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Antioxidant supplementation reduces inter-individual variation in markers of oxidative damage

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Abstract

The aim of this study was to examine the effect of antioxidant supplementation on oxidative damage and chromosome stability in middle-aged men, smokers and non-smokers. A total of 124 men aged 48 ± 6 years from Bratislava and from the rural population near Bratislava were investigated; 64 men (22 smokers and 42 non-smokers) were supplemented for 12 weeks with antioxidants, while 60 (25 smokers and 35 non-smokers) were given placebo. The daily antioxidant supplementation consisted of vitamin C (100 mg), vitamin E (100 mg), β-carotene (6 mg), and selenium (50 μg). Samples of blood were taken on two occasions: At the beginning and at the end of the supplementation trial. Concentrations of dietary antioxidants, ferric reducing ability, malondialdehyde as an indicator of lipid peroxidation in plasma, micronuclei and chromosome aberrations in lymphocytes were measured. Antioxidant supplementation significantly increased the levels of vitamin C, β-carotene, α-tocopherol and selenium in plasma. The overall antioxidant status of plasma measured as ferric reducing ability of plasma (FRAP) increased significantly ($p < 0.001$) after antioxidant supplementation as well. The increase in antioxidant parameters after supplementation were consistently more pronounced in non-smokers than in smokers. There was a significant decrease of malondialdehyde concentration in the non-smokers, while in smokers the decrease of malondialdehyde concentration was not significant. Antioxidant supplementation did not affect the proportion of lymphocytes with micronuclei or the total number of micronuclei; however, there was a significant positive correlation ($p < 0.001$) between the malondialdehyde concentration at the beginning of the supplementation trial and the difference in number of cells with micronuclei before and after the supplementation. The percent of cells with chromosome aberrations decreased significantly after antioxidant supplementation in smokers. These results indicate that a combined antioxidant supplementation (a) is effective even at very moderate doses; (b) significantly diminishes oxidative damage to lipids when it is high initially; and (c) is effective in decreasing chromosomal instability in lymphocytes of middle-aged men.

Keywords: *Antioxidant supplementation, oxidative damage, malondialdehyde, micronuclei, chromosome aberrations*

Abbreviations: *MN, Micronuclei; FRAP, Ferric reducing ability of plasma; MDA, Malondialdehyde; TBA, thiobarbituric acid*

Introduction

Reactive products of oxygen metabolism and the intermediate- and end-products of lipid peroxidation are thought to be involved in the aetiology of many degenerative diseases including atherogenesis, heart disease, cancer and inflammation [1,2]. We have investigated the effect of antioxidant supplementation on various biomarkers related to oxidative stress in middle-aged men, including survivors of myocardial infarction and apparently healthy subjects.

Nutritional antioxidants are thought to play an important role in cellular antioxidative defences.

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Vitamins E, C, β-carotene and selenium belong to the large class of dietary factors with the ability to act as antioxidants. Beneficial effects of antioxidant-rich fruits and vegetables in terms of reducing the risk of cancer and atherogenesis have been found in a number of epidemiological studies [3,4]. On the other hand, three large randomized trials failed to prove any beneficial effect of β-carotene supplementation among healthy men, or even showed a possible harmful effect among heavy smokers and asbestos workers [5].

Cigarette smoking is a recognized risk factor for both cancer and atherosclerosis. Tobacco smoke contains more than 4000 compounds, many of which are known to be genotoxic and carcinogenic agents, including polycyclic aromatic hydrocarbons, N-nitrosamines, and reactive oxygen species [6,7]. It is generally accepted that smoking can accelerate the consumption of stored antioxidant vitamins and increase oxidative stress.

Cytogenetic biomarkers in peripheral lymphocytes are frequently used to assess the exposure of humans to carcinogenic or mutagenic agents. Chromosome aberrations represent structural or numerical chromatid or isochromatid breaks and exchanges. Prospective epidemiological studies, in which higher levels of chromosome aberrations correlated significantly with later risk of cancer, showed that chromosome aberration frequency may be used as a predictive biomarker of cancer risk [8].

Micronuclei (MN) are small, additional nuclei formed by the exclusion of chromosome fragments or of whole chromosomes lagging during mitosis. They represent chromosome damage and may thus provide a marker of increased risk for early-stage carcinogenesis. Despite the well-known presence of carcinogens in tobacco smoke, data about the effect of smoking habit on MN frequency are quite confusing. In some studies there was an increase in the number of MN in smokers [9,10], while others failed to find any effect of smoking on MN frequency [11], or even detected a lower frequency of MN in smokers [12]. Bonassi et al. confirmed that smokers do not experience an overall increase in MN frequency, except of the group of heavy smokers [13].

Recently we published data about the effect of seasonal variation in antioxidant intake in different subgroups of middle aged men in Slovakia on various biomarkers [14]. We also studied the effect of supplementation on genetic stability in these subgroups of men [15]. The aim of this study was to show the effect of antioxidant supplementation on markers of lipid oxidation and genetic stability in middle-aged men, taking account of differences in smoking habits. The underlying hypothesis is that the effectiveness of supplementation is likely to be influenced by the initial values of the biomarkers.

Materials and methods

Subjects and supplementation

The results presented here are part of a large study concerned with the effects of seasonal variations in dietary antioxidant intake in Slovakia. Volunteer middle-aged men from Bratislava and from the rural population near Bratislava participated. The study group, chosen to reflect the Slovak population, comprised 37 normolipidemics (7 smokers and 30 non-smokers), 57 apparently healthy subjects from a rural area, some with mild hyperlipidaemia (34 smokers and 23 non-smokers) and 30 subjects after recovery from myocardial infarction (6 smokers and 24 non-smokers). A total of 124 men with a mean age of 48 ± 6 years were investigated; 64 men were supplemented with antioxidants (22 smokers and 42 non-smokers), while 60 were given placebo (25 smokers and 35 non-smokers) for 12 weeks. The distribution of participants in the subgroups is shown in Table I.

The antioxidant supplement consisted of vitamin C (100 mg), vitamin E (100 mg), β-carotene (6 mg) and selenium (50 μg), while the placebo consisted of 0.5 g glucose each day. Samples of blood were taken on two occasions; at the beginning and at the end of the supplementation period.

Plasma vitamin C

Samples for vitamin C analysis were stored frozen after acidification by addition of an equal volume of 10% metaphosphoric acid. Vitamin C was measured by HPLC using an ionpairing technique [16].

Plasma β-carotene, α-tocopherol

Samples for these analyses were stored frozen and non-acidified. Reverse phase HPLC was performed

Table I. Distribution of subjects according to population source, smokers/non-smokers and receiving placebo or antioxidants.

	Post myocardial infarction	Normolipidemics	Rural subjects
Supplemented smokers	1 (47)	4 (48.3 ± 6.7)	16 (45.3 ± 1.7)
Supplemented non-smokers	16 (55.5 ± 1.8)	13 (49.5 ± 1.5)	14 (46.7 ± 1.7)
Placebo smokers	5 (55.2 ± 2.3)	4 (48.3 ± 4.4)	15 (44.3 ± 1.3)
Placebo non-smokers	8 (53.8 ± 2.4)	16 (48.0 ± 1.5)	12 (44.9 ± 2.5)

Mean ages (± SEM) are shown in parenthesis.

to measure the concentration of β-carotene and α-tocopherol, according to Hess et al. [17].

Serum selenium

Selenium was measured in serum (after dilution with 0.2% Triton X-100, 0.1% Pd and 1% ascorbic acid) by electrothermal atomic absorption spectrometry [18].

Ferric reducing ability of plasma (FRAP)

The index of combined antioxidant status of non-enzymic defences was measured spectrophotometrically according to Benzie and Strain [19]. Ferric to ferrous ion reduction at low pH causes a coloured ferrous-tripyridyltriazine complex to form. FRAP values were obtained by comparing the absorbance changes at 593 nm.

Malondialdehyde (MDA)

MDA levels in plasma were determined by an HPLC method [20]. This assay of plasma lipoperoxides involves hydrolysis in dilute H_3PO_4 at 100°C; complexing of MDA, a hydrolysis product, with thiobarbituric acid (TBA); methanol precipitation of plasma proteins; fractionation of the protein-free extract on a C18 column; and spectrophotometric quantification of the MDA-TBA adduct at 532 nm.

Lymphocyte cultures

Samples of whole blood were taken from all subjects at 0 and 12 weeks. Short-term lymphocyte cultures were set up by adding 0.5 ml whole blood to 4.5 ml of RPMI medium with L-glutamine (Gibco) supplemented with 20% fetal calf serum (Gibco) and antibiotics (penicillin and streptomycin). The cells were stimulated by phytohaemagglutinin (Murex) and incubated at 37°C and 5% CO_2. Two cultures of each sample were set up.

Chromosomal aberrations

Cells were harvested at 48 h following stimulation; colchicine (0,75 μl/ml) (Sigma) was added 2 h before harvest. The cultures were centrifuged and subjected to a hypotonic shock of 20 min in 0.075 M KCl at 37°C. The lymphocytes were fixed twice in acetic acid: methanol (1:3) and air-dried preparations were made. The slides were stained with 5% aqueous Giemsa solution for 5 min. A total of 100 well-spread metaphases per person were examined. All the basic chromosomal abnormalities, chromatid and chromosome gaps, breaks and exchanges were recorded. Owing to controversies over their classification, gaps were not included among aberrant cells. Chromosomal damage was expressed as percent of aberrant cells and number of breaks per cell.

Micronucleus (MN) test

Cytochalasin B (Sigma) (final concentration 6 μg/ml) was added 44 h after the start of culture and at 72 h of incubation, cells were centrifuged, resuspended in 0.075 M KCl and immediately centrifuged again and fixed twice with fixative (acetic acid-methanol, 1:3). The fixed cells were dropped onto slides, air dried and stained with 5% aqueous Giemsa solution for 5 min. Cytochalasin B inhibits cytoplasmic cleavage without preventing mitosis. Thus cells that have divided are readily identified by the presence of two nuclei. MN analysis was performed on 2000 binucleated lymphocytes with preserved cytoplasm for each subject. MN were accepted only when they were morphologically identical to, but smaller than, normal nuclei; had diameters between 1/16 and 1/3 of the main nuclei; were non-refractile; and were not linked to the main nuclei via a nucleoplasmatic bridge though they might sometimes overlap the boundaries of the main nuclei [21].

Statistical analysis

SPSS 11.5 software was used for statistical analysis of data. The data are expressed as means ± SEM. Normality of distribution was tested by the Kolmogorov-Smirnoff test. Equality of variance was tested by Levene's test. Paired samples *T*-test or Wilcoxon Signed-Rank Test (for not normally distributed data) was used for paired values testing (before/after supplementation comparisons). For comparisons between groups we used the Independent samples *T*-test in the case of normally distributed data, and the Mann-Whitney *U*-test for data not normally distributed. A p value < 0.05 was defined as significant.

Multifactorial analysis of variance was applied to identify association between dependent variables (changes in biomarkers after supplementation) and predictors (the 3 different subgroups [post myocardial infarction, normolipidemics, rural], supplementation [yes or no], smoking [yes or no], age, and interactions).

Results

There were no significant differences in the background concentrations of antioxidants or FRAP between the placebo and supplemented group at the beginning of the study. Taking placebo did not influence significantly the levels of antioxidants in the plasma of volunteers, except for the selenium concentration, which increased significantly ($p < 0.05$) in the whole group (smokers and non-smokers combined) (Table II), and in the group

Table II. Effects of supplementation on plasma levels of antioxidants.

Antioxidant		*n*	Before	After	*p* <
Vitamin C (μM)	Supplementation	44	41.78 ± 2.66	61.39 ± 2.95	0.001
	Placebo	36	40.78 ± 3.50	47.80 ± 3.18°	ns
β-Carotene (μg/ml)	Supplementation	42	0.19 ± 0.01	0.32 ± 0.04	0.001
	Placebo	44	0.15 ± 0.02	0.16 ± 0.02°	ns
α-Tocopherol (μg/ml)	Supplementation	33	14.7 ± 0.73	18.63 ± 1.37	0.01
	Placebo	32	13.50 ± 1.09	14.96 ± 1.07°	ns
Selenium (μg/l)	Supplementation	62	86.74 ± 1.87	114.20 ± 2.31	0.001
	Placebo	59	88.87 ± 1.71	93.25 ± 1.91°	0.05
FRAP (μM)	Supplementation	64	1073.68 ± 24.44	1287.10 ± 27.43	0.001
	Placebo	59	1047.45 ± 26.61	1161.90 ± 34.70°	0.001

Mean concentrations are shown, with SEM: n = number of samples, p = probability (before supplementation vs. after supplementation, tested by paired samples t-test for normally distributed data or by Wilcoxon Signed-Rank Test for not normally distributed data), ° = significant difference between supplemented group and placebo ($p < 0.05$), tested by Independent samples t-test in case of normally distributed data, and Mann-Whitney U-test for data not normally distributed.

of smokers (Table III). In placebo-treated subjects, FRAP was significantly increased; this was true for non-smokers when analysed separately ($p < 0.001$, Tables II, IV), though not for smokers. Supplemented subjects had significantly higher concentrations of antioxidants (vitamin C, β-carotene, α-tocopherol, selenium) and FRAP at the end of the study (Table II). When analysed separately, supplemented smokers and non-smokers had also significantly higher concentrations of antioxidants and FRAP at the end of the study, with the exception of α-tocopherol concentrations (Tables III, IV). The increase in antioxidants after supplementation were more pronounced in non-smokers than in smokers. Baseline levels of antioxidants did not differ between smokers and non-smokers, either at the beginning or at the end of supplementation (Tables III, IV).

MDA levels before the intervention period were significantly higher in those non-smokers who subsequently took supplements compared with non-smokers in the placebo group, and compared with supplemented smokers ($p < 0.01$) (Figure 1). Antioxidant supplementation caused a significant decrease in the concentration of MDA in plasma of non-smokers, but did not affect significantly the level of MDA in the smokers (Figure 1). The delta values for the placebo and supplemented groups were also significantly different ($p = 0.02$). Multifactorial analysis of variance showed that the difference in MDA levels was significantly associated with the type of the subgroup but only when vitamin supplementation was included in the model.

The change in proportion of MN-positive cells and in total number of MN in lymphocytes after supplementation was not significant in any group; nor were there significant differencies between smokers and non-smokers (Tables V, VI).

There was a significant correlation ($p < 0.001$) between the MDA concentration before supplementation and the difference in number of MN-positive cells before and after supplementation (Figure 2)—i.e. the higher the MDA concentration before supplementation began, the bigger the decrease in MN apparently resulting from supplementation. This correlation when applied to the placebo group, was not significant ($p = 0.172$).

At the beginning of the study, lymphocytes of the non-smokers contained slightly higher percent of

Table III. Effect of supplementation on plasma levels of antioxidants in smokers.

Antioxidants		*n*	Before	After	*p* <
Vitamin C (μM)	Supplementation	13	37.82 ± 5.44	57.35 ± 5.27	0.05
	Placebo	15	35.12 ± 6.35	40.68 ± 4.86°	ns
β-Carotene (μg/ml)	Supplementation	16	0.20 ± 0.03	0.30 ± 0.05	0.05
	Placebo	18	0.13 ± 0.03	0.12 ± 0.03°	ns
α-Tocopherol (μg/ml)	Supplementation	13	14.39 ± 1.50	17.21 ± 1.66	ns
	Placebo	12	10.37 ± 1.34	13.15 ± 2.05	ns
Selenium (μg/l)	Supplementation	21	87.38 ± 3.27	108.65 ± 3.42	0.001
	Placebo	24	84.05 ± 2.03	93.47 ± 3.24°	0.01
FRAP (μM)	Supplementation	22	1068.91 ± 41.59	1264.71 ± 56.63	0.001
	Placebo	24	1098.90 ± 46.34	1155.54 ± 53.03	ns

Mean concentrations are shown, with SEM: n = number of samples, p = probability (before supplementation vs. after supplementation, tested by paired samples t-test for normally distributed data or by Wilcoxon Signed-Rank Test for not normally distributed data), ° = significant difference between supplemented group and placebo ($p < 0.05$), tested by Independent samples t-test in case of normally distributed data, and Mann-Whitney U-test for data not normally distributed.

Table IV. Effect of supplementation on plasma levels of antioxidant in non-smokers.

Antioxidants		*n*	Before	After	$p <$
Vitamin C (μM)	Supplementation	31	43.45 ± 3.02	63.09 ± 3.56	0.001
	Placebo	21	44.82 ± 3.82	52.88 ± 3.92°	ns
β-Carotene (μg/ml)	Supplementation	26	0.18 ± 0.01	0.35 ± 0.05	0.001
	Placebo	26	0.17 ± 0.02	0.19 ± 0.03°	ns
α-Tocopherol (μg/ml)	Supplementation	20	14.91 ± 0.73	19.56 ± 2.00	0.05
	Placebo	20	15.38 ± 1.42	16.04 ± 1.18	ns
Selenium (μg/l)	Supplementation	41	86.41 ± 2.30	117.04 ± 2.95	0.001
	Placebo	35	92.17 ± 2.39	93.09 ± 2.36°	ns
FRAP– (μM)	Supplementation	42	1076.17 ± 30.56	1298.84 ± 29.86	0.001
	Placebo	35	1012.17 ± 30.82	1166.25 ± 46.43°	0.001

Mean concentrations are shown, with SEM: n = number of samples, p = probability (before supplementation vs. after supplementation, tested by paired samples t-test for normally distributed data or by Wilcoxon Signed-Rank Test for not normally distributed data), ° = significant difference between supplemented group and placebo ($p < 0.05$), tested by Independent samples t-test in case of normally distributed data, and Mann-Whitney U-test for data not normally distributed.

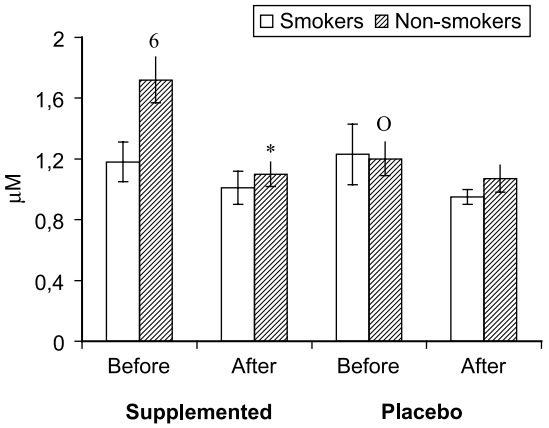

Figure 1. Effect of supplementation on the concentration of MDA in plasma. Open bars represent smokers, hatched bars represent non-smokers. Comparison before vs. after supplementation: *–$p <$ 0.001, tested by paired samples t-test for normally distributed data or by Wilcoxon Signed-Rank Test for not normally distributed data. Comparison smokers vs. non-smokers ^{6}–$p < 0.01$; Comparison supplemented group vs. placebo: °–$p < 0.001$; both comparisons were tested by Independent samples t-test in case of normally distributed data, and Mann-Whitney U-test for data not normally distributed. Mean values are shown, with SEM.

aberrant cells than smoker's lymphocytes, although the difference was not significant. Antioxidant supplementation induced a decrease in the percent of aberrant cells in both groups, but significantly ($p < 0.05$) only in the group of smokers (Figure 3). Placebo smokers had a significantly higher percent of aberrant cells than the supplemented smokers by the end of the study ($p < 0.001$). Changes in percent of aberrant cells were not significant in the group of placebo smokers or non-smokers after taking placebo. Multifactorial analysis of variance showed that the change in percent of aberrant cells was significantly associated with smoking, and with the interaction between vitamin supplementation and smoking.

Discussion

There is much discussion about recommended doses of vitamin supplements. Quite apart from the biological significance of the changes that supplementation can cause, it is difficult to set an exact dose because of the interindividual differencies in antioxidant status caused by differences in nutrition, life style and genetic background. However, as the highly significant increases in plasma antioxidants (vitamin C, β-carotene, α-tocopherol and selenium) in our study show, supplementation with relatively low doses of antioxidants can be very effective. Not only did we find increased concentrations of these supplemented

Table V. Number of cells with micronucleus (per 2000 cells).

		n	Before	After	*p*
Smokers	Supplementation	19	12.53 ± 1.54	14.68 ± 2.31	ns
	Placebo	19	8.26 ± 1.44°	13.95 ± 2.89	ns
Non-smokers	Supplementation	28	16.36 ± 1.32	12.43 ± 1.67	ns
	Placebo	18	12.11 ± 1.38	12.44 ± 1.87	ns

Mean concentrations are shown, with SEM: n = number of samples, p = probability (before supplementation vs. after supplementation, tested by paired samples t-test for normally distributed data or by Wilcoxon Signed-Rank Test for not normally distributed data), ° = significant difference between supplemented group and placebo ($p < 0.05$), tested by Independent samples t-test in case of normally distributed data, and Mann-Whitney U-test for data not normally distributed.

Table VI. Number of micronuclei (per 2000 cells).

		n	Before	After	*p*
Smokers	Supplementation	19	13.95 ± 1.75	17.00 ± 2.93	ns
	Placebo	19	8.90 ± 1.54°	16.16 ± 3.70	ns
Non-smokers	Supplementation	28	18.46 ± 1.63	14.14 ± 1.93	ns
	Placebo	18	14.06 ± 1.72	13.72 ± 2.16	ns

Mean concentrations are shown, with SEM: n = number of samples, p = probability (before supplementation vs. after supplementation tested by paired samples t-test for normally distributed data or by Wilcoxon Signed-Rank Test for not normally distributed data), ° = significant difference between supplemented group and placebo ($p < 0.05$), tested by Independent samples T-test in case of normally distributed data, and Mann-Whitney U-test for data not normally distributed.

vitamins, but there was also a corresponding elevation of FRAP, an indicator of total antioxidant capacity of plasma. It was shown that there are seasonal variations in the natural antioxidant consumption in Slovakia, it being lower in the winter/spring period than in summer/autumn [22,23]. The choice of the period February to March for this supplementation trial was based on that information, and probably accounts for the strength of the effects we observed. According to our expectations, the improvement in antioxidant status in the group of smokers was more moderate than in the group of non-smokers. It suggests that smokers may need to take more supplements than non-smokers to have the same effect. Thus, these results support the general recommendation that smokers should supplement themselves with vitamins or consume more foods rich in antioxidants.

Both human and experimental studies have described beneficial effects of antioxidant supplementation on lipid peroxidation [24,25]. In our study, the decrease in MDA concentration after antioxidant supplementation was significantly lower only in the group of non-smokers, who had significantly higher mean MDA concentration at the beginning of the study than did smokers. Our results are consistent with the report of Chao et al. [26], who found no significant differences in antioxidant enzyme activities or in the amount of LDL lipid peroxides between two groups of hyperlipidemic smokers supplemented with different doses of antioxidants (the higher dose being twice the lower one). The majority of studies report higher MDA concentrations in smokers compared to non-smokers. On the other hand, Jacob et al. [27] did not detect any differences in the biomarkers of lipid peroxidation between groups of healthy non-smokers and heavy smokers, except for TBA-reactive substances measured in urine, which were higher in smokers than in non-smokers. In relatively healthy men, apparently, the endogenous antioxidant defence system supported with a moderate dietary intake of antioxidants is adequate to maintain relatively low levels of lipid peroxidation, even in smokers. Antioxidant supplementation could therefore give significant results only in the case of higher lipid peroxidation—as was the case with the group of non-smokers in this study. In our study, the reason for

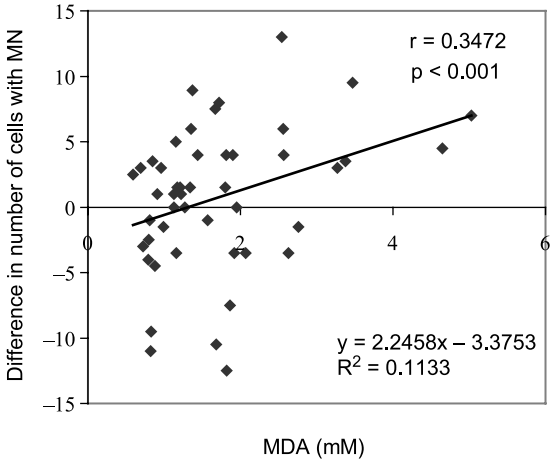

Figure 2. Correlation between the concentration of MDA before supplementation and the difference in number of cells with MN (per 2000 cells) after supplementation, tested by Pearson's correlation for normally distributed data.

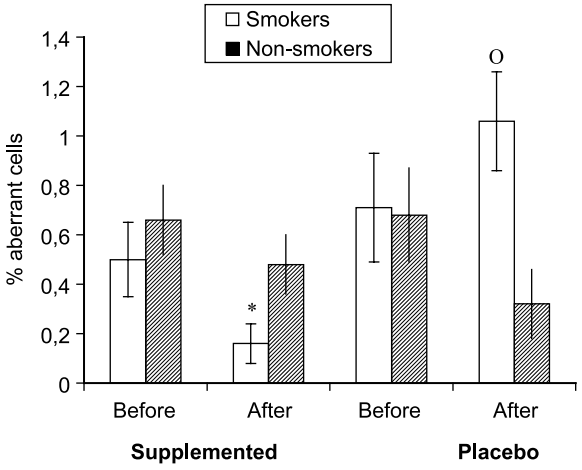

Figure 3. Chromosome aberrations in lymphocytes before and after supplementation Mean values are shown, with SEM. Open bars represent smokers, hatched bars represent non-smokers. Comparison before vs. after supplementation: *–$p < 0.05$, tested by paired samples t-test for normally distributed data or by Wilcoxon Signed-Rank Test for not normally distributed data. Comparison supplemented group vs. placebo: °–$p < 0.001$, tested by Independent samples t-test in case of normally distributed data, and Mann-Whitney U-test for data not normally distributed.

higher MDA concentrations in the group of non-smokers might have been a not entirely even distribution of subjects from the different subgroups: There were more non-smokers among subjects from the subgroup after myocardial infarction. Multifactorial analysis indeed showed that non-smokers from the group of myocardial infarction survivors had higher MDA concentration in the beginning of the study ($p < 0.001$).

Smokers tended to have a lower frequency of MN than non-smokers at the beginning of the study (although it was not significant).This is not entirely contradictory to findings of other researchers: In most reports there were no statistically significant differences in the frequency of MN between smokers and non-smokers, and in many instances smokers had lower MN frequencies [13]. Bonassi et al. [13] performed pooled re-analysis of 24 databases with the aim of understanding the impact of smoking habit on MN frequency and concluded that smokers do not show an overall increase in this parameter unless they are heavy smokers (smoking $\geq$ 30 cigarettes a day). In the present study, MN frequency was in the range of normal values, and that could be the reason why antioxidant supplementation did not affect it significantly in any of the investigated groups.

The significant positive correlation between MDA concentration before supplementation and the difference in number of cells with MN before and after supplementation indicates that the higher the MDA concentration before supplementation, the bigger the change in the MN frequency. Figure 2 shows that there are some values in the negative area of the diagram, which means an increase in MN frequency; however, this occurred only at lower MDA concentrations before supplementation.

Because of the predictive value of chromosome aberrations in terms of cancer risk [28], we regard the decrease in this biomarker after antioxidant supplementation to be a valuable confirmation of the beneficial effect of such moderate supplementation. Although the decrease in chromosomal aberrations was significant only in the group of smokers, in non-smokers the decrease was also quite substantial.

In conclusion, our study shows that supplementation with a combination of several antioxidants (a) is effective already at low doses; (b) diminishes oxidative lipid damage significantly when it is high initially; and (c) is effective in decreasing genetic instability in lymphocytes of middle-aged men.

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