CLINICAL STUDY

Vitamin C protective plasma value

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Abstract

The relationship of plasma concentration and intake of vitamin C was measured in a randomly selected group of 368 apparently healthy adult subjects of two nutritional regimens: traditional mixed diet (general population, n=187) and vegetarians (n=181). The condition of protective plasma concentration over 50 μmol/l (50.3–89.4 μmol/l), the value of which reduces the risk of free radical disease, was found in 87 subjects from the general population group, in whom the average vitamin C intake was 124.2 mg per day in range of 92–181. The recommended dietary allowance for this group in amount of 77 mg of vitamin C daily was calculated from current Slovak recommendations being in amount of 80 mg per day for men and 75 mg for women. Previous epidemiological studies as well as the presented results show that approximately a half of Slovak general population have vitamin C deficient (below 23 μmol/l) or suboptimal (23–50 μmol/l) plasma concentrations with insufficient antioxidative protection. Significantly higher plasma vitamin C concentrations in comparison to non-vegetarians were observed in the vegetarian group. Protective concentrations were noted in 88 % of vegetarians vs 46 % of non-vegetarians. The significantly reduced values of products of oxidative damage of DNA (DNA breaks with oxidised purines and oxidised pyrimidines), lipids (conjugated dienes of fatty acids, malondialdehyde) and proteins (carbonyls) were found in subjects with plasma vitamin C concentrations being over 50 μmol/l vs below 50 μmol/l. The data emphasize the role of vitamin C in free radical disease prevention under the condition of protective, antioxidative concentrations. The results of general population group document the need to revise the recommended dietary allowance for vitamin C as well as to change the nutritional habits including regular consumption of fruit and vegetables several times daily *(Tab. 3, Ref. 28)*. Full Text (Free, PDF) *www.bmj.sk*.

Key words: vitamin C, plasma concentration, general population, vegetarians.

Humans and other primates have lost the ability to synthesize vitamin C as a result of a mutation in the gene coding for L-gulonolactone oxidase, an enzyme required for the biosynthesis of vitamin C via glucuronic acid pathway (Levine, 1986). Thus, vitamin C must be obtained through diet. The vitamin is plentiful especially in fresh fruit, particularly in citrus fruit, and vegetables. Ascorbic acid is the first line of defence against oxygen radicals in the water-soluble compartment. The vitamin serves to prevent lipid hydroperoxide formation in plasma lipoproteins, e.g. LDL, by reducing α-tocopherol radicals formed upon reaction with lipid peroxyl radicals. This is an important role in the prevention of atherosclerosis (Halliwell, 1996). Free radicals are involved in both, cancer initiation and tumor promotion. Ascorbic acid may block some of these processes (Valko et al, 2004). In addition, vitamin C, acting as a nitrite trap, inhibits the formation of carcinogenic nitrosamines from dietary precursors.

Population studies show that individuals with higher intake of vitamin C or those who take supplements have lower risk of a number of chronic diseases, including heart disease, cancer, eye diseases and neurodegenerative conditions (Gey, 1995; Asplund, 2002; Jacob and Sotoudeh, 2002; Padayatty et al, 2003). Data from controlled clinical trials have not established unambiguously that a higher intake of vitamin C alone will help to prevent chronic degenerative diseases and they have suggested that the problem is probably complex (Osganian et al, 2003; Genkinger et al, 2004). It is likely that the amount of vitamin C required to

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Tab. 1. Group characteristics, vitamin C intake and plasma concentration.

	Non-vegetarians	Vegetarians
n (m+w)	187 (78+109)	181 (79+102)
age range (y)	19–68	19–64
average age (y)	39.9±0.5	38.3±0.5
body mass index (kg/m^2)	24.2±0.3	22.6±0.2 *
duration of vegetarianism (y)	–	9.07±0.09
vitamin C intake (mg per day)	90+3	128+7 *
plasma concentration (μmol/l)	53.0±0.9	75.8±1.2 *
>50 μmol/l	46 %	88 %

Results are expressed as means±SEM; * p<0.001

Tab. 2. Vitamin C plasma concentrations in relation to its intake.

	Plasma concentration (μmol/l)		
	<23 deficient	23–50 suboptimal	>50 optimal
n	21	79	87
concentration in plasma			
range	8.3–22.2	23.5–49.7	50.3–89.4
mean±SEM	17.1±0.8	40.2±0.8	70.9±1.2
intake (mg per day)			
range	24–47	42–88	92–181
mean±SEM	37.6±1.6	68.3±1.6	124.2±2.9

prevent scurvy is not sufficient for optimal protection against these diseases. The biochemical, clinical and epidemiological evidence of the role of vitamin C in chronic disease prevention suggests that a change in the currently recommended dietary allowance for vitamin C must be considered (Jacob and Sotoudeh, 2002).

The main goal of this study was to assess the relationship of the intake of vitamin C and its plasma concentrations as well as the values of oxidative damage to DNA, lipids and proteins in adult population under different plasma vitamin C concentrations.

Subjects and methods

Randomly selected group consisted of 368 apparently healthy adult subjects from the region of Bratislava who consumed two distinct types of diets: traditional mixed diet (general population, non-vegetarians, n=187) and vegetarian diet (lacto- and lacto-ovo-vegetarians, who consume plant food, dairy products and eggs, n=181). The group characteristics are showed in Table 1.

The calculation of daily intake of vitamin C was based on the data from dietary questionnaires. The questionnaire contained 146 food items. The frequency of consumption was measured using four categories: almost never, times per day, per week or per month depending on food item. The probands used household measures. Trained workers checked the completeness of questionnaires. The conversion to nutrients was done by using self-developed software Nutrition based on the Slovak food composition database compiled by the Food Research Institute (Slovak Food Data Bank, 1999).

EDTA was used as an anticoagulant. Vitamin C (ascorbic acid) concentrations in plasma were detected by HPLC method (Cerhata et al, 1994). The plasma samples were immediately stabilized by metaphosphoric acid. The alkaline comet assay modified with lesion specific enzymes was used for the detection of DNA strand breaks, oxidised purines and oxidised pyrimidines in isolated lymphocytes (Collins et al, 1996). Values of DNA oxidative damage are expressed in arbitrary units. The intensity of comet tail DNA relative to comet head DNA indicates break frequency. Carbonyl group content in plasma was evaluated by the 2.4-dinitrophenyl-hydrazine assay (Levine et al, 1990). Conjugated dienes of fatty acids in plasma were estimated by spectrophotometric method (Recknagel and Glende, 1984). The plasma malondialdehyde concentration was determined according to the method of Wong et al (1987). The survey was carried out in spring. The intake of vitamin C as well as that of other vitamins, minerals and trace elements only in natural form were considered (no supplementation). Student t-test was used for final statistical evaluation.

Results and discussion

The current recommended dietary allowance for vitamin C, determined in 1980 and reconfirmed in 1989, was 60 mg daily for non-smoking adult men (Young, 1996). The new higher recommended allowance is 75 mg daily for women and 90 mg for men (Jacob and Sotoudeh, 2002). Carr and Frei (1999) suggest that the recommended dietary allowance for vitamin C must be 120 mg per day. Fain (2004) concludes that 110 mg of vitamin C daily is a protective dose. If the antioxidant function of vitamin C is accepted as important for human health, then morbidity and mortality from cancer, cardiovascular disease and cataract in addition to scurvy must be used as the criteria for determining the vitamin C requirements. Available evidence suggests that the intake of 200 mg vitamin C per day saturates the tissues and maintains the fasting plasma levels continuously above the proposed threshold (50 μmol/l) for minimum risk of cardiovascular disease (Gey, 1995; Graumlich et al, 1997). Higher than recommended intakes of vitamin C have been associated with several indices of lowered cardiovascular disease including increasing resistance of lipids and DNA to oxidative damage (Brennan et al, 2000; Krajcovicova-Kudlackova et al, 2001; Osganian et al, 2003; Krajcovicova-Kudlackova and Dusinska, 2004).

The Slovak recommended average dietary allowance is 80 mg for full healthy population or 80 and 75 mg per day for healthy adult men and women, respectively (RDAs, 1997). In our randomly selected group of apparently healthy adult general population we determined the intake of vitamin C leading to the condition of protective plasma concentration being over 50 μmol/l (Tab. 2). Vitamin C intake in 87 subjects (46 % of group) with protective vitamin C plasma value is in amount of 124.2 mg per day (range 92–181 mg). The average recommended dietary al-

Tab. 3. Products of DNA, protein and lipid oxidative damage in relation to vitamin C plasma concentrations.

	Vitamin C plasma concentrations (μmol/l)	
	<50	>50
n	43	47
vitamin C range (μmol/l)	12.8–49.7	50.9–86.8
vitamin C average (μmol/l)	36.6±1.7	65.1±1.6 *
products of oxidative damage		
DNA breaks with oxidised purines (AU)	188±8	157±7 **
DNA breaks with oxidised pyrimidines (AU)	182±7	155±7 **
protein carbonyls (μmol/l)	126±6	87±5 *
conjugated dienes of fatty acids (μmol/l)	1.90±0.14	1.38±0.11 **
malondialdehyde (μmol/l)	1.44±0.10	1.02±0.05 *

Results are expressed as means±SEM; ** p<0.01, * p<0.001

lowance for our selected group (men and women together) calculated from current Slovak recommendations is 77 mg per day. The results suggest that in order to attain the vitamin C plasma concentration that minimizes the oxidative damage it is necessary to increase the vitamin C intake over the current recommendations. The intake of vitamin C in amounts of 37.6 mg (24–47 mg) and 68.3 mg (42–88 mg) (Tab. 2) resulted in its plasma concentrations being either deficient (less than 23 μmol/l) or suboptimal (23–50 μmol/l).

In our repeated studies we showed that vegetarians have a higher antioxidative status (Krajcovicova-Kudlackova et al, 2001, 2003, 2004). As introduced in Table 1, in subjects on alternative nutrition, the vitamin C intake and plasma concentrations were significantly higher. The protective plasma concentrations were found in 88 % of vegetarians in comparison to less than a half of non-vegetarians (Tab. 1). The results in Tables 1 and 2 document the need to revise the Slovak recommended dietary allowance for vitamin C in agreement with other authors. Vitamin C is derived from fruit and vegetables. These food commodities are consumed by vegetarians several times daily, as noted in the frequency dietary questionnaires (Krajcovicova-Kudlackova et al, 2004). Vegetarians consume 463 g of fruit daily vs. 176 g in non-vegetarians. Vegetable consumption is 3.1-fold higher in vegetarians (195 vs 62 g). This indicates that there is a need to change the nutritional habits of the general population.

The effect of different plasma concentrations of vitamin C on the values of products of oxidative damage of DNA, proteins and lipids is showed in Table 3. The 47 subjects from the randomly selected group who were found to have their vitamin C concentrations over 50 μmol/l had significantly reduced values of DNA breaks with oxidized purines or pyrimidines, as well as values of protein carbonyls, conjugated dienes of fatty acids and malondialdehyde when compared to the randomly selected group of 43 persons with their vitamin C concentrations being below 50 μmol/l.

Epidemiological and clinical studies document that a greater fruit and vegetable intake lowers the risk of cancer and cardiovascular death (Genkinger et al, 2004). The daily intake of 700–1000 g of fruit and vegetables produces an improvement in the redox status parameters in diabetic patients (Giammarioli et al, 2004). Observational studies including mainly healthy individuals have shown a favourable relationship between the intake of vitamin C and E and subsequent cardiovascular events. However, most randomized, clinical intervention studies including patients with manifest atherosclerotic disease have not been able to document such a relationship (Landmark and Reikvam, 2005). A high vitamin C intake from supplements was associated with an increased risk of cardiovascular mortality in postmenopausal women with diabetes (Lee et al, 2004). The causes of this adverse effect of high dose of vitamin C are: vitamin C may act as a prooxidant in the presence of free iron and cause damage by glycating proteins or by stimulating lipid peroxidation. High dose of antioxidants that occur in their both oxidised and reduced forms must be connected with sufficient values of their sparring partners for their antioxidative effects to regenerate. The comparison of epidemiological and clinical studies suggests that nutrition prevention from free radical diseases in healthy subjects is more convincing than the effect of antioxidant supplements in an already developed disease (Osganian et al, 2003; Genkinger et al, 2004; Landmark and Reikvam, 2005).

Conclusions

The protective (antioxidative) effect of plasma vitamin C concentration being over 50 μmol/l was found in 88 % of vegetarians, whereas only in 46 % of subjects of general population being on traditional diet. The relationship of vitamin C intake and its plasma concentration in the general population group showed that in order to attain the protective plasma concentration it is necessary to increase the vitamin C intake over the recommended dietary allowance being currently in amount of 80 mg per day in men and 75 mg in women in approximate average amount of 124 mg daily. According to the data of other authors this amount is in the range of 110–200 mg per day. The results document that there is a need to revise the vitamin C recommendations. Subjects on alternative nutrition consume sufficient amounts of fruit and vegetables (approximately 3-fold more than non-vegetarians). This indicated that it is necessary to make a change in the nutritional habits of general population, i.e. to increase the fruit and vegetable consumption.

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