Physiol. Res. 53: 219-224, 2004

SHORT COMMUNICATION

Lipid Peroxidation and Nutrition

M. KRAJČOVIČOVÁ-KUDLÁČKOVÁ, V. SPUSTOVÁ, V. PAUKOVÁ

Institute of Preventive and Clinical Medicine, Bratislava, Slovak Republic

Received February 17, 2003
Accepted June 4, 2003

Summary

Levels of conjugated dienes of fatty acids (first peroxidation product) in relation to their substrates and promotors (triacylglycerols, homocysteine, iron) as well as to their inhibitors (essential antioxidative vitamins) were assessed in a vegetarian group (n=24) and compared with subjects on a mixed diet (traditional nutrition, n=24). Positive significant linear correlation between conjugated dienes and triacylglycerols, homocysteine, iron as well as inverse relationship between conjugated dienes and vitamin E, vitamin C, β-carotene were observed in pooled groups. Lipid peroxidation risk in vegetarians seems to be caused predominantly by hyperhomocysteinemia, whereas in a mixed diet group this was due to a higher supply of substrates or risk iron values. The incidence of only 8 % of risk conjugated diene values in vegetarians in contrast to 42 % in the group with traditional diet indicates that vegetarians have a better antioxidative status as a consequence of regular consumption of protective food.

Key words
Conjugated dienes of fatty acids • Homocysteine • Vitamin C • Vitamin E

Oxidative damage of molecules, e.g. by lipid peroxidation, is mainly caused by the highly toxic hydroxyl radical that is generated by the hyperhomocysteinemia or by the iron-catalyzed Fenton and Haber-Weiss reactions (Linpisarn *et al.* 1991, Dabbagh *et al.* 1994, Loscalzo 1996). Hyperhomocysteinemia may promote the production of hydroxyl radicals through homocysteine autooxidation and thiolactone formation. Damaged endothelium is associated with decreased nitric oxide production resulting in lower formation of S-nitroso-homocysteine. In addition to other favourable effects, this product causes free radical reduction (Zhang *et al.* 2000). Antioxidant vitamins such as vitamins C and E may have an adjunctive role in preventing homocysteine-mediated oxidative vascular injury (Chambers *et al.* 1999). In the Fenton and Haber-Weiss reactions, iron must be released from proteins. Superoxide anion produced during oxidative stress can mobilize iron from ferritin (Biemond *et al.* 1984) and hydrogen peroxide is capable of releasing iron from heme (Gutteridge 1986). Transferrin does not release its iron at a normal pH, while at a low pH, which may be the case in arterial wall, iron can be released from transferrin and induce oxidation of LDL (Leake 1997). Superoxide and hydrogen peroxide have a lower oxidative capacity for lipids, but hydroxyl radicals are produced by their break-up or their mutually-linked reactions catalyzed by iron or copper (mentioned Haber-

Fax +420 241 062 164
E-mail: physres@biomed.cas.cz http://www.biomed.cas.cz/physiolres

Weiss and Fenton reactions). The hydroxyl radical is the most effective oxidant because this radical lacks an enzymatic defense system. Vegetable- and thus antioxidant-rich diets, e.g. of the vegetarian type or the Mediterranean type of diet, are associated with a lower incidence of free radical disease (Gey 1995).

In this report, we evaluated the relationship of lipid conjugated dienes (first peroxidation product) to levels of homocysteine, iron as well as to essential antioxidant levels in the dependence on nutrition (alternative – subjective healthy vegetarians vs. traditional mixed diet – general subjective healthy population). Group characteristics are presented in Table 1. The plasma levels of conjugated dienes of fatty acids were measured by spectrophotometry (Recknagel and Glende 1984). Total plasma homocysteine was assessed using HPLC with fluorescence detection and SBD-F (7-fluorobenzofurazane-4-sulfonic acid) as a derivation agent (Vester and Rasmussen 1991). Levels of vitamins C, E and β-carotene in plasma were detected by HPLC (Lee *et al.* 1992, Cerhata *et al.* 1994). Serum levels of iron, cholesterol and triacylglycerols were measured by an automatic Vitros analyzer using standard laboratory methods. Food habits were assessed from questionnaires focused on the frequency of consumption of selected food items. The intake of vitamins, mineral and trace elements was considered exclusively in the natural form. The survey was carried out in the spring. Basic statistic methods, Student´s t-test and regression analysis were used for the final data evaluation.

We observed a positive linear correlation between lipid conjugated dienes and triacylglycerols in relevant groups of traditional and alternative nutrition. This relation documents a higher supply of substrates (polyunsaturated fatty acids) for lipid peroxidation at higher triacylglycerol values. The general population group had significantly higher triacylglycerol levels with 42 % of risk values vs. 8 % in vegetarians (Table 1).

The prooxidative activity of homocysteine suggested that elevated plasma homocysteine levels might be associated with lipid peroxidation. In hyperhomocysteinemic animals, significantly higher tissue concentrations of malondialdehyde and reduced contribution of linoleic and linolenic acids to the total fatty acid content were found in the heart (Young *et al.* 1997).

Table 1. Selected prooxidative and antioxidative parameters, incidence of risk values and consumption of selected food constituents.

	Nutrition	
	Traditional	**Alternative**
Number (M/W)	24 (12/12)	24 (11/13)
Age span (years)	21-69	20-62
Average age (years)	41.2±2.0	40.0±1.7
Body mass index (kg/m²)	25.6±0.7	22.2±0.5 xxx
Period of vegetarianism (y)	–	9.6±0.9
Smokers	0	0
Conjugated dienes of fatty acids (μmol/l)	2.38 0.14	1.59±0.08 xxx
>2.4 A	42 %	8 %
Triacylglycerols (mmol/l)	1.79±0.35	1.16±0.07 x
>1.8 B	42 %	8 %
Homocysteine (μmol/l)	9.61±0.32	13.8±0.64 xxx
>15 C	4 %	29 %
Iron (μmol/l)	18.2±1.0	14.6±1.2 x
>27 – M, >24 – W) B	17 %	4 %
Vitamin C (μmol/l)	44.4±2.7	61.3±2.8 xxx
<50 D	58 %	8 %
Vitamin E (μmol/l)	25.9±1.2	30.2±1.0 xx
<30 D	67 %	33 %
Vitamin C/vitamin E	1.71±0.10	2.03±0.09 x
<1.0 E	21 %	0 %
Vitamin E/cholesterol (μmol/mmol)	5.08±0.26	6.79±0.21 xxx
<5.2 D	50 %	13 %
Vitamin E/triacylglycerols (μmol/mmol)	20.1±2.0	29.4±1.6 xxx
<16 F	38 %	4 %
β-carotene (μmol/l)	0.26±0.02	0.41±0.02 xxx
<0.4 D	83 %	33 %
Consumption (g/day)		
Fruit	176±11	463±22 xxx
Vegetables	62±3	195±12 xxx
Whole grain products	65±2	242±14 xxx
Grain sprouts	0	3.6±0.5 xxx
Oil seeds	7±1	29±3 xxx
Plant oils	32±2	63±3 xxx
Soy products	6±1	38±3 xxx

Results are expressed as mean ± S.E.M. x P<0.05 xx P<0.01 xxx P<0.001. Risk limits according to A Krajčovičová-Kudláčková et al. (1995b), B Wagner (1996), C Ueland et al. (1993), D Gey (1995), E Gey (1998), F Krajčovičová-Kudláčková et al. (2003)

Simple significant correlation between homocysteine levels and F_2-isoprostane values in humans was reported by Voutilainen *et al.* (1999). We demonstrated a positive linear relationship between homocysteine and conjugated dienes of fatty acids (Fig. 1). Hyperhomocysteinemia was found in 29 % of vegetarians but only in 4 % of subjects on a mixed diet (Table 1). The higher occurrence of mild hyper-homocysteinemia in vegetarians is a consequence of vitamin B_{12} deficiency as plant food lacks vitamin B_{12} (Krajčovičová-Kudláčková *et al.* 2000, Krajčovičová-Kudláčková and Blažíček 2002).

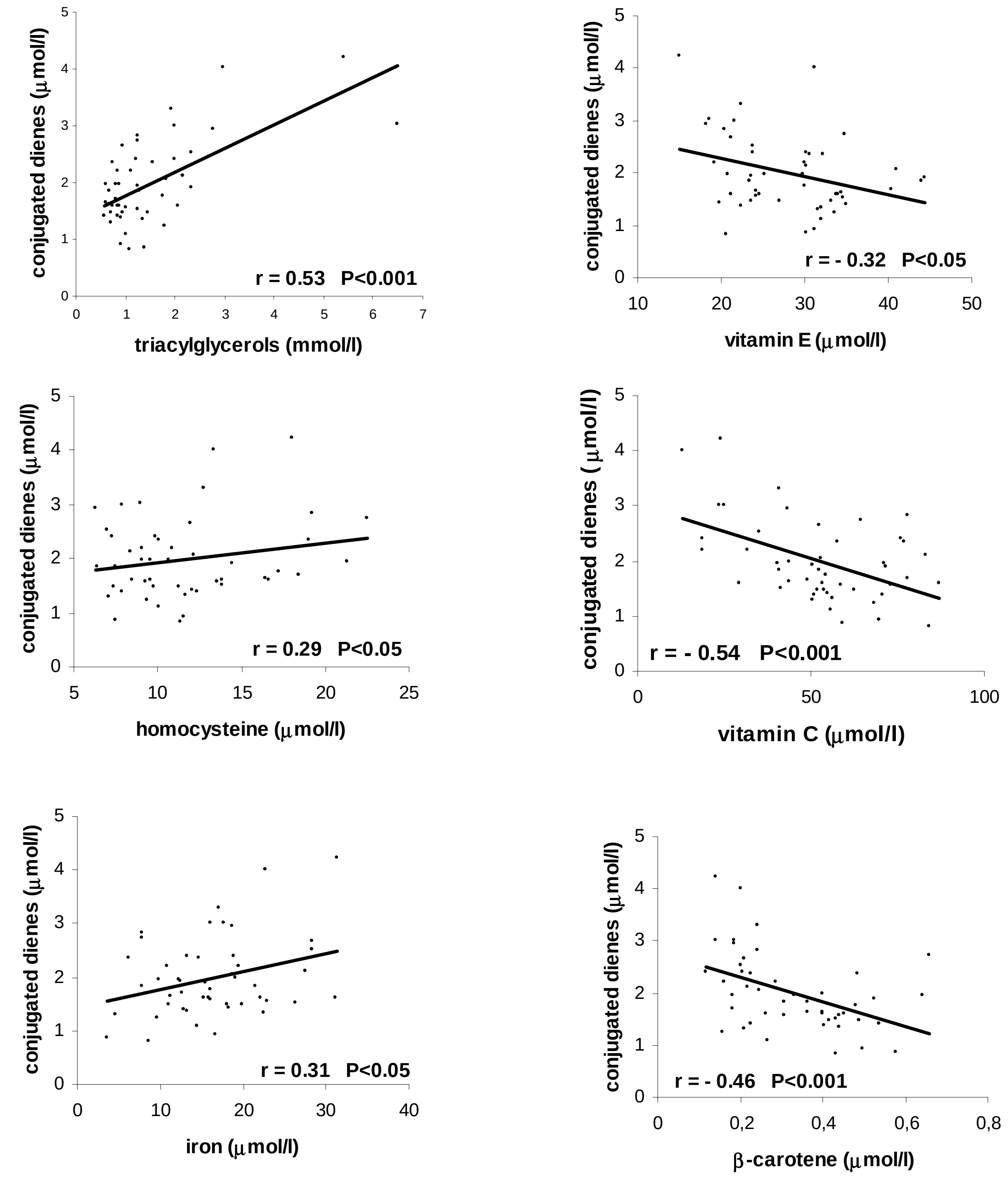

Fig. 1. Correlation between levels of conjugated dienes of fatty acids and levels oftriacylglycerols, homocysteine, iron, vitamin E, vitamin C and β-carotene in pooled vegetarian and traditional nutrition group

It is evident that high iron values after iron release from the protein bond might serve as a stimulator of lipid peroxidation (Valk and Marx 1999). Serum ferritin accounted for only 2 % of the variability in serum lipid peroxide levels, while the copper concentrations accounted for 21 % after multivariate analysis. Figure 1 shows a positive linear correlation of conjugated dienes and iron levels in the corresponding group. Iron levels were significantly reduced in vegetarians (Table 1). The iron utilization from plant food is approximately five times lower as compared to animal food (Herbert 1987). Phytic acid (cereals, pulses) is an intensive antagonist of iron absoption (Fairweather-Tait 1998). High iron values were found in 4 % of vegetarians vs. 17 % in the mixed diet group.

It may thus be concluded that with the exception of the known and hitherto unknown exogenic or endogenic factors, the lipid peroxidation risk in vegetarians can be caused by hyperhomocysteinemia (higher radical production) and in the general population, lipid peroxidation values can be elevated by a higher supply of substrates (risk triacylglycerol values) together with higher radical production, e.g. by risk iron values. This conclusion, without antioxidative evaluation, might be considered as a theoretical implication, because we found only 8 % of risk values of lipid peroxidation product in vegetarians but 42 % in general population (Table 1). A sufficient antioxidative capacity of the organism is a key problem in free radical defense. Improved antioxidant status helps to minimize oxidative damage and thus to delay or prevent pathological changes (Benzie 1996). This suggests a possible utility of antioxidant-based dietary strategies for lowering the risk of chronic age-related free radical disease (Diplock *et al.* 1998). Threshold values of plasma essential antioxidants have been determined (Gey 1995). Overthreshold values implicate a reduced risk of free radical diseases.

Figure 1 expresses a significant inverse linear relationship of lipid conjugated dienes and most important essential (dietary derived) antioxidants (vitamin E, vitamin C, β-carotene) in a relevant group of vegetarian and mixed nutrition subjects. Vegetarians have a higher level of antioxidants in comparison to non-vegetarians with high incidence of overthreshold values (92 % vs. 42 % for vitamin C, 67 % vs. 33 % for vitamin E, 100 % vs. 79 % for vitamin C/vitamin E, 87 % vs. 50 % for vitamin E/cholesterol, 96 % vs. 62 % for vitamin E/triacylglycerols and 67 % vs. 17 % for β-carotene) (Table 1). These results document a better antioxidant status of vegetarians as a consequence of higher consumption of protective food (Krajčovičová-Kudláčková *et al.* 1995a, 2002).

Epidemiological data strongly support the idea that high, and most importantly regular, consumption of fruit and vegetables, dark or whole grain products, grain sprouts, plant oils and oil seed rich in minerals, trace elements, mono- and polyunsaturated fatty acids, antioxidant vitamins, fibers, complex carbohydrates, flavonoids and nutrients together with an otherwise healthy lifestyle may protect against degenerative diseases (Gey 1995, Benzie 1996, Diplock *et al.* 1998, Krajčovičová-Kudláčková *et al.* 1995a, 2000, Key *et al.* 1999). Our results indicate that vegetarian nutrition might provide an effective prevention of free radical diseases. This favourable effect can also be provided by optimized traditional nutrition with sufficient consumption of protective food constituents. Nevertheless, nutritional research has shown that nutrition of the Slovak population is incorrect (Krajčovičová-Kudláčková *et al.* 2000, Babinská and Béderová 2002).

References

BABINSKÁ K, BÉDEROVÁ A: Changes in nutrient intake in the adult population of Slovak Republic. *J Food Comp Anal* **15:** 359-365, 2002.

BENZIE IFF: Lipid peroxidation, a review of causes, consequences, measurement and dietary influences. *Int J Food Sci Nutr* **47:** 233-262, 1996.

BIEMOND P, Van EIJK HG, SWAAK AJG, KOSTER JF: Iron mobilization from ferritin by superoxide derived from stimulated polymorphonuclear leukocytes, possible mechanism in inflammatory diseases. *J Clin Invest* **73:** 1576-1579, 1984.

CERHATA D, BAUEROVÁ A, GINTER E: Ascorbic acid determination in serum by high performance liquid chromatography and its correlation with spectrophotometric determination. *Ces Slov Farm* **43:** 166-168, 1994.

CHAMBERS JC, McGREGOR A, JEAN-MARIE J, OBEID OA, KOONER JS: Demonstration of rapid onset of vascular endothelial dysfunction after hyperhomocysteinemia, an effect reversible with vitamin C therapy. *Circulation* **99:** 1156-1160, 1999.

DABBAGH AJ, MANNION T, LYNCH SM, FREI B: The effect of iron overload on rat plasma and liver oxidant status in vivo. *Biochem J* **300:** 799-803, 1994.

DIPLOCK AT, CHARLEUX JL, CROZER-WILLI G, KOK FJ, RICE-EVANS C, ROBERFROID M, STAHL W, VINA-RIBES J: Functional food science and defense against reactive oxidative species. *Brit J Nutr* **80:** 77-112, 1998.

FAIRWEATHER-TAIT SJ: Trace element bioavailability. *Bibl Nutr Dieta* **54:** 29-39, 1998.

GEY KF: Longterm adequacy of all major antioxidants, presumably in synergy with other vegetable-derived nutrients, may help to prevent early stages of cardiovascular disease and cancer respectively. *Int J Vitamin Nutr Res* **65:** 65-69, 1995.

GEY KF: Vitamin E plus C and interacting conutrients required for optimal health. A critical and constructive review of epidemiology and supplementation data regarding cardiovascular disease and cancer. *Biofactors* **7:** 113-174, 1998.

GUTTERIDGE JMC: Iron promotors of the Fenton reaction and lipid peroxidation can be released from haemoglobin by peroxides. *FEBS Lett* **201:** 291-295, 1986.

HERBERT V: Recommended dietary intakes (RDI) of iron in humans. *Am J Clin Nutr* **45:** 679-686, 1987.

KEY TJ, GWYNETH KD, APPLEBY PN: Health benefits of a vegetarian diet. *Proc Nutr Soc* **58:** 271-275, 1999.

KRAJČOVIČOVÁ-KUDLÁČKOVÁ M, BLAŽÍČEK P: Effect of nutrition at homocysteine levels. *Biologia* **57:** 395-399, 2002.

KRAJČOVIČOVÁ-KUDLÁČKOVÁ M, ŠIMONČIČ R, BABINSKÁ K, BÉDEROVÁ A, BRTKOVÁ A, MAGÁLOVÁ T, GRANČIČOVÁ E: Selected vitamins and trace elements in blood of vegetarians. *Ann Nutr Metab* **39:** 334-339, 1995a.

KRAJČOVIČOVÁ-KUDLÁČKOVÁ M, ŠIMONČIČ R, BABINSKÁ K, BÉDEROVÁ A: Levels of lipid peroxidation and antioxidants in vegetarians. *Eur J Epidemiol* **11:** 207-211, 1995b.

KRAJČOVIČOVÁ-KUDLÁČKOVÁ M, BLAŽÍČEK P, BABINSKÁ K, KOPČOVÁ J, KLVANOVÁ J, BÉDEROVÁ A, MAGÁLOVÁ T: Traditional and alternative nutrition-levels of homocysteine and lipid parameters in adults. *Scand J Clin Lab Invest* **60:** 657- 664, 2000.

KRAJČOVIČOVÁ-KUDLÁČKOVÁ M, ŠEBEKOVÁ K, SHINZEL R, KLVANOVÁ J: Advanced glycation end products and nutrition. *Physiol Res* **51:** 313-316, 2002.

KRAJČOVIČOVÁ-KUDLÁČKOVÁ M, URSÍNYOVÁ M, BLAŽÍČEK P, SPUSTOVÁ V, GINTER E, HLADÍKOVÁ V, KLVANOVÁ J: Free radical disease prevention and nutrition. *Bratisl Lek Listy* **104:** 64-68, 2003.

LEAKE DS: Does an acidic pH explain why low density lipoprotein is oxidized in atherosclerotic lesions? *Atherosclerosis* **129:** 149-157, 1997.

LEE BL, CHUA SC, ONG HY, ONG CN: High performance liquid chromatographic method for routine determination of vitamins A and E and beta-carotene in plasma. *J Chromatogr* **581:** 41-43, 1992.

LINPISARN S, SATOH K, MIKAMI T, ORIMO H, SHINJO S, YOSHINO Y: Effects of iron on lipid peroxidation. *Int J Hematol* **54:** 181-188, 1991.

LOSCALZO J: The oxidant stress of hyperhomocysteinemia. *J Clin Invest* **98:** 5-7, 1996.

RECKNAGEL R, GLENDE EA: Spectrophotometric detection of lipid conjugated dienes. In: *Methods in Enzymology*. COLOWICK SP, KAPLAN NO (eds), Academic Press, San Diego, 1984, pp 331-337.

UELAND PM, REFSUM H, STABLER SP, MALINOW MR, ANDERSSON A, ALLEN RH: Total homocysteine in plasma or serum, methods and clinical applications. *Clin Chem* **39:** 1754-1779, 1993.

VALK B, MARX JJM: Iron, atherosclerosis and ischemic heart disease. *Arch Intern Med* **159:** 1542-1548, 1999.

VESTER B, RASMUSSEN K: High performance liquid chromatography method for rapid and accurate determination of homocysteine in plasma and serum. *Eur J Clin Chem Clin Biochem* **29:** 549-554, 1991.

VOUTILAINEN S, MORROW JD, ROBERTS LJ, ALFTHAN G, ALHO H, NYYSSONEN K, SALONEN JT: Enhanced in vivo lipid peroxidation at elevated plasma total homocysteine levels. *Arterioscler Thromb Vasc Biol* **19:** 1263-1266, 1999.

WAGNER P: *Laboratory Reference Values*. Praha, Triton, 1996.

YOUNG PB, KENNEDY S, MOLLOY AM, SCOTT JM, WEIR DG, KENNEDY KG: Lipid peroxidation induced in vivo by hyperhomocysteinemia in pigs. *Atherosclerosis* **129:** 67-71, 1997.

ZHANG X, LI H, JIN H, EBIN Z, BRODSKY S, GOLIGORSKY MS: Effect of homocysteine on endothelial nitric oxide production. *Am J Physiol Ren Physiol* **279:** F671- F678, 2000.

Reprint requests

M. Kudláčková, Ph.D., Institute of Preventive and Clinical Medicine, Limbová 14, SK-833 01 Bratislava, Slovak Republic. E-mail: kudlackova@upkm.sk