EXPERIMENTAL STUDY

Free radical disease prevention and nutrition

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Abstract

An improved antioxidant status (overthreshold plasma values of essential antioxidants) minimizes the oxidative damage. The levels of antioxidant vitamins C and E, „antioxidant" trace elements selenium, zinc, copper and iron were measured in two groups of adults with different nutritional habits – alternative (vegetarians; n=110) and traditional (mixed diet, control, n=101). The prevalence of iron and zinc deficiencies was found in the alternative group (20 % vs 11 % – iron, 13 % vs 9 % – zinc) as a consequence of higher intake of plant trace element absorption inhibitors. As opposed to the latter, the control group had higher findings of iron and copper levels over the optimal range (18 % vs 8 % – iron, 11 % vs 2 % – copper). The subjects on mixed diet was showed a significant negative linear correlation between serum zinc and iron levels. This favourable relationship means a decrease in Fenton reaction by indirect zinc effect. Average plasma values of vitamin C, vitamin C/vitamin E, vitamin E/cholesterol (LDL protection), vitamin E/triacylglycerols (polyunsaturated fatty acid protection) in vegetarians are over the threshold with high number of individual overthreshold values (94 % vs 17 % – vitamin C, 100 % vs 58 % – vitamin C/vitamin E, 89 % vs 68 % – vitamin E/cholesterol, 100 % vs 64 % – vitamin E/triacylglycerols). Homocysteine levels in vegetarians (36 % atherogenic levels) correlate significantly inversely to vitamin C levels, the fact of which means a positive vitamin C effect in free radical remove also in hyperhomocysteinemia. Plant food is a rich source of antioxidants. A correct vegetarian nutrition or optimized mixed diets with regular and frequent consumption of protective food commodities may be an effective contribution to the age-related chronic degenerative disease prevention. ***(Tab. 2, Fig. 2, Ref. 31.)***

Key words: zinc, copper, iron, vitamin C, vitamin E, homocysteine, nutrition.

Free radicals have been implicated in the etiology of chronic degenerative diseases, especially including cardiovascular diseases and cancer (Esterbauer et al, 1993; Harman, 1996; Halliwell, 1996 a). An extremely short half-life of most aggressive radical species prevents direct investigation. But indirect information is available from the measuring of antioxidants, which are crucial in the body multilevel protection from radicals. There are determined threshold values of plasma essential antioxidants (Gey, 1993). Overthreshold values mean a reduced risk of free radical diseases. An improved antioxidant status helps to minimize the oxidative damage and thus to delay or prevent pathological changes. This suggests the possible utility of antioxidant-based dietary strategies for lowering the risk of age-related chronic diseases (Halliwell, 1996b; Diplock et al, 1998). Lines of defense include enzymes (e.g. superoxide dismutase, catalase, glutathione peroxidase, their active centres include micronutrients zinc, copper, manganese, iron, selenium), non-essential endogenous antioxidants (glutathione, uric acid, proteins, ubiquinol 10 etc.) and essential radical scavengers (vitamins C, E, A and carotenoids – vitamin A precursor ß-carotene and non-vitamin A precursors e.g. lycopene). Although vitamin C and vitamin E are diet-derived antioxidants of major physiological importance, the optimal level of each antioxidant is required for the maintenance of optimal health. Hence, optimal "total" antioxidant status is desirable (Gey, 1993, 1998; Halliwell, 1996 a,b; Diplock et al,

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Tab. 1. Group characteristics and trace element levels.

	Nutrition	
	alternative	traditional
n (m+w)	110 (48+62)	101 (49+52)
age span (years)	23-60	19-55
average age (years)	37.5±0.3	35.0±0.4
BMI (kg/m2)	22.2±0.2[c]	24.9±0.4
period of vegetarianism (years)	5.6±0.2	–
smokers	0 %	25 %
selenium (μmol/L)	1.08±0.01	1.18±0.02
>0.57	99 %	100 %
zinc (μmol/L)	15.1±0.2	14.5±0.3
<10.7	13 %	9 %
>18.4	4 %	8 %
copper (μmol/L)	17.4±0.3	18.3±0.7
<12.5	1 %	8 %
>27.5	2 %	11 %
iron (μmol/L)	16.9±0.2[a]	18.8±0.8
<12-m, 10-w	20 %	11 %
>27-m, 24-w	8 %	18 %

results are expressed as mean±SEM
[a] $p<0.05$, [c] $p<0.001$
limit for "antioxidant" selenium effect (Gey, 1993)

1998). Here, the correct nutritional regime with high intake of antioxidants plays an important role.

This study compares the antioxidant status in adult subjects with two nutritional regimes – alternative (vegetarians) and traditional (omnivores, mixed diet). Out of the antioxidant vitamin group vitamins C and E are evaluated, as well as the relationship between vitamin E and lipids. Out of "antioxidant" trace elements levels of zinc, copper, iron and selenium are measured. Pro-oxidant homocysteine is correlated with antioxidant vitamins.

Materials and methods

The randomly selected group of alternative nutrition subjects consisted of 110 subjectively healthy adult vegetarians (plant food, milk, dairy products, eggs), who were investigated in coincidence with an epidemiological study of health and nutrition of the vegetarian population in Slovakia (A group). The control group of 101 subjects consuming traditional mixed diet was randomly selected from 396 Slovak individuals who were examined within an epidemiological study of nutrition and health of ethnic groups in Slovakia (T group). All subjects live in Bratislava and its surroundings. The characteristics of groups are presented in Table 1.

Blood samples were collected in the standard way. EDTA was used as an anticoagulant. Zinc, copper and selenium levels were determined by the method of atomic absorption spectrometry (Minor electrolytes in serum, Philips,UK; Usrinyova and Hladikova, 1998). Serum iron, cholesterol and triacylglycerol values were measured by standard laboratory methods using the automatic analyzer Vitros 250 (Johnson & Johnson, USA). Vitamin C and vitamin E levels in plasma were detected by HPLC (Lee et al, 1992; Cerhata et al, 1994). Total homocysteine in

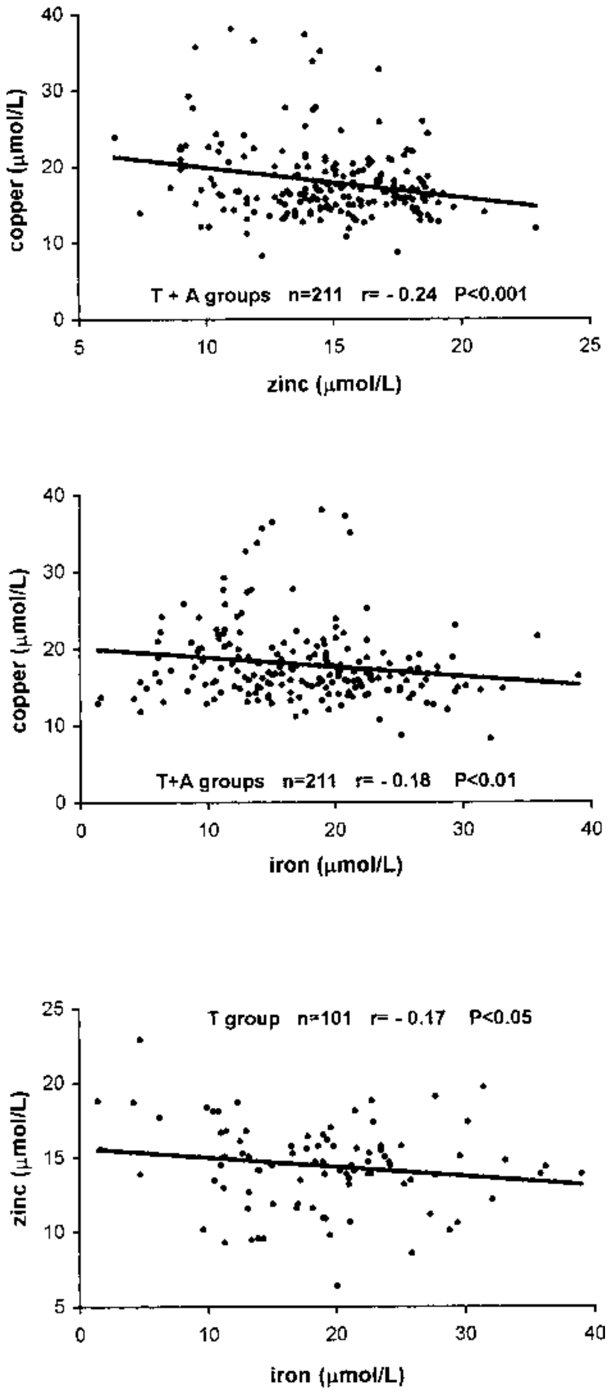


Fig. 1. Linear relationship between copper – zinc levels and copper – iron levels in both traditional mixed nutrition and vegetarians (T+A groups) and zinc – iron levels in subjects on mixed diet (T group).

plasma was assessed by HPLC method with fluorescence detection and SBD-F as derivation agent (Vester and Rasmussen, 1991). Serum folic acid and vitamin B12 levels were measured by Elecsys-immunoassay test (Boehringer).

The survey was carried out in spring. Only the intake of vitamins, mineral and trace elements in natural form was considered

Tab. 2. Vitamin and homocysteine levels.

	Nutrition	
	alternative	traditional
vitamin C (μmol/L)	81.5±1.1[C]	31.5±2.2
<50[A]	6 %	83 %
vitamin E/cholesterol (μmol/mmol)	6.71±0.06[C]	5.00±0.09
<5.2[A]	11 %	32 %
vitamin E/triacylglycerols (μmol/mmol)	30.6±0.3[C]	20.4±1.0
<16	0 %	36 %
vitamin C/vitamin E	2.72±0.06[C]	1.31±0.10
<1.0[B]	0 %	42 %
homocysteine (μmol/L)	13.6±0.3[C]	9.4±0.2
>15	36 %	5 %
folic acid (nmol/L)	26.2±0.4[C]	15.3±0.8
<9.5	2 %	25 %
vitamin B12 (pmol/L)	239±3[C]	324±9
<179	52 %	7 %

[C] p<0.001
[A, B] limit for antioxidant effect (AGey, 1993; B Gey, 1998; limit value of vitamin E/triacylglycerols = threshold vitamin E value/upper reference limit for triacylglycerols)

(no supplementation). The basic statistic method, Student t-test and regression analysis were used in the final evaluation.

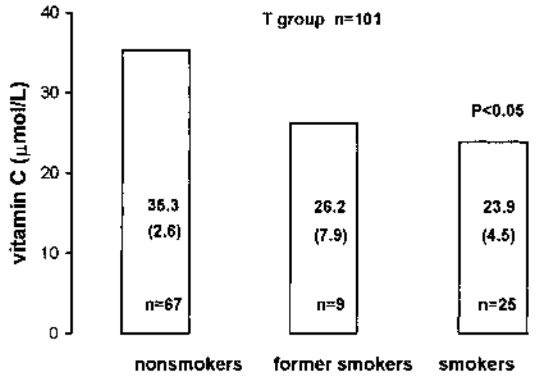
Results and discussion

“Antioxidant” trace elements are localized in the active centre of detoxificant metalloenzymes (superoxide dismutase – zinc and copper, catalase – iron, glutathione peroxidase – selenium). Serum selenium levels are optimal in both investigated groups (Tab. 1). The significant positive linear correlation between plasma selenium and glutathione peroxidase activity was introduced (Plecko et al, 1998). Similar correlation is described in case of superoxide dismutase activity and levels of zinc and copper (Paik et al, 1999). In the vegetarian group we found 83 % of zinc levels and 97 % of copper levels in the optimal range vs 83 % zinc and 81 % copper values in the traditional group (Tab. 1). Vegetarians regularly consume various nuts and other oil seeds, soy, whole grain products, grain sprouts, in which the concentration of selenium is higher and these food commodities contain also more zinc and copper (Krajcovicova-Kudlackova et al, 1995). On other hand, plant food contains trace element availability inhibitors (phytic acid, oxalic acid, fibre) (Fairweather-Tait, 1998). The average bioavailability of selenium is 50–95 % and it is better than that of other trace elements (Fairweather-Tait, 1998). Copper availability is 25–70 % of intake, zinc from food is utilized at 5–50 % and iron 0–15 %. In the vegetarian group we recorded higher prevalence of deficient iron and zinc values (Tab. 1) (iron 20 % vs 11 % in control group, zinc 13 % vs 9 %) as a consequence of mainly higher phytate intake (Fairweather-Tait, 1998; Krajcovicova-Kudlackova, 2001). Phytic acid (cereals, pulses) is an intensive antagonist of iron and zinc absorption (Fairweather-Tait, 1998). The iron utilization from plant food is approximately 5-times lower as compared with animal food (Herbert, 1988).

Beside phytates the copper availability is affected by zinc and iron (Fairweather-Tait, 1998). We recorded an inverse significant correlation between copper – zinc levels and copper – iron levels (Fig. 1). The inhibited absorption of iron, zinc or copper by phytates and other plant inhibitors means that vegetarians have not over limit serum values of these micronutrients as opposed to subjects on traditional diet, in whom we found an 18 % prevalence of high iron levels and 11 % copper values over the reference range (Tab. 1). Zinc may be also an iron antagonist (Fairweather-Tait, 1998). On Figure 1 we document the negative significant linear relationship of zinc – iron levels in the traditional nutrition group. This is a favourable finding because zinc indirectly decreases the Fenton reaction and thus inhibits the hydroxyl radical formation (Bray and Bettger, 1990). Hydroxyl radicals are known lipid peroxidation initiators. From the data given on literature it is evident that high iron values may stimulate the lipid peroxidation, but not normal – physiological iron values (Valk and Marx, 1999). Copper concentrations accounted for 21 % of the variability in serum lipid peroxide levels while serum ferritin accounted for only 2 % after multivariate analysis.

Vitamin C, which is a water-soluble antioxidant, reacts directly with superoxide, hydroxyl radical and singlet oxygen, and it reduces the tocopheryl radical back to α-tocopherol (Chan, 1993). Vitamin E, which is a lipid-soluble chain breaking antioxidant converts the peroxyl radical to the much less reactive hydroperoxide, thus inhibiting the propagating step in lipid peroxidation (Esterbauer et al, 1993). The relationships between lipid peroxidation products and on other hand the total antioxidant status, vitamin C and vitamin E have a significant inverse linear trend (Krajcovicova-Kudlackova et al, 1997). The risk of oxidative stress increases at the ratio of vitamin C/vitamin E being below 1.0 (Gey, 1998). LDL oxidation is inhibited at the ratio of vitamin E/cholesterol being over 5.2 μmol/mmol (Gey, 1993). Peroxidation of polyunsaturated fatty acids is decreased at the ratio of vitamin E/triacylglycerols being over 16 μmol/mmol. Average values of mentioned parameters are over the threshold in the vegetarian group (Tab. 2) with predominance of individual values for antioxidant effect (94 % vs 17 % in control group – vitamin C, 89 % vs 68 % – vitamin E/cholesterol, 100 % vs 64 % – vitamin E/triacylglycerols, 100 % vs 58 % – vitamin C/vitamin E).

Vitamin C is derived from fruit and vegetables. These food commodities are consumed by vegetarians several times daily, thus the vitamin C intake represents 122 mg (Krajcovicova-Kudlackova et al, 2000). This intake ensures the plasma vitamin C to reach the value with antioxidant effect, as introduced also in the study of Carr and Frei (1999). 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. The vegetarian nutrition regime is significantly a richer source of antioxidants, as shown in Table 2. The low average vitamin C concentration in subjects on traditional diet can be caused by 25 % contribution of smokers. Figure 2 expresses an inverse relationship of vitamin C and smoking. Similar dependence for vitamin E was not recorded. Overthreshold value of vitamin E/triacylglycerols as well as an in-



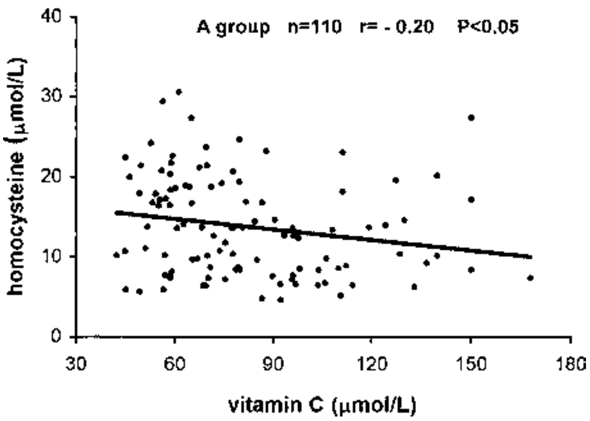


Fig. 2. Smoking dependence of vitamin C in subjects on traditional mixed nutrition (T group) and linear relationship between homocysteine – vitamin C levels in vegetarians (A group).

verse linear correlation of this parameter to lipid peroxidation product (Krajcovicova-Kudlackova et al, 2002) are inevitable in vegetarians for higher plasma content of polyunsaturated fatty acids as substrates of lipid peroxidation (Krajcovicova-Kudlackova et al, 1996). In spite of the higher substrate supply, protection of fatty acids in vegetarians was found to be 2.3 times more effective than in the general population. This is due to the higher antioxidant status (Krajcovicova-Kudlackova et al, 1996).

Hyperhomocysteinemia is a lipid independent risk cardiovascular factor. Homocysteine with pro-oxidative activity may cause atherosclerosis by damaging the endothelium either directly or by altering the oxidative status (Loscalzo, 1996). It has been suggested that hyperhomocysteinemia may promote the production of hydroxyl radicals through homocysteine autooxidation and thiolactone formation (Heinecke, 1988; Loscalzo, 1996). Damaged endothelium has a decreased nitric oxide production and thus less S-nitroso-homocysteine is formed, the fact of which reduces free radicals. Antioxidant vitamins may have an adjunctive role in preventing homocysteine mediated oxidative vascular injury (Chambers et al, 1999). In the vegetarian group we found 36 % of subjects with hyperhomocysteinemia (Tab. 2) as a consequence of vitamin B12 deficiency. Plant food lacks vitamin B12 and thus subjects with exclusively or prevailing consumption of plant food may suffer from vitamin B12 deficiency (Krajcovicova-Kudlackova and Blazicek, 2002). The significant inverse linear correlation between homocysteine and vitamin B12 levels in vegetarians is described (Krajcovicova-Kudlackova and Blazicek, 2002). In general population hyperhomocysteinemia is caused by folic acid deficiency (Tab. 2) (Krajcovicova-Kudlackova and Blazicek, 2002). In investigated alternative group with high prevalence of risk homocysteine levels we found an inverse significant linear correlation of homocysteine and vitamin C levels (Fig. 2). Similar relationship in case of vitamin E in vegetarians or in case of both vitamins in the control group was not significant. Folic acid and vitamin B12 represent a metabolic reducing homocysteine level effect and vitamin C and other antioxidants remove free radicals formed also from homocysteine mainly at condition of hyperhomocysteinemia.

Hyperhomocysteinemia, evaluated separately, may partly counteract the beneficial lifestyle of vegetarians. In connection with high antioxidant levels and low levels of risk lipid parameters (Krajcovicova-Kudlackova et al, 1996, 2000; Key et al, 1999) the correct vegetarian nutrition might represent an effective prevention for degenerative diseases. This favourable effect provides also optimized traditional nutrition with sufficient consumption of protective food commodities. The strict vegetarian (vegan) nutrition with a more health risks (Krajcovicova-Kudlackova, 2001) may be not implicated into the group of protective diets.

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