elevated levels of Cu could have influence on inflammatory mechanisms. Our results suggest that GPx and CRP, as biomarkers of oxidative stress and chronic inflammation, respectively, have significant role in the pathogenesis of metabolic syndrome.

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