SHORT COMMUNICATION

Effects of diet and age on oxidative damage products in healthy subjects

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Short title: Oxidative damage in relation to diet and age

Summary

Damage of molecules as a consequence of oxidative stress has been implicated in the pathogenesis of chronic diseases related to aging. Diet is a key environmental factor affecting the incidence of many chronic diseases. Antioxidant substances in diet enhance the DNA, lipid and protein protection by increasing the scavenging of free radicals. Products of oxidative damage of DNA (DNA strand breaks with oxidised purines or oxidised pyrimidines), lipids (conjugated dienes of fatty acids) and proteins (carbonyls) in relation to nutrition (vegetarian diet vs. non-vegetarian, traditional mixed diet) were measured in young women aged 20-30 years (46 vegetarians, 48 non-vegetarians) vs. older women aged 60-70 years (33 vegetarians, 34 non-vegetarians). In young subjects, no differences in values of oxidative damage as well as plasma values of antioxidative vitamins (C,β-carotene) were observed between vegetarian and non-vegetarian groups. In older vegetarian group vs. non-vegetarian group significantly reduced values of DNA breaks with oxidised purines, DNA breaks with oxidised pyrimidines and lipid peroxidation and on the other hand significantly increased plasma values of vitamin C and β-carotene were found. Significant age dependences of measured parameters (increase in all oxidative damage products and decrease in plasma vitamin concentrations in older women) were noted only in non-vegetarians. Vegetarian values of older women vs. young women were similar or non-significantly changed. The results suggest that increase of oxidative damage in aging may be prevented by vegetarian nutrition.

Key words

Oxidative damage . Nutrition . Antioxidative vitamins . Age

Oxidative stress has been implicated in the pathogenesis of chronic diseases related to aging such as cancer and cardiovascular disease (Benzie 2000). It was proposed 50 years ago that free radicals are the major factor involved in the aging process (Harman 1956). The main idea was that aging is caused by the accumulation of free radical-elicited oxidative damage to various biological molecules in tissue cells. Miquel (1991) provided first experimental support to this theory in early years by showing that oxidative damage to mitochondrial DNA and lipofuscin pigment formation in animal tissues are concurrently increased during aging. This "mitochondrial theory of aging" emphasized that enhanced production of reactive oxygen species and accumulation of mitochondrial DNA mutations are a contributory factor to human aging (Linnane *et al*. 1989).

Evidence that diet is a key environmental factor affecting the incidence of many chronic diseases is overwhelming (Block *et al*. 1992; Gallus *et al*. 2004; Key *et al*. 2006). Antioxidant substances in such a diet enhance the DNA, protein and lipid protection by increasing the scavenging of radical oxidative species that occur during metabolic reactions. The lack of balance between the amount of "unhealthy" and "healthy" food leads to the accumulation of unrepaired damage, initiating DNA instability and inducing cancer development (Kapiszewska 2006).

The main goal of this study was to assess the products of oxidative damage to DNA, lipids and proteins in relation to nutrition and age. Here were compare two nutritional regimens: a vegetarian diet with predominance of plant food with consumption of dairy products, eggs as well as ≤ 2 times monthly consumption of white meat (poultry or fish) vs. a non-vegetarian traditional mixed diet (general population). The other comparison was young vs. older subjects at condition of a vegetarian nutrition and a traditional non-vegetarian nutrition. A randomly selected 161 adult non-smoking women from the same region (Bratislava and surroundings) was divided into four groups: vegetarian young women aged 20-30 years (n=46), non-vegetarian young women aged 20-30 years (n=48), vegetarian older women aged 60-70 years (n=33) and non-vegetarian older women aged 60-70 years (n=34). The probands have an approximately similar physical activity (no sports). The group characteristics are presented in Table 1. Plasma concentrations of vitamins C, E, A, β-carotene were detected by HPLC (Cerhata *et al*. 1994; Lee *et al*. 1992). EDTA was used as an anticoagulant. The alkaline comet assay modified with lesion specific enzymes was used for detection of DNA strand breaks, oxidised purines and oxidised pyrimidines in isolated lymphocytes (Collins *et al*. 1996). The plasma concentrations of conjugated dienes of fatty acids and protein carbonyls were measured by spectrophotometric methods (Recknagel and Glende 1984; Levine *et al.* 1990). The intake of vitamins, mineral and trace elements only in natural form was considered (no supplementation).

Values of oxidative damage products as well as plasma antioxidative vitamins are introduced in Table 1. In groups of young women, no differences in values of oxidative damage to DNA, lipids and proteins were observed between vegetarians and non-vegetarians. The plasma values of antioxidative vitamins also were similar in both nutritional groups. In older vegetarian group the significantly reduced values of DNA breaks with oxidised purines, DNA breaks with oxidised pyrimidines as well as lipid peroxidation product were found if these values were compared with those in older non-vegetarians. Older vegetarians vs. older non-vegetarians have the significantly increased plasma values of vitamin C and β-carotene (Table 1).

Current knowledge of nutrition suggests that regular and sufficient consumption of fruit and vegetables, whole grain products, grain sprouts, pulses, plant oils and oil seeds together with healthy life style protect against degenerative diseases (Rajaram and Sabaté 2000; Krajčovičová-Kudláčková 2005; Key *et al*. 2006). These food commodities are crucial components of vegetarian diets. In previous studies we found significantly reduced values of DNA breaks with oxidised purines and lipid peroxidation products in small group of 24 vegetarians aged 20-69 years in comparison to 24 non-vegetarians of the same age range (Krajčovičová-Kudláčková and Dušinská, 2004; Krajčovičová-Kudláčková *et al*. 2004). The inverse relationships between DNA breaks with oxidised purines and plasma vitamin C or β-carotene concentrations as well as between plasma values of conjugated dienes and vitamins C, E, β-carotene were recorded. Vegetarian DNA breaks as well as DNA breaks with oxidised pyrimidines were non-significantly reduced. The young women in presented study have the similar values of body mass index and plasma antioxidative vitamins. These results suggest an approximately similar consumption of protective food in young vegetarians and non-vegetarians. This conclusion is supported by optimal plasma concentrations (with high antioxidative effect) of vitamin C and β-carotene as main vitamins derived from fruit and vegetables. The significantly higher values of vitamins (C, β-carotene) and significantly reduced values of products of oxidative damage (DNA breaks with oxidised purines, DNA breaks with oxidised pyrimidines, conjugated dienes of fatty acids, protein carbonyls) in older vegetarian women vs. older non-vegetarian women suggest an incorrect composition of non-vegetarian diet and on the other hand a protective effect of vegetarian diet against age-related degenerative diseases.

A significant age dependence (the increase in older subjects) of DNA damage with oxidised purines, DNA damage with oxidised pyrimidines, lipid peroxidation and protein oxidation was found in non-vegetarian women. The vegetarian values were age independent. Plasma values of antioxidative vitamins, vitamin C and β-carotene significantly decreased in older non-vegetarian women. In vegetarian groups, these vitamin values were similar in young and older subjects. Age-nutrition changes in case of vitamin A were not observed (Table 1). The significantly increased plasma values of lipid soluble vitamin E in older subjects on both diets vs. young subjects are a consequence of its accumulation at higher body fat value (Table 1). This assumption is supported by increased values of body mass index in older subjects of both nutritional regimens (Table 1). The significantly higher value of body mass index in older non-vegetarians vs. older vegetarians (overweight upper limit – obesity beginning) confirms an incorrect composition of non-vegetarian diet. The same conclusion from view of body mass index and incorrect nutrition in general population we obtained in our previous epidemiological studies (e.g. Krajčovičová-Kudláčková *et al.* 2005).

Oxidative DNA damage may be important in mutagenic, carcinogenic, and aging processes. Although it is plausible that antioxidant vitamins may reduce oxidative DNA damage, evidences for favourable effects of vitamin supplements in human studies are partly inconsistent (Moller and Loft 2006). The result of introduced article is that antioxidant intake through consumption of antioxidant-rich foods was more effective at lowering DNA damage than supplementation with single antioxidants. The benefits of fruit and vegetable intake became evident with the consumption being at least three servings/day (Huang *et al.* 2000). Supplementation of diet with 500 mg of vitamin C daily and 400 IU d-α-tocopherylacetate/day had no significant main effect or interaction effect on oxidative DNA damage as measured by urinary 8-hydroxy-2´-deoxyguanosine in non-smoking adults (Huang *et al.* 2000). On the other hand, Moller *et al.* (2004) found that long-term vitamin C supplementation at high dose 500 mg together with vitamin E in moderate dose 182 mg decreases the steady state level of oxidative DNA damage in mononuclear blood cells of smokers. Various aspects of the Mediterranean diet or the vegetarian diet are considered favourable with regard to cancer risk (Rajaram and Sabaté 2000; Gallus *et al.* 2004). For most epithelial cancers, the risk decreased with increasing vegetable and fruit consumption, with odds ratios between 0.3 and 0.7 for the highest versus the lowest tertile. Subjects reporting frequent red meat intake showed odds ratios above unity for several common neoplasms. Whole grain food intake was related to reduced risk of several types of cancer, particularly of the upper digestive tract, probably on account of its high fiber content. Intake of legumes was inversely associated with risk of colon cancer and pancreatic cancer (Fraser 1999). Several phytochemicals such as flavonoids, isothiocyanates and allylsulfides derived from fruit and vegetables are potent modulators of the enzyme system responsible for metabolizing carcinogens (Rajaram and Sabaté 2000). Antioxidant vitamins C and E and polyphenols inhibit formation of N-nitroso compounds, which are potential carcinogens (Bartsch and Frank 1996). Phytochemicals in whole grains and legumes have the ability to block initial DNA damage and suppress post-initiation, which in turn can halt the carcinogenic process. The increased glucagon activity as a consequence of higher intake of arginine and pyruvigenic amino acids from plant protein sources may lead to a decrease in insulin-like growth factor activity that can be expected to retard cancer development (McCarty 1997). Our previous studies shown that vegetarian consumption of protective food is significantly higher in comparison to non-vegetarians (fruit 463 vs. 176 g/day, vegetables 195 vs. 62 g, whole grain products 242 vs. 65 g, nuts 29 vs. 7 g, plant oils 63 vs. 32 g, legumes 38 vs. 6 g) (Krajčovičová-Kudláčková *et al*. 2004).

It is known that oxidative damage to tissue macromolecules increases during aging (Barja 2002). The results of presented study suggest that increase of oxidative damage in aging may be prevented by vegetarian nutrition.

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Reprint requests

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Table 1. Products of DNA, protein, lipid oxidative damage and concentrations of antioxidative vitamins in young and older vegetarians and non-vegetarians

	Young		Older	
	vegetarians	non-vegetarians	vegetarians	non-vegetarians
Number of subjects	46	48	33	34
Age range (years)	20-30	20-30	60-70	60-70
Age average (years)	25.3±0.4	23.8±0.3	65.3±0.5	64.7±0.5
Body mass index (kg/m^2)	21.4±0.4	21.1±0.5	25.0±0.7 ^{C,Z}	29.8±0.9 ^{Z}
Smokers	0	0	0	0
Duration of vegetar. (years)	9.6±0.6	-	11.2±0.9	-
DNA breaks (AU)	45.5±2.0	47.3±3.0	53.4±3.7	54.8±3.2
DNA breaks with oxidised purines (AU)	94.7±5.6	94.8±5.7	96.3±6.6 ^{A}	114.3±5.6 ^{X}
DNA breaks with oxidised pyrimidines (AU)	88.8±4.7	95.8±5.1	95.2±5.5 ^{A}	116.9±6.4 ^{X}
Conjugated dienes of fatty acids (µmol/l)	1.12±0.10	1.40±0.10	1.31±0.16 ^{A}	1.78±0.14 ^{X}
Protein carbonyls (µmol/l)	72.7±5.7	70.7±7.4	80.5±9.2	95.4±9.7 ^{X}
Vitamin C (µmol/l)	55.3±2.2	56.7±2.2	56.2±3.1 ^{B}	40.3±3.6 ^{Z}
β–carotene (µmol/l)	0.46±0.04	0.38±0.06	0.55±0.08 ^{C}	0.24±0.02 ^{X}
Vitamin A (µmol/l)	1.45±0.07	1.43±0.08	1.52±0.07	1.41±0.08
Vitamin E (µmol/l)	22.4±0.7	24.3±0.8	32.8±1.5 ^{Z}	36.5±1.5 ^{Z}

Results are expressed as means ± S.E.M.

^{A,B,C} vegetarians vs. non-vegetarians ^{A} $P<0.05$ ^{B} $P<0.01$ ^{C} $P<0.001$

^{X,Z} young vs. older subjects ^{X} $P<0.05$ ^{Z} $P<0.001$