Oxidative DNA damage in relation to nutrition

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Oxidative DNA damage in humans could arise also from incorrect nutritional habit and life style. DNA strand breaks with apurinic/apyrimidinic sites, oxidized purines and oxidized pyrimidines were assessed in 24 subjectively healthy vegetarians (plant food, dairy products, eggs) and compared with 24 non-vegetarians (traditional diet, general population).

DNA strand breaks + oxidized purines are significantly reduced in vegetarians ($p<0.05$), DNA strand breaks are nonsignificantly decreased. The sufficient antioxidative status (overthreshold values of natural essential antioxidants, which mean a reduced risk of free radical disease) is crucial in free radical defense. Intake of protective food commodities (fruit, vegetables, dark grain products, grain sprouts, oil seeds) is significantly higher in vegetarians. Alternative nutrition subjects have a significantly increased plasma levels of vitamin C, vitamin E, β-carotene with high incidence of overthreshold values (92% vs. 42% – vitamin C, 67% vs. 33% – vitamin E, 67% vs. 17% – β-carotene).

There is recorded a significant inverse linear correlation between values of DNA strand breaks + oxidized purines and vitamin C or β-carotene levels ($p<0.01$, $p<0.05$).

Vegetarian diet is significantly more rich source of antioxidants. The results of reduced endogenous DNA damage and higher antioxidative status in vegetarians document that a correct vegetarian nutrition might represent an effective cancer prevention.

Key words: DNA damage, vitamin C, β-carotene, vegetarians, non-vegetarians

The damaging effect of reactive oxygen species on cellular biomolecules (proteins, lipids, nucleic acids) are well documented and the consequences of such damage have been implicated in the ethiology of number of human health disorders. Through a variety of mechanism, free radicals produce a number of lesions in DNA and nucleoprotein such as base lesions, sugar lesions, single-strand breaks, abasic sites and DNA-protein cross-links [12]. Some oxidative DNA lesions are promutagenic and oxidative damage is proposed to play a role in the development of certain cancers [16]. DNA damage in humans could arise from a variety of causes, such as exposure to harmful chemicals [25], environmental pollution [2], dietary habits and life style [4, 11, 17] as well as different diseases [9, 13].

In presented study was assessed DNA damage in dependence to different dietary habit.

Subjects and methods

Randomly selected group of alternative nutrition subjects consisted of 24 subjectively healthy adult vegetarians, who consumed plant food, milk, dairy products and eggs. Control group of 24 subjects of general population consumed a traditional mixed diet. All subjects live in Bratislava and surroundings, they were non-smokers and non-consumers of alcohol. The probands had approximately a similar physical activity and a higher education level (secondary and university). The characteristic of groups is presented in Table 1.

Blood samples were collected in the standard way. EDTA was used as an anticoagulant. The alkaline comet assay modified with lesion specific enzymes was used for detection of strand breaks, oxidized purines and oxidized pyrimidines in isolated lymphocytes [8]. For detection of oxidized purines, especially 8-oxo-guanine, 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 [8, 9]. Vitamins C and E as well as β-carotene levels in plasma were detected by HPLC methods [6, 23]. Conjugated dienes of fatty acids were estimated by spectrophotometric method [24].

Intake of selected food commodities was calculated from frequency dietary questionnaires. The survey was carried out in spring. Intake of vitamins, mineral and trace elements only natural form was considered (no supplementation). Basic statistic method, chi-square test and regression analysis were used for final evaluation.

Results and discussion

DNA damage and mutation arise from endogenous products of cellular metabolism, as it was documented in the past 20 years. Oxygen radical can attack DNA bases or deoxyribose residues to produce damaged bases or strand breaks [12]. Alternatively, free radicals can oxidize lipid or protein molecules to generate intermediates that react with DNA to form adducts [14]. Figure 1 represents a positive linear relationship between DNA damage expressed as DNA breaks + oxidized purines and lipid peroxidation expressed as fatty acid conjugated dienes. Value of strand breaks of DNA with apurinic/apyrimidinic sites is nonsignificantly reduced in vegetarians vs. non-vegetarians (Tab. 1). Value of DNA breaks + oxidized purines is significantly decreased in vegetarians and value of breaks + oxidized pyrimidines is similar in both investigated groups.

Epidemiological studies have demonstrated a strong link between the nutrition habit with high fruit and vegetable consumption and a reduced incidence of degenerative diseases including many types of cancer [3, 5]. Protection dietary components include mainly the essential antioxidative vitamins, such as ascorbic acid (vitamin C), α-tocopherol (vitamin E) and carotenoids. The sufficient antioxidative capability of organism is a crucial problem in free radical defense. Vegetarian diets are significantly more rich source of antioxidants [18–20, 22] (Tab. 1). The levels of three mentioned vitamins are significantly increased in plasma of vegetarians with high incidence of overthreshold values (92% vs. 42% – vitamin C, 67% vs. 33% – vitamin E, 67% vs. 17% – β-carotene). Overthreshold values mean a reduced risk of free radical disease. The results document a more effective antioxidant status of vegetarians as a consequence of higher consumption of protective food (Tab. 1).

Vitamin C and β-carotene are derived from fruit and vegetables. Rich sources of vitamin E are dark or whole grain products, grain sprouts, soy and other pulses, oil seeds whereas animal products are generally poor sources of this vitamin. Relationship between lipid peroxidation product and on the other hand total antioxidant status, vitamin C,

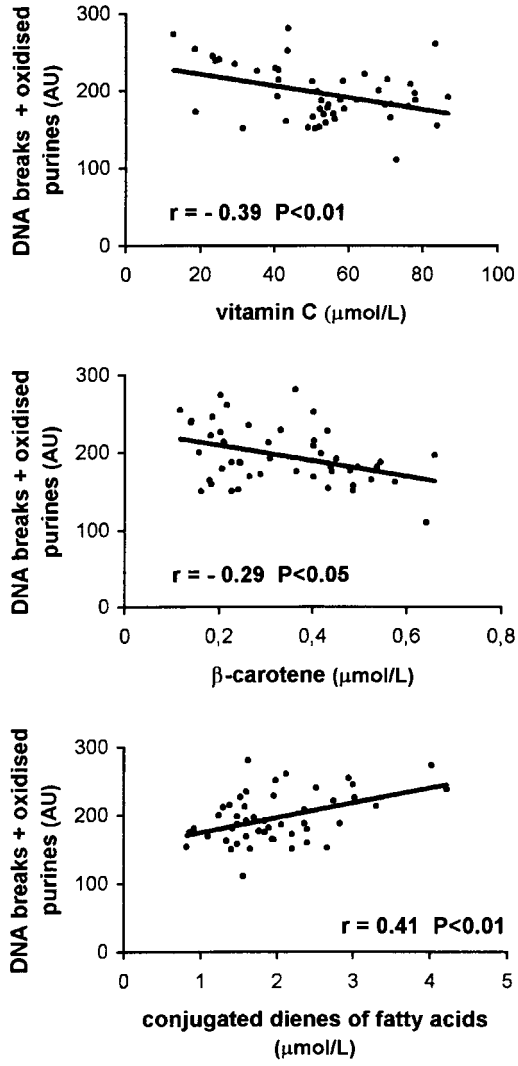

Figure 1. Relationship between DNA damage values and vitamin C, β-carotene, fatty acid conjugated diene levels in connected group of vegetarians and non-vegetarians.

vitamin E have a significant inverse linear trend [21]. Risk values of fatty acid conjugated dienes had only 8% of vegetarians vs. 42% of non-vegetarians (Tab. 1). A comparison of reduced DNA damage (significantly DNA strand breaks + oxidized purines) and higher antioxidative status (significantly increased plasma levels of essential antioxidants) in vegetarians expresses that a correct vegetarian nutrition might represent an effective prevention for free radical disease. Figure 1 presents a significant inverse linear correlation between DNA damage values and vitamin C or β-carotene plasma levels. In case of vitamin E this correlation was nonsignificant because this lipid-soluble chain breaking antioxidant is probably more effective in lipid peroxidation [14]. A favourable effect could provide also optimised traditional nutrition with sufficient consumption of protective food commodities. Epidemiological research clearly de-

Table 1. Characteristic of groups, values of DNA damage, levels of antioxidative vitamins and conjugated dienes of fatty acids and nutritional habit

	Non-vegetarians	Vegetarians
n (m+w)	24 (12+12)	24 (11+13)
age range (y)	21–69	20–62
average age (y)	41.2 ± 2.0	40.0 ± 1.7
body mass index (kg/m^2)	25.6 ± 0.7	22.2 ± 0.5***
period of vegetarianism (y)	–	9.6 ± 0.9
smokers	0	0
DNA damage (AU)		
DNA breaks	116.3 ± 10.5	99.8 ± 7.7
DNA breaks+oxidized purines	207.6 ± 8.5	185.2 ± 5.8*
DNA breaks+oxidized pyrimidines	193.4 ± 9.6	188.9 ± 7.4
Vitamin C (μmol/l)	44.4 ± 2.7	61.7 ± 2.8***
>50	42%	92%
Vitamin E (μmol/l)	25.9 ± 1.2	30.2 ± 1.0**
>30	33%	67%
β-carotene (μmol/l)	0.26 ± 0.02	0.41 ± 0.02***
>0.4	17%	67%
conjugated dienes of fatty acids (μmol/l)	2.38 ± 0.14	1.59 ± 0.08***
>2.4	42%	8%
Food consumption (g/day)		
fruit	176 ± 11	463 ± 22***
vegetables	62 ± 3	195 ± 12***
whole grain products	65 ± 2	242 ± 14***
grain sprouts	0	3.6 ± 0.5***
oil seeds	7 ± 1	29 ± 3***
plant oils and spreads	32 ± 2	63 ± 3***
soy products	6 ± 1	38 ± 3***

Results are expressed as mean ± SEM; $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$

monstrate that nutrition of greater part of Slovak population is incorrect [1, 22], which we recorded also in small study group.

Our beneficial results of reduced DNA damage in vegetarians as compared to non-vegetarians are in agreement with the study of DHAWAN et al [11] for Indian lacto-vegetarians (n=26, reduced parameters of comet assay expressed as tail DNA, tail length, tail moment). Food antioxidants reduce cancer risk by providing a degree of protection for DNA from damage by reactive oxygen species as well as also may stimulate DNA repair. The study of FILLION et al [15] demonstrated enhanced removal of DNA strand breaks and oxidized bases in lymphocytes from volunteers with high plasma β-carotene concentrations or volunteers whose plasma β-carotene levels increased 24 hours after taking 45 mg of β-carotene isolate. Another study has examined the role of vitamin C in regulating the DNA damage/DNA repair balance [7]. Dietary supplementation with vitamin C (500 mg/day) was found to reduce levels of 8-oxo-2'-deoxyguanosine in human mononuclear cell DNA. Sufficient intake of vitamin C can lead to a DNA protection as well as to a stimulation of the removal of oxidized bases from DNA [10].

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