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Antioxidative vitamins and oxidative lipid and DNA damage in relation to nutrition

Marica Krajcovicova-Kudlackova, Martina Valachovicova, Csilla Mislanova, Jana Pribojova

Slovak Medical University, Bratislava, Slovakia

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Corresponding Author
Marica Krajcovicova-Kudlackova
Slovak Medical University,
Limbova 12,
833 03 Bratislava, Slovakia.
marica.kudlackova@szu.sk

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Abstract
Nutrition is a key environmental factor influencing the incidence of chronic age related free radical induced diseases. The better antioxidant status by a sufficient consumption of food commodities with antioxidant compounds helps to minimize oxidative damage, and thus can delay or prevent pathological changes. In three groups of adult apparently healthy non-smoking population of different nutritional habits (93 persons of general population, non-vegetarians, NV; 77 semi-vegetarians, V-S; 64 lacto-ovo-vegetarians, V-LO) plasma concentrations of vitamins B9, C, E and β-carotene, potential prooxidant homocysteine and products of oxidative damage of lipids (total peroxides, malondialdehyde, oxidized LDL) as well as and oxidative DNA damage in lymphocytes (strand breaks, oxidized purines, oxidized pyrimidines) were measured. Vegetarian (V-S, V-LO) *vs* NV plasma concentrations of vitamin C, vitamin E, lipid standardized vitamin E, β-carotene and vitamin B9 were significantly higher. Plasma total homocysteine values are significantly higher in both vegetarian groups as a consequence of deficient vitamin B12 concentrations. Hyperhomocysteinemia, a marker of systemic or endothelial oxidative stress may promote the production of hydroxyl radicals which are the initiators of lipid oxidation. In spite of 28.5% and 40.6% incidence of hyperhomocysteinemia in V-S and V-LO, the concentrations of total peroxides, malondialdehyde as well as values of oxidized LDL were significantly reduced as a consequence of a better antioxidant status. Parameters of DNA damage as biomarkers of cancer risk in epidemiological studies are significantly reduced in two groups of vegetarians. The findings suggest that dominant consumption of plant food in vegetarians, higher intake of dietary antioxidants and their higher plasma concentrations in comparison to general population may have protective effects against oxidative damage of molecules and a preventive effect on incidence of chronic age related diseases.

INTRODUCTION

The global prevalence of chronic diseases such as atherosclerosis, diabetes mellitus, hypertension, cancer is continually increasing. These diseases, which constitute the major causes of death globally, are associated with oxidative stress. Severe oxidative stress progressively leads to cell dysfunction and ultimately cell death. Oxidative stress is defined as an imbalance between pro-oxidants or free radicals on one hand, and anti-oxidizing systems on the other. Deleterious effects of oxygen are due to the production of free radicals, which are toxic for the cells (superoxide anions, hydroxyl radicals, peroxyl radicals, hydrogen peroxide, hydroperoxides and peroxinitrite anions). Free radical attacks are responsible for cell damage and the targeted cells are represented by the cell membranes, which are particularly rich in polyunsaturated fatty acids sensitive to oxidation; DNA is also the target of severe attacks by the reactive oxygen species [1].

Antioxidant is any substance that delays, prevents or removes oxidative damage to a target molecule [2]. Humans have evolved a variety of mechanisms to protect from the potentially deleterious effects of free radicals. These include endogenous antioxidants (superoxide dismutases, hydrogen peroxide-removing enzymes, metal binding proteins) which are inadequate to prevent damage completely, so that diet-derived antioxidants are important in maintaining health. From nutrition view, the antioxidant vitamins play a significant protective role. In long-term, epidemiological and clinical studies determined the protective plasma values of antioxidant vitamins on the base of correlations between occurrence of disease or risk markers for its genesis on one hand and plasma vitamin values on the other. The lower limit of protective values was called as a threshold value [3]. The plasma vitamin concentrations can be easily favourable influenced by correct nutritional habit to attainment and maintenance of protective values [4].

The nutrition is a key environmental factor implicated in health and disease [5, 6]. Improved antioxidant status helps to minimize oxidative damage, thus can delay and prevent pathological changes [7, 8]. This suggests the possible utility of antioxidant-based dietary strategies for lowering of chronic age-related, free radical induced disease risk. Food naturally containing antioxidants but not super-rich in calories, namely fruit, vegetables, nuts, seeds and cereal grains help to maintain human health and delay disease onset.

The aim of this study was to assess the blood concentrations of antioxidative vitamins in relation to selected prooxidants and oxidative damage products in healthy population of three different nutritional habits.

SUBJECTS AND METHODS

Randomly selected apparently healthy adult non-smoking non-obese 234 subjects (100 men, 134 women) aged 20-60 years were divided into three groups depending on the nutritional habit, which was detected according to the data from standardized and validated dietary questionnaires (intake of 146 food items).

Group I: 93 subjects of general population on traditional mixed diet (non-vegetarians, NV; 42 men, 51 women).

Group II: 77 long-term semi-vegetarians who consumed plant food, dairy products, eggs, white meat and fish (V-S; 32 men, 45 women).

Group III: 64 long-term lacto-ovo-vegetarians who consumed dominantly plant food with addition of dairy products and eggs (V-LO; 26 men, 38 women).

All subject lived in the same region (Bratislava, Slovakia, and surroundings). The volunteers have an approximately similar physical activity (no sports). The group characteristic is showed in Table 1. The results were produced by realization of project about health benefits and risks of alternative forms of nutrition vs. general population as the control group. The Regional Ethic Committee approved this study, and all participants gave their written informed consent.

Blood was sampled after an overnight fasting by a standard procedure. Plasma concentrations of vitamins C, E and β-carotene were measured by high-performance liquid chromatography (HPLC) [9, 10]. EDTA was used as an anticoagulant. Serum concentration of total cholesterol was measured by standard laboratory method on Vitros 250 autoanalyzer (Johnson & Johnson, New York, NY, USA). Serum folic acid concentration was determined using Elecsys 2010 System (Roche Diagnostics, Basel, Switzerland). Plasma concentrations of total homocysteine were measured by HPLC method with electrochemical detection [11]. Total peroxides in plasma (direct correlation between free radicals and circulating biological peroxides) were detected by OxyStat colorimetric assay for the quantitative determination of peroxides in EDTA plasma, serum and other biological fluids (reaction of biological peroxides with peroxidase and photometric measurement of produced coloured liquid) (Biomedica Medizinprodukte, Vienna, Austria). Malondialdehyde in plasma were assessed by HPLC [12]. Oxidized LDL concentration in serum was measured by a commercial oxidized LDL ELISA kit (Mercodia, Uppsala, Sweden).

Table 1. Characteristic of groups

	NV	V-S	V-LO
n (men + women)	93 (42 + 51)	77 (32 + 45)	64 (26 + 38)
Average age (year)	40.1 ± 1.2	41.6 ± 1.2	39.2 ± 1.6
BMI (kg/m^2)	24.3 ± 0.5	23.3 ± 0.5	22.7 ± 0.5*****
Range	18.4-29.9	17.8-29.3	17.7-28.8
>25	31%	18%	16%
>30	0	0	0
Duration of vegetarianism (year)	-	12.1 ± 0.8	12.0 ± 0.9

NV, non-vegetarians; V-S, semi-vegetarians; V-LO, lacto-ovo-vegetarians; *****$p < 0.05$

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The alkaline comet assay modified with lesion-specific enzymes was used for detection of DNA strand breaks, oxidized purines and oxidized pyrimidines in isolated lymphocytes. For detection of oxidized purines slides were incubated with formamidopyrimidine glycosylase; oxidized pyrimidines were detected after incubation with endonuclease III. Comets were analyzed by visual scoring of 100 randomly selected images per gel, classifying them into five categories representing relative tail intensity and thus increasing degrees of damage. This method was calibrated by reference to computer image analysis based on fluorometric measurement of DNA intensities in head and tail [13, 14].

The intake of vitamins, mineral and trace elements in natural form only was allowed (no supplementation). The study was carried out during spring and the similar amounts of NV, V-S, V-LO were investigated weekly. The quantitative data are presented as means ± SEM. The significance of differences in measured values between groups was determined by unpaired Student´s t-test; p values less than 0.05 were considered to indicate statistical significance.

RESULTS AND DISCUSSION

Plasma values of antioxidant vitamins are introduced in Table 2. We recorded the significantly higher plasma concentrations of vitamin C, vitamin E, lipid standardized vitamin E and β-carotene in both alternative nutrition groups V-S and V-LO *vs* NV. Vegetarian values of vitamin C, vitamin E/cholesterol and β-carotene are over-threshold, protective from view of free radical risk (> 50 μmol/l vitamin C, > 0.4 μmol/l β-carotene, > 5.2 μmol/mmol vitamin E/cholesterol). They may mean a sufficient antioxidant capacity together with other substances with antioxidant properties derived from intake of fruit and vegetables as well as other commodities containing antioxidants.

In a previous study we calculated from dietetic questionnaires [4], that vegetarians *vs* non-vegetarians consume daily 463 g *vs* 176 g fruit, 195 g *vs* 62 g vegetables, 242 g *vs* 65 g whole grain products, 29 g *vs* 7 g oil seeds, 63 g *vs* 32 g plant oils, and 38 g *vs* 6 g soya products. In the work of Waldmann *et al* [15] a high value of the mean vitamin E/cholesterol ratio in vegans (5.97) was reported. In the V-S and V-LO groups of our study these values are also protective (> 5.2) indicating a good protection of LDL against peroxidation.

New literature data demonstrate an antioxidant activity of folates and their beneficial effects in free radical scavenging [16]. Folic acid and, in particular, its reduced derivates produce antioxidant effects directly or indirectly. Folates interact with the endothelial enzyme nitric oxide (NO) synthase, influence the cofactor bioavailability of NO and peroxynitrite formation. The present results revealed that both groups of vegetarians (V-S, V-LO) have significantly increased blood vitamin B9 concentrations in comparison to NV (Table 2).

Table 2. Concentrations of antioxidant vitamins

	NV	V-S	V-LO
Vitamin C (μmol/l)	42.7 ± 2.4	55.6 ± 2***	64.2 ± 1.9***
Vitamin E (μmol/l)	22.6 ± 0.8	25.1 ± 0.9*	24.4 ± 0.7**
Total cholesterol (mmol/l)	5.24 ± 0.09	4.88 ± 0.09**	4.63 ± 0.14***
Vitamin E/total cholesterol (μmol/mmol)	4.37 ± 0.15	5.27 ± 0.18***	5.61 ± 0.2***
β-carotene (μmol/l)	0.43 ± 0.06	0.78 ± 0.08***	0.87 ± 0.08***
Vitamin B9 (nmol/l)	15.2 ± 0.6	26.8 ± 1***	27.4 ± 0.9***

*p < 0.05; **p < 0.01; ***p < 0.001

Table 3. Concentrations of homocysteine and products of oxidative lipid and DNA damage

	NV	V-S	V-LO
Homocysteine (μmol/l)	11.3 ± 0.3	13.5 ± 0.4**	15.4 ± 0.7***
Total peroxides (μmol/l)	413 ± 25	347 ± 18*	334 ± 14**
Malondialdehyde (μmol/l)	0.94 ± 0.03	0.84 ± 0.03*	0.81 ± 0.03**
Oxidized LDL (U/l)	32.3 ± 0.8	30.7 ± 0.8	28.8 ± 1*
DNA strand breaks (AU)	54.3 ± 2.1	55.1 ± 2.5	53.7 ± 3
Oxidized purines (AU)	56.8 ± 3.6	44.8 ± 2.8**	45.6 ± 4.1*
Oxidized pyrimidines (AU)	54.2 ± 2.9	42.5 ± 2.4**	41.7 ± 3.1**

AU, arbitrary units; *p < 0.05; **p < 0.01; ***p < 0.001

One of many functions of vitamin B9 as a metabolic regulator is its presence in homocysteine (HCy) metabolism. In spite of high folic acid concentrations, significantly increased HCy plasma concentrations were found in vegetarian groups (Table 3) as a consequence of insufficient or deficient vitamin B12 concentrations, a vitamin which is not contained in plants. Mean HCy value in V-LO is over limit for mild hyperhomocysteinemia (15 µmol/l). In our previous study we recorded 78% of deficient vitamin B12 serum values in vegans as well as 53% incidence of mild hyperhomocysteinemia [17]. It was long believed that the beneficial effects of folate and vitamin B12 on vascular function and disease are related directly to the HCy decrease [18, 19].

Recent reports confirmed that hyperhomocysteinemia is not directly responsible for cardiovascular disease, but is merely present in subjects suffering for acute or chronic cardiovascular events, as a collateral finding. Reduced methylation potential due to decreased S-adenosyl-methionine/S-adenosyl-homocysteine ratio, induced by the elevated plasma HCy concentrations seems to be the true responsible for cardiovascular diseases [18]. Other data suggest that risk values of HCy not only promote cellular and protein injury via oxidant mechanisms, but are also a marker for the presence of pathological oxidative stress. Thus, it is possible that hyperhomocysteinemia is not a common primary cause of atherothrombotic disorders, but rather a marker of systemic or endothelial oxidative stress that is a major mediator of these disorders [19]. It has been suggested that hyperhomocysteinemia may promote the production of hydroxyl radicals which are the initiators of lipid oxidation. In spite of 28.5% incidence of hyperhomocysteinemia in V-S and 40.6% in V-LO in the present study, the concentrations of total peroxides, malondialdehyde as well as value of oxidized LDL were significantly reduced as a consequence of a better antioxidative status in comparison to subjects of general population (Table 3). Value of oxidized LDL in V-S was non-significantly decreased. Oxidized LDL damage artery wall, and a diet such as vegetarian diets rich in vitamins and low in saturated fat and cholesterol may reduce this risk [20] (Table 3).

The lack of balance between “unhealthy” and “healthy” food leads to the accumulation of unrepaired damage, initiating DNA instability and inducing cancer development. Parameters of DNA damage are used as biomarkers of cancer risk in epidemiological studies [5]. In Table 3 significantly reduced values of oxidized purines and oxidized pyrimidines in two groups of vegetarians *vs* general population are presented.

CONCLUSION

The presented findings suggest that dietary antioxidants may have protective effects against oxidative damage of molecules and thus they may have a beneficial effect on many age related diseases. High-dose antioxidant supplements generally do no good and may cause harm [2, 21]. The widespread use of supplements is hampered by several factors: lack of prospective and controlled studies, insufficient knowledge about pro-oxidant, oxidant and anti-oxidant properties of various supplements, growing evidence that free radicals are not only by-products, but also play an important role in cell signal transduction, apoptosis and infection control [1].

The results of our epidemiological study showed that dominant consumption of key vegetarian commodities such as fruit, vegetables, whole grains, nuts, oil seeds, plant oils, legumes, which are rich in antioxidants, produces significantly higher values of plasma antioxidants in comparison to general population on traditional mixed diet and the significantly lower concentrations of pro-oxidative homocysteine, lipoperoxidation products in plasma as well as values of oxidized purines and oxidized pyrimidines in lymphocytes.

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COMPETING INTEREST STATEMENT

The authors declare that they have no conflict of interest.

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