Z Ernährungswiss 37: 178–182 (1998)

Z. Kováčiková
D. Čerhata
J. Kadrabová
A. Madarič
E. Ginter

Antioxidant status in vegetarians and nonvegetarians in Bratislava region (Slovakia)

Der Antioxidative Status bei Vegetariern und Nichtvegetariern der Region Bratislava (Slovakei)

Summary The level of antioxidant vitamins (C, A, E) and the activity of a selenium-dependent glutathione peroxidase as indicators of antioxidant status and lipid peroxides as markers of oxidative damage were investigated in blood of 196 healthy middle-aged inhabitants of Bratislava region. The group consisted of 78 vegetarians (32 men and 46 women) and 118 nonvegetarians (61 men and 57 women). The aim of the study was to compare the effect of the diet on the antioxidant status in vegetarians and nonvegetarians. The most important differences were found in the level of ascorbic acid and in the activity of selenium-dependent glutathione peroxidase; the ascorbic acid in serum was significantly higher, while the selenium and the activity of selenium-dependent glutathione peroxidase was lower both in plasma and erythrocytes of vegetarians.

Zusammenfassung Im Blut von 196 gesunden Bewohnern der Region Bratislava, im reifen Alter, wurden der Spiegel der Antioxidationsvitamine (C, A, E) und der Selen-abhängigen Glutathion-Peroxidase als Indikatoren für den Antioxidationszustand festgestellt, und der Spiegel der Lipidperoxide als Zeichen für die Oxidationsschädigung untersucht. Die Teilnehmer dieser Gruppe waren 78 Vegetarier (32 Männer und 46 Frauen) und 118 Nichtvegetarier (61 Männer und 57 Frauen). Ziel dieser Arbeit war es den Antioxidationszustand der Vegetarier und der Nichtvegetarier zu vergleichen. Die signifikantesten Unterschiede wurden beim Askorbinsäurespiegel und der Selen-abhängigen Glutathion-Peroxidase gefunden; im Serum war der Askorbinsäurespiegel statistisch signifikant erhöht, während Selen und die Selen-abhängigen Glutathion-Peroxidase in Plasma und auch in der Erythrozyten der Vegetarier erniedrigt war.

Key words Vegetarians – antioxidant status – vitamins – selenium – lipid pexoxides

Schlüsselwörter Vegetarier – Antioxidationszustand – Vitamine – Selen – Lipidperoxide

Abbreviations *GSH-Px* = glutathione peroxidase · *Hb* = hemoglobin *LPO* = lipid peroxides · *MDA* = malonedialdehyde · *TBA* = thiobarbituric acid

Received: 26. April 1996
Accepted: 23. October 1997

Z. Kováčiková (✉) · D. Čerhata
J. Kadrabová · A. Madarič · E. Ginter
Institute of Preventive and Clinical Medicine
Limbová 14
833 01 Bratislava
Slovak Republic
E-mail: kovacik@enigma.upkm.sanet.sk

Introduction

According to our today's knowledge we assume that the reactive oxygen radicals play an important role in the pathogenesis of cardiovascular diseases and cancer. They are derived from normal physiological and metabolic processes that are essential to the cell. The evolutionary process has provided aerobic organism with antioxidant, defense system formed by some vitamins (A, C, E) glutathione, uric acid, and antioxidant scavenging enzymes (glutathione peroxidase, superoxide dismutase, catalase) (1).

The epidemiological data from Slovakia and the former Czechoslovakia show a short life expectancy and one

of the highest premature mortality from "free radical diseases" (cancer, cardiovascular diseases) in Europe (3). The cause of this unpropitious phenomenon has not been clearly identified. Usually three main casual factors are considered: nutritional imbalances, wrong life style, and environmental pollution. The insufficient supply of Slovak population with vitamin C (5) and the extremely low selenium status (selenium is an integral part of glutathione peroxidase) in plasma (7) have been actually proved.

Man can regulate his antioxidant intake through his diet. Questions about optimal dietary patterns in this regard are interesting scientifically and have important dietetic and economic implications. The aim of this study was to ascertain the effect of vegetarian diet on the antioxidant status of the Slovak population.

Materials and methods

Recruitment of the subjects was carried out in the spring of 1993 with the cooperation with Slovak Vegetarian Society among its members and other volunteers, mostly employees in the medical sphere with a medical education. All participants of this study were inhabitants of Bratislava and its surroundings or they were studying there and living at a college. Everybody signed an informed-consent form before taking part in the search. A questionnaire was used to collect information concerning health history and dietary habits, amount, and frequency of food consumption. All participants were examined by the practitioner. The study protocol was approved by the Ethical Commission of the Institute of Preventive and Clinical Medicine.

Fasting blood samples were drawn from the forearm between 8.00 and 9.00 a.m. One part of the blood was collected in tubes containing sodium heparin as an anticoagulant. The blood was promptly centrifuged for 15 min at 4 °C (800–850 xg) to separate the plasma. The erythrocytes were rewashed three times in PBS. The second part of the blood was allowed to clot for about 20 min. Serum was separated after centrifugation at room temperature. The samples were analysed immediately (lipid peroxides) or they were refrigerated (-20 °C).

Chemical analyses

Vitamin C (ascorbic acid), vitamin E (α-tocopherol), and vitamin A (retinol) were determined by high-performance liquid chromatography, the lipophilic vitamins by the methods of Nierenberg and Lester (10) and Taylor et al. (17), vitamin C by the method of Cerhata et al. (2). Hemoglobin was estimated using the Drabkins' reagent. Selenium was determined by atomic absorption spectrometry in plasma and erythrocytes (7).

Glutathione peroxidase activity (GSH-Px) was estimated in plasma and erythrocytes according the method of Paglia and Valentine (12). Lipid peroxides (LPO) were estimated as the amount of malondialdehyde (MDA), a breakdown product of lipid peroxidation, after the reaction with thiobarbituric acid (TBA) (11) and after the chromatographic separation of the MDA-TBA adduct (19).

The results were statistically evaluated by the analysis of variance and the regression analysis (Statgraphics 5.0).

Results and discussion

The total sample consisted of 239 individuals. After consulting the practitioner and evaluating the questionnaires, 43 persons were rejected because of a health problem. 196 subjects were left in the study. The participation in this study was voluntary. The subjects, who proclaimed themselves vegetarians, formed a heterogenous group with respect to dietary practice. The majority of vegetarians were actually semivegetarians, only some of them were pure vegetarians or vegans. The semivegetarians limited their consumption of red meat, but they eat seldom fish and chicken (maximum once a week), eggs or diary products. The consumption of diary products was restricted to low-fat ones; they use only vegetable fat. The eating pattern of the whole vegetarian group contained significantly greater amount of fruits, fresh, and boiled vegetables than was the practice in the nonvegetarian group. Table 1 gives the number, age, body mass index, educational level, and number of smokers separately for vegetarian and nonvegetarian men and women, respectively. The differences between average age of vegetarian and nonvegetarian men and nonvegetarian women were not statistically significant. Mean body mass index was significantly lower both in vegetarian men and women than in their nonvegetarian counterparts. The educational level was higher in vegetarian subjects.

Table 2 summarizes the levels of some antioxidant factors in the blood. The concentration of ascorbic acid was significantly elevated in the serum of vegetarians and this enhancement was more pronounced in women than in men. The relation was similar in the study of Millet et al. (8) in French vegetarians and nonvegetarians. Their absolute values were higher (from 65.9 in nonveg-etarian men to 88.0 μmol/l, U/g in vegetarian women), but it is difficult to draw any conclusions from this fact, since a different analytical method for ascorbic acid determination was used Plasma concentration of about 40 μmol AA/l, U/g corresponds to half-saturation of the body. This degree of saturation gives a healthy person a resistance against scurvy for about 60 days. Full-saturation of the body means plasma concentration of about 80 μmol/l U/g (4). Accepting these data we have to state that even the vegetarians, whose vitamin C intake and level in organism is higher, are only in the lower region of AA saturation.

Table 1 Characteristics of the study subjects

	Men		Women	
	vegetarian n = 32	nonvegetarian n = 61	vegetarian n = 46	nonvegetarian n = 57
age (year)	32.2 ± 1.7[a]	35.9 ± 1.2[ab]	33.6 ± 1.7[ab]	37.1 ± 1.3[b]
BMI (kg/m^{-2})	21.77 ± 0.40[a]	24.29 ± 0.40[b]	21.93 ± 0.55[a]	23.48 ± 0.56[b]
educational level*				
primary	0 (0)	1 (1.63)	1 (2.17)	3 (5.26)
secondary	9 (28.12)	24 (39.34)	18 (39.13)	26 (45.61)
graduated	23 (71.87)	36 (59.01)	27 (58.69)	28 (49.12)
smokers	0	16	3	12

Data represent mean ± standard error; BMI = body mass index; * = percent of subjects for the category in parentheses; [a,b] = different superscripts indicate significantly different means ($P < 0.05$) in the same line

Table 2 Mean levels of antioxidant factors in vegetarians and nonvegetarians

	Men		Women	
	vegetarian n = 32	nonvegetarian n = 61	vegetarian n = 46	nonvegetarian n = 57
Vitamin C µmol/l, U/g	58.56 ± 2.79[a]	46.96 ± 2.94[b]	64.23 ± 3.08[a]	49.64 ± 2.98[b]
Vitamin E µmol/l, U/g	17.77 ± 1.08[a]	20.67 ± 0.83[b]	20.80 ± 0.84[b]	18.69 ± 0.76[ab]
Vitamin A µmol/l, U/g	1.22 ± 0.08[ac]	1.32 ± 0.06[a]	1.10 ± 0.05[bc]	0.99 ± 0.04[b]
Se in plasma µg/l, U/g	49.53 ± 2.47[a]	57.42 ± 1.17[b]	48.65 ± 1.29[α]	57.15 ± 1.18[b]
Se in erythrocytes ng/g, U/g Hb	248.71 ± 14.91[a]	296.61 ± 8.08[b]	240.27 ± 6.10[a]	286.74 ± 7.53[b]
GSH-Px in U/l, U/g plasma	241.37 ± 14.13[a]	333.01 ± 9.48[b]	245.69 ± 13.07[a]	339.54 ± 8.81[b]
GSH-Px in erythrocytes U/g, U/g Hb	28.07 ± 1.01[a]	35.55 ± 0.97[β]	28.45 ± 1.21[a]	37.20 ± 0.98[b]

Data represent mean ± standard error; GSH-Px = glutathione peroxidase; Hb = hemoglobin; [a,b,c] = different superscripts indicate significantly different mean ($P < 0.05$) in the same line

The level of ascorbic acid in plasma is negatively influenced by smoking (4). Deleting the smokers from the studied set did not change the relation between the tested groups.

The level of vitamin A was higher in men, which is in concordance with the study from Finland (13). The concentration of retinol in vegetarian women was enhanced comparing to their nonvegetarian counterparts but there was no effect of the eating pattern in men. It differs from the French study about vegetarians (8), where no difference was found. The plasma vitamin A concentration is subject to homeostatic regulation; therefore, it is nearly constant and is independent on vitamin A intake, so long as the liver reserve is sufficient. In adults, a plasma (serum) level of less than 1 µmol/l, U/g is indicative of an inadequate liver reserve of the vitamin A (18). Our results show sufficient vitamin A store in studied subjects.

Vitamin E is the major lipid-soluble antioxidant in plasma protecting lipids against peroxidative damage. Concentration of vitamin E was lower in serum of vegetarian men comparing to nonvegetarian men and vegetarian women. The level of α-tocopherol in human plasma varies between 15–40 µmol/l, U/g (15). The vitamin E concentration estimated in participants of our study was in the lower part of this interval, but according

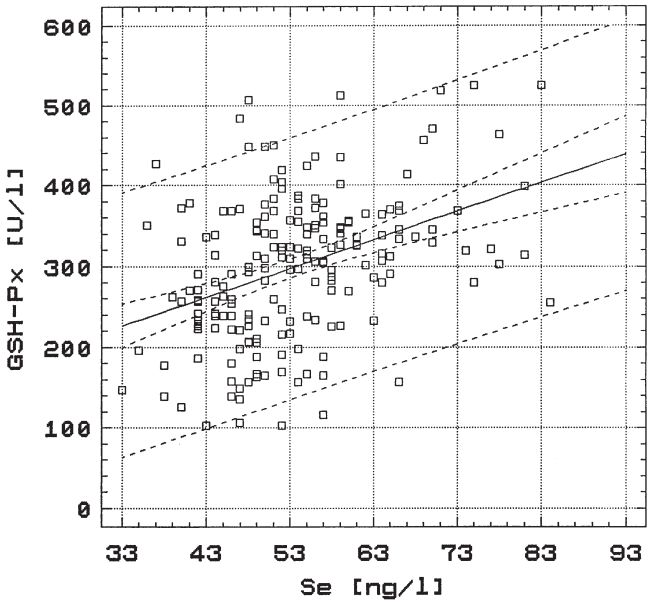

Fig. 1 Correlation between plasma selenium and GSH-Px activity (linear model, r = 0.388, P = 0.00000). Confidence and prediction limits appear on the regression plot as the pair of dotted lines closest to and farthest from the regression line, respectively.

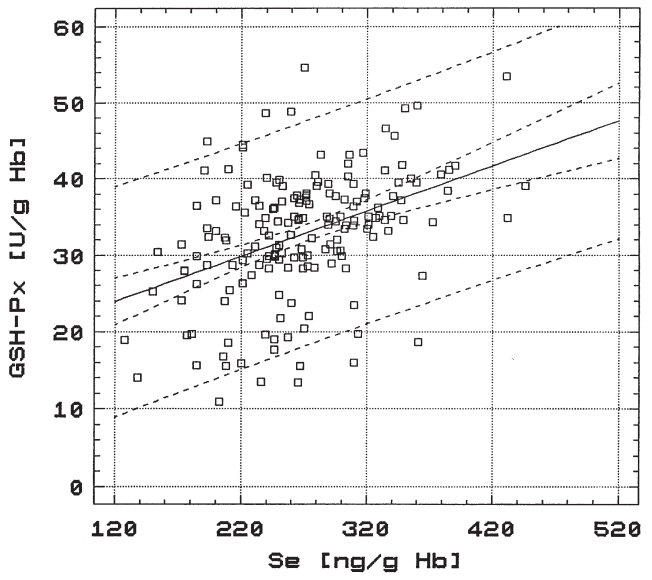

Fig. 2 Correlation between erythrocyte selenium and GSH-Px activity (linear mode, r = 0.419, P = 0.00000). Confidence and prediction limits appear on the regression plot as the pair of dotted lines closest to and farthest from the regression line, respectively.

the guidelines for interpreting vitamin E status (14), the protective role of vitamin E becomes optimal when the plasma concentration of α-tocopherol is ≥ 11.6 μmol/l, U/g. In our study the effect of the eating pattern in women was lacking but the level in nonvegetarian men was significantly higher than in vegetarians. There is a high degree of correlation between plasma (serum) level of tocopherol and the total plasma lipids (4). According the questionnaires our nonvegetarian subjects had higher intake of fat than vegetarians. This could be the reason for lower serum vitamin E concentration in our vegetarian participants.

Selenium was decreased both in vegetarian men and women comparing to their nonvegetarian counterparts. The tendency was the same for selenium in plasma and in erythrocytes. The activity of glutathione peroxidase, a selenoenzyme, was correlated with the selenium concentration both in plasma and in erythrocytes (Figs. 1 and 2). Lower selenium level in lactovegetarians (6) and 40 % decrease in plasma selenium after the diet shift from mixed to lactovegetarian (16) were already reported. The primary source of selenium is wheat and the secondary is meat. The mean Se concentration in wheat in Slovakia is 21 μg/kg, U/g, which represents the lower range comparing with the wheat from other countries. The Se status of the organism is reflected in its level in the blood. Plasma Se responds rapidly to the changing of Se status, while erythrocyte Se responds more slowly. It is due to the time required for the erythropoieses and due to erythrocyte halflife time of about 120 days (9). The selenoenzyme GSH-Px plays a role in protection from lipid peroxidation. Our results show clear correlation between the Se status and GSH-Px activity both in plasma and erythrocytes.

The level of lipid peroxides was significantly higher in serum of nonvegetarians than in vegetarians. After dividing subjects according to sex we have found out the same tendency, but the differences between the LPO-levels in vegetarians and nonvegetarians of the same sex were no longer statistically significant (Fig. 3). Regardless of eating patterns, the concentration of MDA was higher in men than in women. 31 participants of the study were smokers; 28 of them declared they were smoking from 1 cigarette in a week to 5 cigarettes daily. Deleting them from the study did not significantly change the relation between the tested groups. This result is in concordance with that mentioned above that deleting the smokers did not change the differences in ascorbic acid level in plasma between the tested groups.

Conclusion

On the whole, we have found a positive effect of the vegetarian diet on serum ascorbic acid level, but vitamin C saturation was still not achieved. Previous results (7)

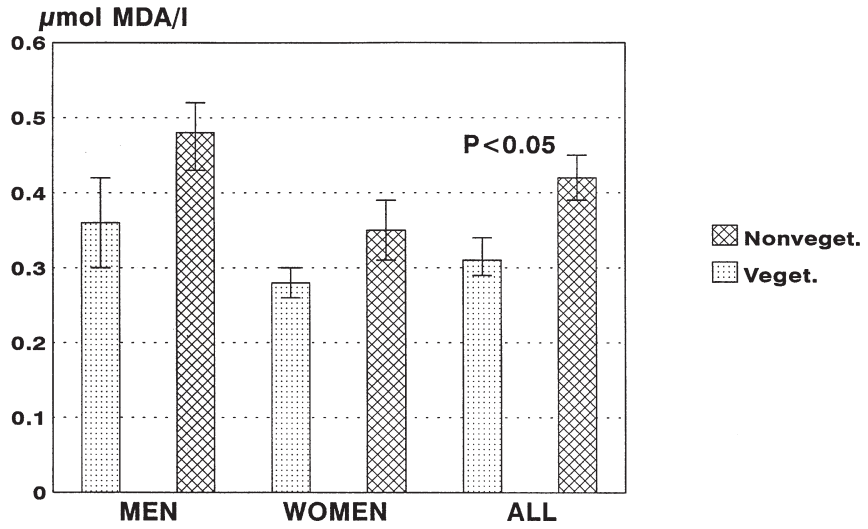

Fig. 3 The level of lipid peroxides in plasma

indicate a low selenium status in Slovak population. Vegetarianism even enhanced this nutritional disbalance. The level of lipophilic vitamins A and E was sufficient in both vegetarians and nonvegetarians. The concentration of lipid peroxides corresponded with these results as a response to antioxidant status. The presented results do not allow us to make a definite conclusion about the effect of vegetarianism on the antioxidant status in the examined population.

Acknowledgments We wish to thank M. Valentová, D. Meká, M. Trtalová, A. Javorská, J. Bernadičová for their technical assistance and B. Hatalová for statistical evaluation of the results. We appreciate the willingness of the Slovak Vegetarian Society to take part in this study.

References

1. Byung PY (1994) Cellular defenses against damage from reactive oxygen species. Physiological Reviews 74:139–161
2. Cerhata D, Bauerová A, Ginter E (1994) Determination of ascorbic acid in blood serum by high-performance liquid chromatography and its correlation with spectrophotometric determination. Czech and Slovak Pharmacy 43:166–168
3. Food and Health Indicators in Europe (1990) WHO Regional Office for Europe. Copenhagen, Denmark
4. Friedrich W (1988) Vitamins. Walter de Gruyter, Berlin/New York, pp 929–1003
5. Ginter E (1988) Participation of chronic vitamin C deficiency in cardiovascular mortality. Cas Lék Ces 127:804–809
6. Kumpusalo E, Karinpaa A, Jauhiainen M, Laitinen M, Lappetelainen R, Maenpaa PH (1990) Multivitamin supplementation of adult omnivores and lactovegetarians: circulating levels of vitamin A, D and E, lipids, apolipoproteins and selenium. Int J Vitam Nutr Res 60:58–66
7. Madaric A, Kadrabová J, Ginter E (1994) Selenium concentration in plasma and erythrocytes in healthy Slovak population. J Trace Element Electrolytes Health Dis 8:43–47
8. Millet P, Guilland JC, Fuchs F, Klepping J (1989) Nutrient intake and vitamin status of healthy French vegetarians and nonvegetarians. Am J Clin Nutr 50:718–727
9. Néve J, Vertongen F, Capel P (1988) Selenium supplementation in healthy Belgium adults: response in platelet glutathione peroxidase activity and other blood indices. Am J Clin Nutr 48:139–143
10. Nierenberg DW, Lester DC (1985) Determination of vitamins A and E in serum and plasma using a simplified clarification method and high-performance liquid chromatography. J Chromat 34:5275–5284
11. Ohkawa H. Ohishi N, Yagi K (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analyt Biochem 95:351–358
12. Paglia D, Valentine W (1967) Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med 70:158–169
13. Parviainen MT, Kumpusalo E, Halonen P, Neittaanmäki L, Pekkarinen H (1992) Epidemiology of vitamins A, E, D, and C in rural villages in Finland: biochemical, nutritional and socioeconomical aspects. Internat J Vit Nutr Res 62:238–243
14. Sauberlich HE, Dowdy RP, Skala JH (1979) Laboratory tests for the assessment of nutritional status. CRC Press, Cleveland, pp 74–80
15. Sies H, Stahl W, Sundquist AR (1992) Vitamins E and C, beta-carotene, and other carotenoids. Beyond Deficiency 699:7–20
16. Srikumar TS, Johansson GK, Ockerman PA, Gustafsson JA, Akesson B (1992) Trace element status in healthy subjects switching from a mixed to a lactovegetarian diet for 12 months. Am J Clin Nutr 55:885–890
17. Taylor SL, Lamden MP, Tapel AL (1976) Sensitive fluorometric method for tissues tocopherol analysis. Lipids 11:530–538
18. Wolf G (1982) Is dietary β-carotene an anticancer agent? Nutrition Reviews 40:257–261
19. Wong SHZ, Knight JA, Hopfer SM, Zaharia O, Leach CN, Sunderman FW (1987) Lipoperoxides in plasma as measured by liquid-chromatographic separation of malondialdehyde-thiobarbituric acid adduct. Clinical Chemistry 33:214–220