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Lower Plasma Levels of Antioxidant Vitamins in Patients with Metabolic Syndrome: A Case Control Study*

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Abstract

Background. Metabolic syndrome (MS) is a coexistence of metabolic risk factors affecting the development of cardiovascular diseases. Reactive oxygen species, which are excessively produced in MS, participate in its pathogenesis. Vitamins A, C and E are an important part of the non-enzymatic antioxidative barrier in humans.

Objectives. The aim of the study was to estimate plasma vitamin A, C and E levels and the intake of these vitamins from the diet in patients with MS.

Material and Methods. The study included 182 patients with MS, 94 men and 88 women, aged 30–65 years (mean 57.31 ± 8.28 years). The control group was comprised of 91 subjects, 56 men and 35 women, aged 41–65 years (mean 57.75 ± 5.84 years). The MS diagnosis was based on IDF criteria. The determination of the serum level of vitamin A, C and E was performed using the spectrophotometric method. The food intake was assessed by 24-h dietary recall.

Results. The mean plasma vitamin A, C and E levels were significantly lower in MS patients than in the controls ($p = 0.05$). No correlation was found between vitamin A, C and E intake from the diet and their plasma concentrations in MS patients. Plasma vitamin A, C and E deficiency was observed significantly more often in MS patients than in the control group (15.38% vs. 2.19%, 79.12% vs. 8.79% and 60.45% vs. 5.49%, $p < 0.0001$, respectively). BMI was the one factor significantly affecting the mean value of vitamin A, C and E levels in MS patients.

Conclusions. MS patients demonstrated significantly lower plasma levels of vitamin A, C and E compared to the healthy subjects. Lower plasma levels of antioxidant vitamins with their high intake from the diet indicate antioxidant barrier impairment in MS patients (*Adv Clin Exp Med* 2016, 25, 4, 689–700).

Key words: metabolic syndrome, diet, oxidative stress, antioxidant vitamins.

Metabolic syndrome (MS) is defined as the co-occurrence of metabolic risk factors that create favorable conditions for the development of cardiovascular diseases. Its components include elevated

blood pressure or hypertension treatment, fasting hyperglycemia or diagnosed type 2 diabetes, abnormal lipid profile (hypertriglyceridemia and/or low HDL-cholesterol) or the treatment of these

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disorders, and central obesity, which is thought to play a key role in its pathogenesis. It has been proven that adipocytes are metabolically active cells regulating the energy balance due to the secretion of adipokines (leptin, resistin, adiponectin) and cytokines (TNF- α , IL-1, IL-6) [1]. In the context of MS, it is important to note that cytokines and resistin can induce insulin resistance, adipocytes produce more of these substances in obese subjects and visceral adipose tissue produces more of these cytokines than subcutaneous tissue. Because of this, glucose intolerance is more frequent in obese subjects and/or in those with triglyceridemia, and tissue insulin resistance correlates with their intracellular content of triglycerides as well as with abdominal adipose tissue volume. Therefore, glucose intolerance remains in the cause-and-effect relationship with lipid disorders. MS is probably connected with an excessive cellular accumulation of triglycerides and/or the adverse effects of hormonal-humoral factors produced by overdeveloped adipose tissue [2].

Reactive oxygen species (ROS) are products of natural cell metabolism, which in addition to exerting a beneficial effect on cell functioning, may cause excessive lipid peroxidation and damage to proteins and DNA. Their positive or negative effect on the organism depends on their amount and on the efficiency of antioxidant mechanisms. Among them there are distinguished enzymatic (superoxide dismutase, glutathione peroxidase, catalase) and non-enzymatic mechanisms, in which antioxidant vitamins play a very important role [3, 4].

Oxidative stress occurs when the cellular redox balance is disturbed. This leads to excessive production of ROS in hyperglycemia, insulin resistance (due to cell respiration disorders) and obesity (a positive correlation was demonstrated between the volume of adipose tissue and the rate of ROS production), which are elements of MS. Thus, subjects with MS are particularly exposed to excessive ROS and they require special care in the range of efficient functioning of the antioxidant barrier [5, 6].

The level of antioxidant compounds in cells is supported by their appropriate intake in the diet or by *de novo* synthesis. The results of large population studies indicate that subjects with MS are characterized by low consumption of vegetables, fruits and products typical of the Mediterranean diet, rich in antioxidant ingredients, including vitamin A, C and E [3]. On the other hand, as shown in numerous studies, patients with MS are characterized by a higher level of oxidative stress and weakened antioxidant barrier as compared to those without MS [7]. Thus, a properly balanced diet not only determines the homeostasis of the

internal environment of the body, but can significantly contribute to strengthening the antioxidant barrier in patients with MS.

The aim of the study was to estimate plasma vitamin A, C and E levels in patients with MS according to their intake from the diet.

Material and Methods

Study Population

The study, conducted in the years 2013–2014, included 182 patients with MS recruited from the Department of Internal Medicine and Nephrodiabetology, Medical University of Lodz, 94 men and 88 women, aged 30–65 years (mean 57.31 ± 8.28 years).

The control group was comprised of 91 subjects, 56 men and 35 women, aged 41–65 years (mean 57.75 ± 5.84 years), clinically healthy, without MS.

All of them were nonsmokers and in the last year they had not taken any dietary supplements.

Metabolic Syndrome (Definition)

The MS diagnosis was based on IDF (International Diabetes Federation) criteria, stating the type of central obesity (waist circumference in women ≥ 80 cm, in men ≥ 94 cm) and two of the following risk factors: triglycerides ≥ 1.7 mmol/L or treatment of this disorder, low HDL cholesterol (in women < 1.3 mmol/L, in men < 1.0 mmol/L) or treatment of the disorder, fasting glucose level ≥ 6.1 mmol/L or treated type 2 diabetes, blood pressure $\geq 130/85$ mm Hg or treatment of hypertension [1]. All subjects were nonsmokers and they had not taken any dietary supplements in the last year.

Biochemical Analyses

Three blood pressure (systolic and diastolic) measurements were taken using a mercury sphygmomanometer and the mean was calculated.

Fasting blood glucose was determined with a reaction between glucose and ATP catalyzed by hexokinase; TG concentration was enzymatically measured with coupled reactions in which TG was hydrolyzed to produce glycerol; TC was measured with reactions using cholesteryl ester hydrolase, cholesterol oxidase, and peroxidase; HDL was measured using a heparin-manganese precipitation method; LDL was assessed using the Friedewald equation.

Blood samples (5 mL) were drawn from the basilic vein of the MS patients and the controls for laboratory examinations. In all patients, the determinations of the serum level of vitamin E, A and C were performed by spectrophotometric method using a spectrophotometer T60V (PG Instruments) according to the modified Rutkowski et al. method [8–11]. Plasma levels of the investigated vitamins were given in $\mu\text{mol/L}$.

Plasma vitamin deficiency was stated for vitamin A $< 0.9 \mu\text{mol/L}$, for vitamin C $< 36.1 \mu\text{mol/L}$ and for vitamin E $< 12 \mu\text{mol/L}$ [8–11].

Nutritional Evaluation

The food intake was assessed using a questionnaire concerning ingestion within the 72 h prior to the examination, in accordance with the guidelines of the National Food and Nutrition Institute in Warszawa [6, 13]. A total of 546 24-h dietary recalls (three 24-h dietary recalls for each individual) were obtained from the subjects with MS by an interviewer and the means of consumption were calculated for each nutrient. The “Album of photographs of food products and dishes” of the National Food and Nutrition Institute in Warszawa was used to determine the normal size of the consumed portions [13].

The vitamin content in the diet was determined using DIET 5.0 software (license No: 52/PD/2013), worked out by the National Food and Nutrition Institute in Warszawa, based on the “Charts of nutritive values of products and foods. Third edition expanded and updated” and “Standards of Human Nutrition” [12, 14]. The program takes into account the loss of vitamins during technological and culinary food processing at the levels of 25% for vitamin E, 25% for vitamin A and 55% for vitamin C [12–14].

Anthropometric Analyses

Height was measured using a fixed stadiometer and weight was taken with individuals wearing light clothes and no shoes on a digital scale with a capacity of 200 kg and accurate to the nearest 100 g. Body mass index (BMI) was calculated as weight (kg) divided by height in m^2 . Waist circumference was measured at the midpoint between the bottom of the rib cage and above the top of the iliac crest during minimal respiration.

Statistical Analyses

Statistical analysis was performed using STATISTICA 7.1 PL and OFFICE 2010 software. The

normal distribution was determined using the Shapiro-Wilk test. The variables not normally distributed underwent logarithmic transformation (\log_{10}) before statistical analysis. The comparison between the means of two independent groups was performed using the Student's *t* test and Mann-Whitney *U* test for continuous variables, and the χ^2 and Fisher's exact tests were applied for dichotomic ones. Correlations were assessed by Spearman's and Pearson's coefficient. One-way analysis of variance (ANOVA) with the *post-hoc* Bonferroni test for multiple comparisons was used to determine if differences existed between the means of patients belonging to different plasma vitamin A, C and E groups according to sex and BMI at the level of significance $p < 0.05$. In order to determine the association between the clinical and biochemical factors and plasma vitamin A, C and E levels, we constructed a Multivariate Adaptive Regression Splines model with selected variables having *p*-values < 0.05 in the analysis.

The study was approved by the Bioethics Committee of the Medical University in Lodz (No: RNN/556/10/KB). A written consent was obtained from all research participants.

Results

In the group of patients with MS, the tested parameters (including vitamin A, C and E levels) did not differ markedly between men and women, except for waist circumference, which was significantly greater in men than in women (118.98 vs. 108.26 cm, $p = 0.046$), and HDL-cholesterol, which was significantly higher in women than in men (1.129 mmol/L vs. 1.040 mmol/L, $p = 0.042$).

The mean plasma vitamin A, C and E levels were significantly lower in MS patients than in the controls ($p < 0.0001$) (Table 1). The greatest decrease in plasma antioxidant vitamin levels was found for vitamin E in MS patients in relation to subjects without MS (Fig. 1).

A plasma deficiency of vitamin A was observed in 15.38% of patients with MS, vitamin C in 79.12% and vitamin E in 60.45% of the cases, significantly more often than in the control group ($p < 0.0001$).

Serum vitamin C concentration in MS patients inversely correlated with SBP (Pearson's coefficient: -0.31 , $p < 0.0001$), DBP (Pearson's coefficient: -0.1689 , $p = 0.042$) and HDL (Pearson's coefficient: -0.19 , $p = 0.018$), but was not associated with TC, LDL or TG. Serum vitamin E concentration inversely correlated with DBP (Pearson's coefficient: -0.36 , $p = 0.044$) in patients with MS and positively with HDL (Pearson's coefficient: 0.46 ,

Table 1. Selected baseline characteristics of study participants

	MS (n = 182)	Without MS (n = 91)	p-value
	mean \pm SD/% (n)	mean \pm SD/% (n)	
Age [years]	57.31 \pm 8.28	57.75 \pm 5.84	0.2414 ^a
Sex [% women]	59.89	56.04	0.3489 ^a
T2D [%]	46.70	27.47	< 0.001 ^c
HT [%]	79.67	56.04	0.0467 ^c
BMI [kg/m ²]	34.75 \pm 5.29	27.21 \pm 2.75	< 0.0001 ^a
Waist [cm]	113.71 \pm 12.66	96.34 \pm 10.64	< 0.0001 ^a
SBP [mm Hg]	145.97 \pm 15.62	128.33 \pm 12.61	< 0.0001 ^b
DBP [mm Hg]	87.57 \pm 10.31	81.25 \pm 7.87	< 0.0001 ^b
Plasma concentration			
Glc [mmol/L]	8.06 \pm 3.08	5.52 \pm 0.55	< 0.0001 ^b
TG [mmol/L]	1.93 \pm 0.9	1.41 \pm 0.25	< 0.0001 ^b
TC [mmol/L]	4.57 \pm 1.28	4.55 \pm 0.95	0.8261 ^a
HDL [mmol/L]	1.08 \pm 0.25	1.26 \pm 0.27	< 0.0001 ^a
LDL [mmol/L]	2.98 \pm 0.99	2.93 \pm 0.84	< 0.0001 ^a
Vitamin A [μ mol/L]	1.37 \pm 0.37	1.80 \pm 0.51	< 0.0001 ^a
Vitamin C [μ mol/L]	31.18 \pm 9.21	58.43 \pm 18.15	< 0.0001 ^a
Vitamin E [μ mol/L]	12.47 \pm 2.66	25.49 \pm 3.01	< 0.0001 ^a
Vitamin A deficiency [%]	15.38 (28)	2.19 (2)	<0.0001 ^c
Vitamin C deficiency [%]	79.12 (144)	8.79 (8)	<0.0001 ^c
Vitamin E deficiency [%]	60.44 (110)	5.49 (5)	<0.0001 ^c
Dietary intakes			
Total energy [kcal/d]	2152.31 \pm 977.16	1880.19 \pm 594.35	0.0753 ^b
Proteins [g/d]	100.34 \pm 44.01	92.775 \pm 32.60	0.5595 ^b
Fats [g/d]	83.36 \pm 56.92	78.647 \pm 40.22	0.8045 ^b
Carbohydrates [g/d]	281.40 \pm 146.12	265.515 \pm 119.10	0.5625 ^b
Cholesterol [mg]	370.83 \pm 215.69	276.181 \pm 151.19	0.0002 ^b
SFA [g/d]	34.29 \pm 23.94	33.248 \pm 17.55	0.8652 ^b
MUFA [g/d]	32.27 \pm 25.016	30.346 \pm 18.14	0.8531 ^b
PUFA [g/d]	10.76 \pm 7.22	11.517 \pm 6.54	0.7964 ^b
Fiber [g]	23.21 \pm 9.02	21.999 \pm 7.53	0.8997 ^b
Vitamin A [μ g of retinol equivalent]	1300.14 \pm 1043.86	1173.00 \pm 535.32	0.9446 ^b
Vitamin C [mg]	103.99 \pm 139.14	93.30 \pm 127.95	0.3454 ^b
Vitamin E [mg of α -tocopherol equivalent]	8.85 \pm 5.59	9.33 \pm 5.09	0.2695 ^b

a – Student's t-test; b – Mann-Whitney U test; c – χ^2 test.

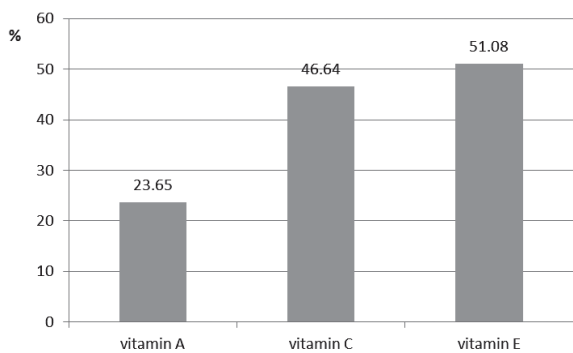


Fig. 1. The percentage value of the fall in vitamin A, C and E concentrations in the plasma of MS patients compared to subjects without MS

$p = 0.017$), but it was not associated with SBP, TC, LDL or TG. Serum vitamin A concentration positively correlated with HDL (Pearson’s coefficient: 0.42, $p = 0.021$) and it was not associated with SBP, DBP, TC, LDL or TG in patients with MS.

Multivariate analysis of variance showed that in MS patients BMI was the factor significantly affecting the mean value of vitamin A, C and E concentration. Significantly lower levels of antioxidant vitamins were found in obese patients than in patients with normal weight or overweight. Furthermore, an interaction was observed between gender and BMI in the case of vitamin A and C (Table 2, Figs. 2–4). Significant differences between the

mean levels of vitamin A and C were found in men and women with normal weight and overweight, but in the group of women the level of vitamin A and C decreased with the increase of BMI from normal to overweight values and in the group of men it increased.

The consumption of energy, protein, fat, carbohydrates, fiber, PUFA, MUFA and SFA, as well as vitamin A, C and E did not differ significantly between patients with MS and controls, except that a higher consumption of cholesterol was noted in MS patients (370.829 ± 215.699 vs. 276.181 ± 151.193 , $p = 0.0002$).

In the group of patients with MS, no correlation was found for any of the tested antioxidant vitamins between their consumption with the diet and plasma concentration. Such a correlation was observed in the control group for vitamin E (Pearson’s coefficient: 0.28, $p = 0.012$) and vitamin C (Pearson’s coefficient: 0.31, $p = 0.026$). However, in the MS group a correlation was noted between the plasma level of vitamin E and the consumption of dietary MUFA ($Rho = 0.26$, $p < 0.001$), total fat ($Rho = 0.22$, $p < 0.001$) and total protein ($Rho = 0.28$, $p < 0.001$). Furthermore, multivariate adaptive regression (MARSplines – Multivariate Adaptive Regression Splines) demonstrated a correlation between plasma levels of vitamin A, C and E and their consumption with a diet, taking into account gender, the prevalence of over-

Table 2. Univariate tests of significance for vitamins A, C and E. Sigma-restricted parameterization

	SS	Partial eta-squared	MS	F	p
Vitamin A					
Intercept	404.90	0.89	404.91	2258.78	0.0000
Sex	0.35	0.01	0.35	1.93	0.1656
BMI	5.11	0.09	1.70	9.50	0.0000
Sex*BMI	6.34	0.12	2.11	11.79	0.0000
Vitamin C					
Intercept	283728.7	0.81	283728.7	1117.67	0.0000
Sex	158.2	0.00	158.2	0.62	0.4305
BMI	16278.0	0.19	5426.0	21.37	0.0000
Sex*BMI	3814.3	0.05	1271.4	5.01	0.0021
Vitamin E					
Intercept	52846.05	0.88	52846.05	1922.73	0.0000
Sex	41.96	0.01	41.96	1.52	0.2177
BMI	4816.33	0.40	1605.44	58.41	0.0000
Sex*BMI	60.75	0.01	20.25	0.74	0.5309

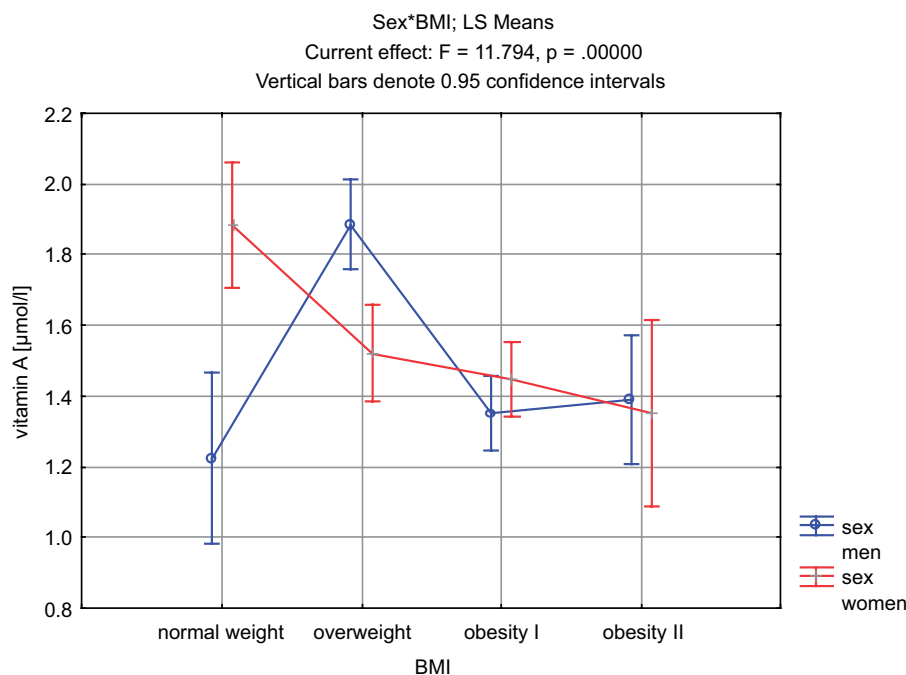


Fig. 2. Association between plasma vitamin A concentration, sex and BMI in patients with MS

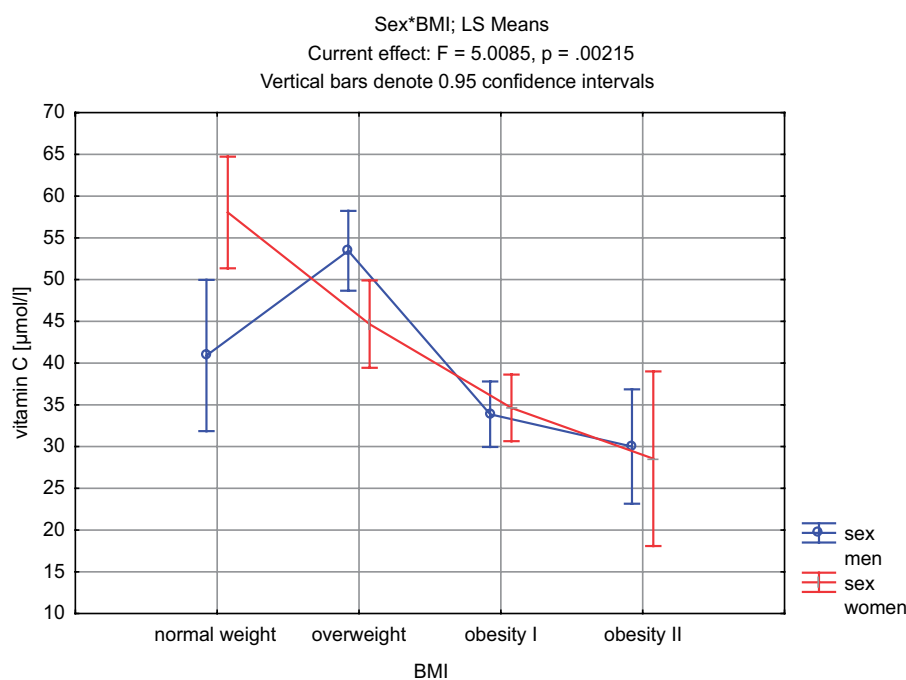


Fig. 3. Association between plasma vitamin C concentration, sex and BMI in patients with MS

weight or obesity according to BMI, diabetes, and hypertension (correlation coefficients at the level of 0.374, 0.412 and 0.497, respectively, $p < 0.001$) (Figs. 5–7).

Discussion

The prevalence of MS in Poland, and Europe in general, is high. It has been estimated that according to IDF criteria, 26% of people in Poland suffer from MS, and thus they are at increased risk of cardiovascular diseases which have been

the leading cause of death in this country for years [2, 15–17].

Patients with MS are at high risk of developing micro- and macrovascular abnormalities. Macroangiopathy-type complications are most often clinically manifested as coronary artery disease and stroke. These complications result from the damage to small and medium-sized vessels and this damage results from atherosclerotic lesions. MS, understood as a set of co-occurring risk factors for atherosclerosis, predisposes in a special way to the development of macrovascular disease [6]. The majority of recent reports, including those

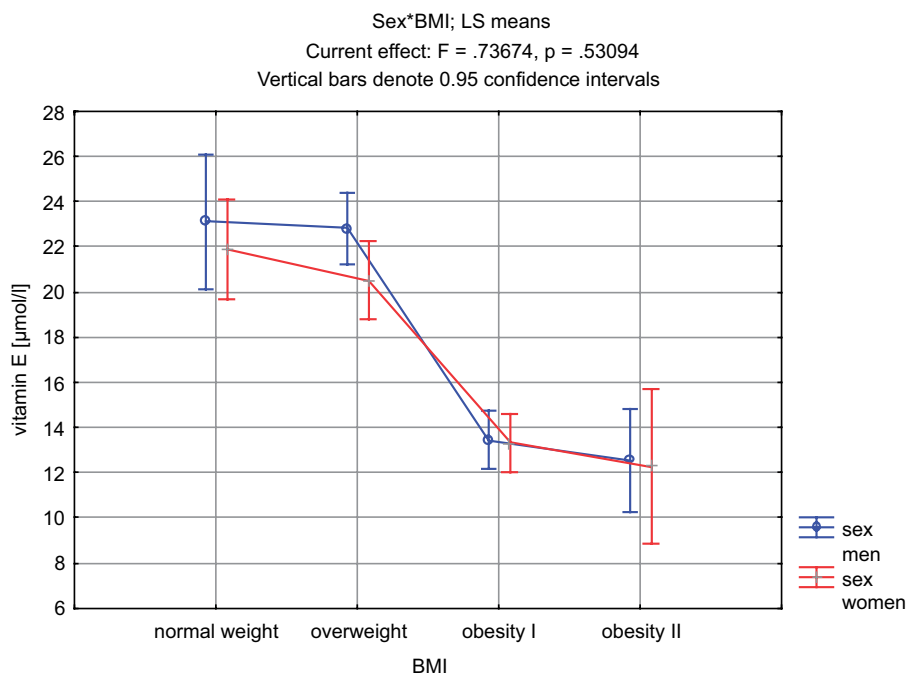


Fig. 4. Association between plasma vitamin E concentration, sex and BMI in patients with MS

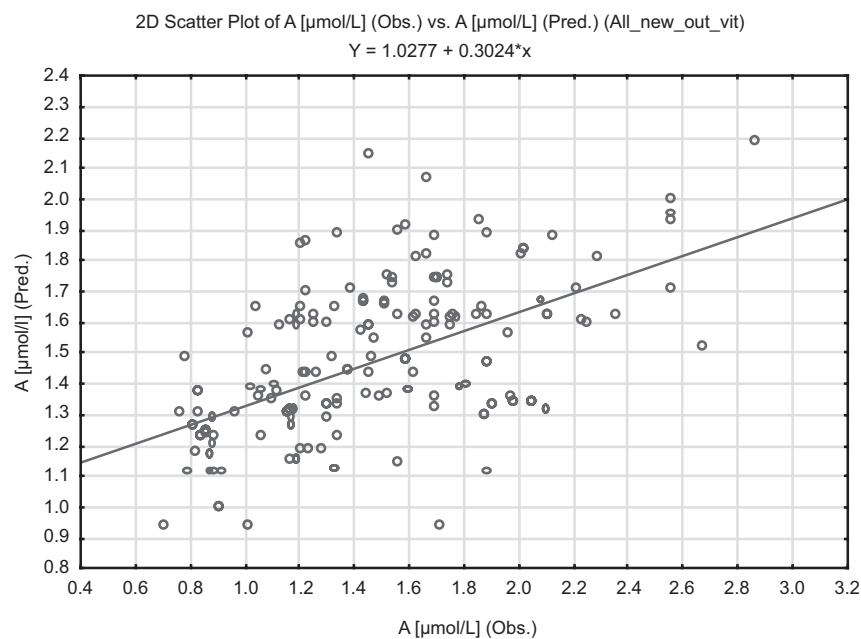


Fig. 5. Multivariate adaptive regression for the plasma level of vitamin A and its consumption with a diet, taking into account gender, the prevalence of overweight or obesity, diabetes, and hypertension in patients with MS

from large population studies such as the Framingham Offspring Study and the San Antonio Heart Study, indicate that the prevalence of MS is most associated with an increased risk of coronary artery disease. Similarly, reports are appearing more and more often about a significant role of MS and its components in the pathogenesis of microvascular complications so far associated mainly with diabetes. Changes in the fundus of the eye are the most common among microvascular complications in patients with MS. However, Kowalski et al. found a relationship between the severity of vascular lesions in the fundus of the eye in patients with MS and the incidence of coronary ar-

tery disease [18]. Thus, in the light of recent reports, early diagnosis and treatment of MS may be important in the prevention and delay of vascular complications.

In the circulatory system, ROS are generated by endothelial cells, vascular smooth muscle cells, fibroblasts and leukocytes. The negative effects of ROS in arterial vessels result from the change in endothelial permeability and lipid peroxidation. Moreover, free radicals induce hypertension due to the decreased synthesis and availability of NO which plays a role in the regulation of vascular smooth muscle tension. The mechanisms leading to endothelial dysfunction are complex and their

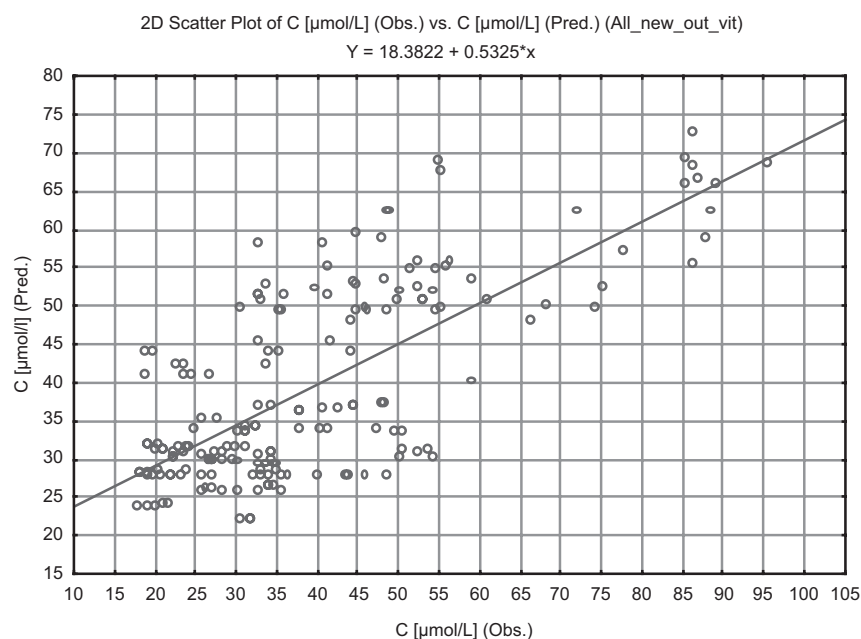


Fig. 6. Multivariate adaptive regression for the plasma level of vitamin C and its consumption with a diet, taking into account gender, the prevalence of overweight or obesity, diabetes, and hypertension in patients with MS

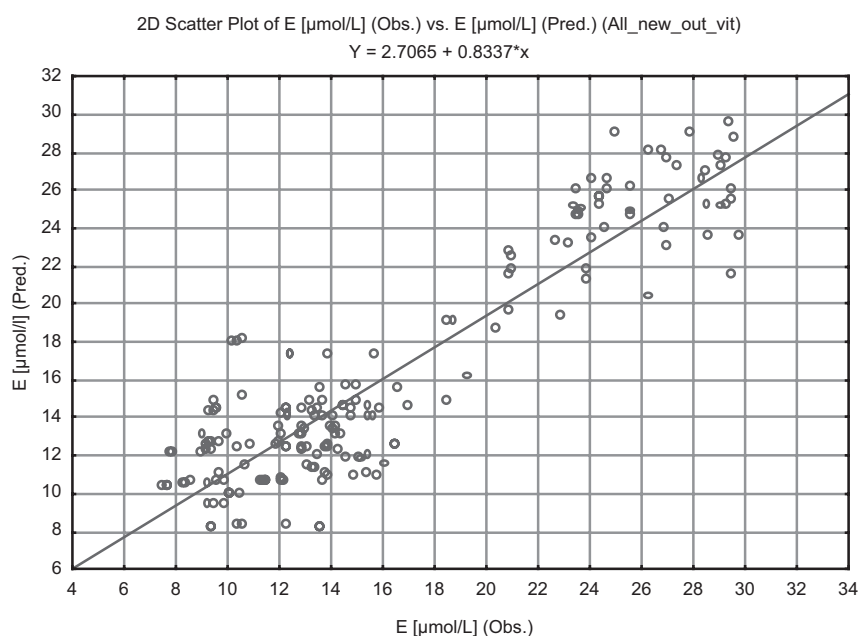


Fig. 7. Multivariate adaptive regression for the plasma level of vitamin E and its consumption with a diet, taking into account gender, the prevalence of overweight or obesity, diabetes, and hypertension in patients with MS

activity is multidirectional. In MS patients their effect may accumulate due to the impact of individual components of the metabolic syndrome and impair NO-dependent vasodilation [19]. There are reports confirming significantly lower NO concentration in patients with MS than in healthy controls, which indicates functional endothelial damage. Since antioxidant compounds, including vitamin E, A and C, belong to the first line of defense against ROS and can play a key role in the pathophysiology of these diseases, the determination of the concentration of antioxidants in patients with MS is justified [3–6].

In our study, MS patients demonstrated significantly lower plasma levels of vitamins E, A and C

compared to healthy subjects without MS. These results found confirmation in the research of other authors.

Analyzing the concentration of antioxidant vitamins in the plasma of patients with MS, Ford et al. observed significantly lower plasma levels of vitamins A, C and E in these patients than in healthy subjects without MS [20].

Moreover, the American NHANES study also confirmed significantly lower levels of vitamin C in patients with diagnosed MS than in the control group. Furthermore, this study demonstrated insignificantly higher plasma vitamin E levels in patients with MS compared to healthy subjects [21].

Sharma et al., in their study on an Indian population, showed significantly lower levels of vitamin A, C and E in the plasma of patients with MS, both in relation to the patients with two MS components and to clinically healthy subjects without MS [22].

These observations were confirmed by Odum et al. In the group of 100 Nigerian patients with MS they obtained significantly lower plasma concentrations of vitamin A, C and E compared to healthy individuals [23].

Examining the Brazilian population, in patients with type 2 diabetes, Illison et al. detected significantly lower plasma levels of vitamin E compared to the control group. Moreover, they demonstrated that low vitamin E levels correlated with waist circumference in the investigated group [24].

Singh et al. came to similar conclusions studying a group of 595 patients with diabetes and coronary artery disease. They observed a significantly lower plasma concentration of vitamin A, C and E in both groups than in the group of patients with no risk factors for cardiovascular diseases [25].

Cahill et al., examining Canadian adults, found a vitamin C deficiency in the plasma which correlated with the components of MS such as waist circumference and arterial blood pressure [26].

Obesity, especially the visceral type, is considered a classic risk factor for cardiovascular diseases, which was first demonstrated in the Framingham study [27]. It accelerates the development of atherosclerosis, modulates immunity through the production of pro-inflammatory substances and thus contributes to the development of chronic inflammation, which is one of the elements of atherogenesis. A lot of studies have confirmed the reverse correlation between antioxidant vitamin levels and BMI [16, 28–30]. There are works which have evaluated the concentrations of antioxidant vitamins in the plasma of subjects with an excessive content of adipose tissue, particularly located in the abdomen, which is considered a key element of MS.

Galan et al. reported significantly lower plasma levels of vitamin A and C and insignificantly lower plasma levels of vitamin E in the French adult population in a group of obese subjects compared to healthy individuals [31].

Ohrvall et al. drew similar conclusions in a study of patients with central obesity who presented significantly lower plasma vitamin E levels than those with normal body weight [32].

This was confirmed by Knekt et al. who found significantly lower levels of vitamin E in the plasma of an obese Finnish adult population in comparison to those with normal body weight [33].

Similarly, Sinha et al. found lower plasma levels of vitamin E in obese subjects compared to those of normal body weight [34].

In the study of Hebert et al., obese subjects had a significantly lower plasma level of vitamin E than those with normal body weight irrespective of balanced diet [35].

Furthermore, Wallstrom et al. also observed a plasma level of vitamin E which was significantly lower in the group of obese subjects than slim ones [36].

Our study confirmed the results of other authors, who showed decreased plasma levels of vitamin A, C and E in MS patients compared to healthy subjects, which resulted in a weakening of the body's defenses against ROS. The question whether the low plasma levels of antioxidant vitamins of patients with MS are the cause of metabolic disorders or their consequence still remains unanswered. In any case, from the prophylaxis point of view, this is an important problem in the prevention of complications associated with MS mainly of the cardiovascular system.

Epidemiological studies conducted in Poland and around the world have pointed to a correlation between the components of a daily diet and the incidence of cardiovascular diseases, including MS [37]. Although no set of specific therapeutic and prophylactic doses of vitamins A, C and E have been established in various diseases, most clinical studies indicate a beneficial effect of antioxidant vitamins on the quality and duration of life. Thus, a properly balanced diet in terms of energy content and nutrients, including antioxidant vitamins, can contribute to the improvement of one's health condition and to a decrease of the incidence of cardiovascular diseases.

However, taking into account the results of our studies, in which we showed a high mean intake of vitamins A, C and E in patients with MS (similar to the control group), the question should be asked about the cause of the low plasma level of antioxidant vitamins in these patients. In the literature there are studies indicating a reduced plasma level of vitamin A, C and E in these patients [31, 38–40].

There are several hypotheses explaining this phenomenon in patients with MS. Metabolic disorders characteristic of MS are accompanied by systemic increased oxidative stress, which is counteracted by vitamins or supplements consumed in the diet as well as other enzymatic mechanisms. Thus, in a group of patients characterized by severe oxidative stress, reduced levels of these vitamins can be expected due to their greater consumption in the process of neutralization of reactive oxygen species (ROS).

Oxidative stress also plays a role in the formation of atherosclerotic plaque. The concept of the mechanism of formation of atherosclerotic deposits in blood vessels assumes that plaques are formed by cells filled with lipids taken up from plasma lipoproteins damaged by ROS. As lipid disorders are one of the diagnostic criteria in patients with MS, it therefore can be assumed that despite the proper intake of antioxidant vitamins in the diet, their plasma concentration may be decreased [41].

Moreover, increased oxidative stress is a component of the pathogenesis of disorders of carbohydrate metabolism specific to patients with MS. Glucose, the molecule of which contains an aldehyde group, reacts with amino groups. As a result of this reaction, long-lived proteins undergo progressive non-enzymatic glycosylation. The amount of O₂⁻ produced due to oxidation of glucose and protein glycation end products is higher in the body of a diabetic patient [41].

In the literature, reports are also found indicating that antioxidants, such as vitamin A, C and E, may interact to protect against degradation or to promote regeneration. Thus, ascorbic acid participates in the regeneration of hydrophobic antioxidants, such as α -tocopherol and β -carotene from their radical forms. It is understood that this reaction takes place on the surface of cell membranes. Ascorbate reduces the tocopheryl radical, creating at the same time an ascorbyl radical. This reaction is possible because the tocopherol chroman group is oriented towards the surface of the cell membrane and can react with ascorbate in an aqueous medium [42]. Thus, in MS patients the reduced plasma concentration of vitamin C may result from the regeneration of other antioxidant vitamins.

The fact that the adipose tissue is the one that stores fat-soluble vitamins should also be taken into account. Thus, in patients with MS, among

whom obesity is the most common component, vitamins A and E move from plasma to the adipose tissue and their plasma concentration is reduced [31, 37, 38].

Clinical studies have confirmed that the use of antioxidant vitamin supplementation and therefore an increase in their plasma concentration leads to an inhibition of lipid oxidation, a decrease of the incidence of coronary heart disease and to a reduction of the death rate due to cardiovascular diseases [40, 43]. It is also known that a combination of these antioxidants may provide better antioxidant protection than a single antioxidant [44, 45].

Thus, compounds with an antioxidant effect can prevent cardiovascular diseases associated with atherosclerotic changes. Vitamin E saturating LDL particles were found to be more resistant to oxidation *in vitro* and endothelial cells and macrophages enriched with vitamin E were resistant to the cytotoxic effect of oxidized LDL. Thus, vitamin E may have a major impact on the stabilization of atherosclerotic plaque [46]. The World Health Organization conducted research studies which clearly showed that low levels of vitamin A, C and E could be even more important in the pathogenesis of cardiovascular diseases than classic risk factors for atherosclerosis, such as hypercholesterolemia and hypertension [47]. This is extremely important from the point of view of the observed cardiovascular complications in patients with MS.

MS patients demonstrate significantly lower plasma levels of vitamin E, A and C compared to healthy subjects; especially vitamin E. The lower plasma level of antioxidant vitamins with their high intake from the diet indicates an impairment of the antioxidant barrier in MS patients. The introduction of vitamin A, C and E supplementation in order to improve the antioxidative barrier should be considered in these patients.

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