CASE REPORT

Nutritional determinants of plasma homocysteine

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Abstract

The total Hcy, methionine, vitamin B12, folic acid and vitamin B6 blood concentrations were measured in apparently healthy adult subjects aged 20–30 years with three types of nutrition – 52 normal weight subjects of general population on traditional mixed diet (non-vegetarians), 52 normal weight vegetarians and 24 overweight and obese non-vegetarians. In the groups with lower methionine intake (vegetarians, normal weight non-vegetarians; methionine intake 0.45–2.12 g/day), Hcy values are dependent on vitamin B12 and folic acid. Vegetarian Hcy concentration is significantly increased and hyperhomocysteinemia was found in 35 % of vegetarians vs 10 % of non-vegetarians. Elevated Hcy values in vegetarians are the consequence of vitamin B12 deficiency – 31 % of vegetarians with deficient serum values vs 2 % of non-vegetarians (vitamin is not contained in plant food). Non-vegetarians are more deficient in folic acid (8 % vs 0 % in vegetarians) due to of lower consumption of food rich in folic acid (vegetables, whole grain products, pulses, seeds).

The results suggest that in healthy population, a correct nutritional regime with an optimal intake of nutritional Hcy determinants is crucial for the maintenance of Hcy concentration in normal range and for the prevention of hyperhomocysteinemia *(Tab. 2, Fig. 2, Ref. 27)*. Full Text (Free, PDF) *www.bmj.sk*.

Key words: homocysteine, B-group vitamins, general population, vegetarians.

Homocysteine (Hcy) is a sulfur-containing amino acid produced within the metabolism of the essential amino acid methionine. Hcy is metabolized by two vitamin-dependent pathways – remethylation (requiring folate and vitamin B12) which converts Hcy back to methionine, and transsulfuration (requiring vitamin B6), which converts Hcy to cysteine and taurine. An alternative remethylation pathway in the liver and kidney utilizes betaine instead of folate (1). Total serum Hcy reflects the combined pool of free, bound, reduced and oxidized forms of Hcy in the blood. Normal Hcy concentrations range between 5 and 15 μmol/l; the increase from 16 to 30 μmol/l, from 31 to 100 μmol/l, and >100 μmol/l is classified as mild, moderate and severe hyperhomocysteinemia, respectively (2). Several factors can cause the Hcy increase (3, 4): genetic defects (thermolabile variant of methylene tetrahydrofolate reductase, cystathionine-synthase deficiency), dietary deficiencies (folic acid, vitamin B12, vitamin B6), lifestyle factors (chronic alcohol intake, smoking, high coffee intake), health state (renal failure, end-stage diabetes, systemic lupus erythematosus, hyperproliferative disorders) and medications (methotrexate, sulfonamides, antacids). This evidence suggests that in healthy population, B-group vitamin deficiencies accounts for the majority of cases of the increased Hcy.

The Hcy "hypothesis of atherosclerosis" was first proposed by McCully in 1969 (5), when he observed premature atherothrombosis in children with homocystinuria, an inborn defect in methionine metabolism. In 1976, Wilcken and Wilcken (6) provided the first evidence of a relationship between an abnormal Hcy metabolism and coronary artery disease in the general population. During the last decade, the use of Hcy in predicting the risk for atherosclerosis has been evaluated in several observational studies. These studies showed that the overall risk for vas-

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Tab. 1. Group characteristics, concentrations of homocysteine and its determinants, concentration of vitamin C and cholesterol.

	Normal weight vegetarians	Normal weight non-vegetarians	Overweight and obese non-vegetarians
n (m+w)	52 (0+52)	52 (0+52)	24 (12+12)
mean age (y)	25.2±0.4	23.9±0.3	26.6±0.6
range	20–30	20–29	20–30
BMI (kg/m^2)	21.0±0.3	20.9±0.3	32.0±0.8^3
range	16.9–24.9	17.7–25.0	27.3–40.2
duration of vegetarianism (y)	9.7±0.5	–	–
methionine (µmol/l)	26.2±0.8^2	30.0±0.8	ND
range	15.1–39.1	20.8–44.4	ND
homocysteine (µmol/l)	14.1±0.6^2	12.3±0.4	15.0±0.9^2
range	5.4–32.1	6.2–22.0	7.3–22.2
>15	35 %	10 %	46 %
vitamin B12 (pmol/l)	246±14^2	320±18	363±19
range	85–518	174–680	226–562
<179	31 %	2 %	0 %
folic acid (nmol/l)	22.6±1.2^3	17.6±0.6	17.4±1.3
range	14.0–45.4	8.6–26.5	7.9–32.1
<9.5	0 %	8 %	29 %
vitamin B6 (µg/l)	4.73±0.41^2	3.41±0.21	2.66±0.23^1
range	2.22–19.87	1.51–10.92	1.16–5.11
<3	10 %	40 %	67 %
vitamin C (µmol/l)	58.0±1.6	54.9±2.4	42.7±3.6^2
<50	13 %	38 %	54 %
cholesterol (mmol/l)	4.52±0.10	4.54±0.15	5.72±0.20^3
>5.2	13 %	21 %	67 %

The results are expressed as mean±SEM. ^{1} – p<0.05, ^{2} – p<0.01, ^{3} – p<0.001 – compared to normal weight non-vegetarians, BMI – body mass index; ND – non-detected

cular disease is smal. In prospective longitudinal studies, a weak association between Hcy and atherothrombotic vascular disease was reported, compared to retrospective case-control and cross-sectional studies with a stronger association (4, 7, 8).

The aim of this study was to evaluate the total Hcy plasma concentration during different nutritional regiments (vegetarians, non-vegetarians, overnutrition).

Subjects and methods

Randomly selected young adults, apparently healthy, aged 20–30 years were divided into the three groups: group 1 – 52 normal weight vegetarians consuming predominantly plant food, but also dairy products, eggs and ≤2 times monthly white meat (poultry or fish), group 2 – 52 normal weight non-vegetarians (general population on traditional mixed diet) and group 3 – 24 overweight or obese non-vegetarians (body mass index BMI >25). All subjects lived in the same region (Bratislava and neighbourhood). They were non-smokers and consumed normal or low amount of coffee and alcohol (according to data in dietary questionnaires). The groups characteristic is showed in Table 1.

Blood was sampled after an overnight fasting using a standard procedure. Plasma levels of total Hcy and methionine were measured by HPLC method (9, 10). EDTA was used as an anticoagulant. Serum vitamin B12 and folic acid were determined using Elecsys immunoassay (Roche tests). Serum vitamin B6 values were detected by HPLC method (Chromsystems test). Plasma concentrations of vitamin C were measured by HPLC method (11). Serum cholesterol levels were measured by a standard laboratory method using Vitros 250 autoanalyzer (Johnson & Johnson, USA). The intake of vitamins, mineral and trace elements in natural form only was allowed (no supplementation). The Student t-test was used for the final evaluation.

Results and discussion

The Hcy plasma concentration was significantly higher in vegetarians vs non-vegetarians, and hyperhomocysteinemia was observed in 35 % of vegetarian subjects vs 10 % in non-vegetarians (Tab. 1). Methionine concentration was significantly reduced in vegetarians (Tab. 1). Figure 1 showed a significant inverse correlation between Hcy and methionine concentrations. In 1980, Mudd (12) reported a strong inverse correlation between remethylation and methionine intake. Low methionine intake leads to activation of remethylation cycle, decrease of Hcy concentration and increase of methionine concentration. Remethylation is activated by methionine intake <3 g (0 % remethylation at 3 g and more methionine intake) and percentage of remethylation decreases proportionally to the increase of methionine intake. In previous study, (13) we have found that a daily methionine intake was lower than 3 g in general population with normal body mass index as well as in alternative nutrition subjects (1.46 g (range 0.68–2.12) – general population, 0.9 g (range 0.45–1.6) – lacto-ovo-vegetarians, 0.77 g (range 0.41–1.51) – vegans). Methionine content in plant proteins is significantly reduced (14) compared to reference protein of hen egg: e.g. soya flour 41 %, lentil seed 21 %, hazelnut 25 %, walnut 31 %, oat flakes 49 %. At optimal protein intake of 100–110 % of RDA (recommended dietary allowance), we found a deficiency in sulphur amino acid intake in 22 % of vegetarian subjects vs 6 % in normal weight general population (14).

These results suggest than remethylation cycle of Hcy metabolism dependent on vitamin B12 and folic acid prevails in subjects of general population with correct nutrition and in vegetarians. This assumption is expressed in Figure 1 by a significant inverse correlation between Hcy and vitamin B12 or folic acid concentrations. No correlation (horizontal trend line) was found in vitamin B6 (Fig. 1). Vitamin B12 serum value is significantly reduced in vegetarian group in comparison to non-vegetarians and 31 % of vegetarians vs 2 % of non-vegetarians are vitamin B12 deficient (Tab. 1). This vitamin is not contained in plant food (15). Concerning the healthy individuals on traditional mixed diet, vitamin B12 plays a minor role as a determinant of Hcy concentration with an exception in old persons, who often develop vitamin B12 deficiency due to atrophic gastritis (16, 17). Serum folate concentration was significantly increased

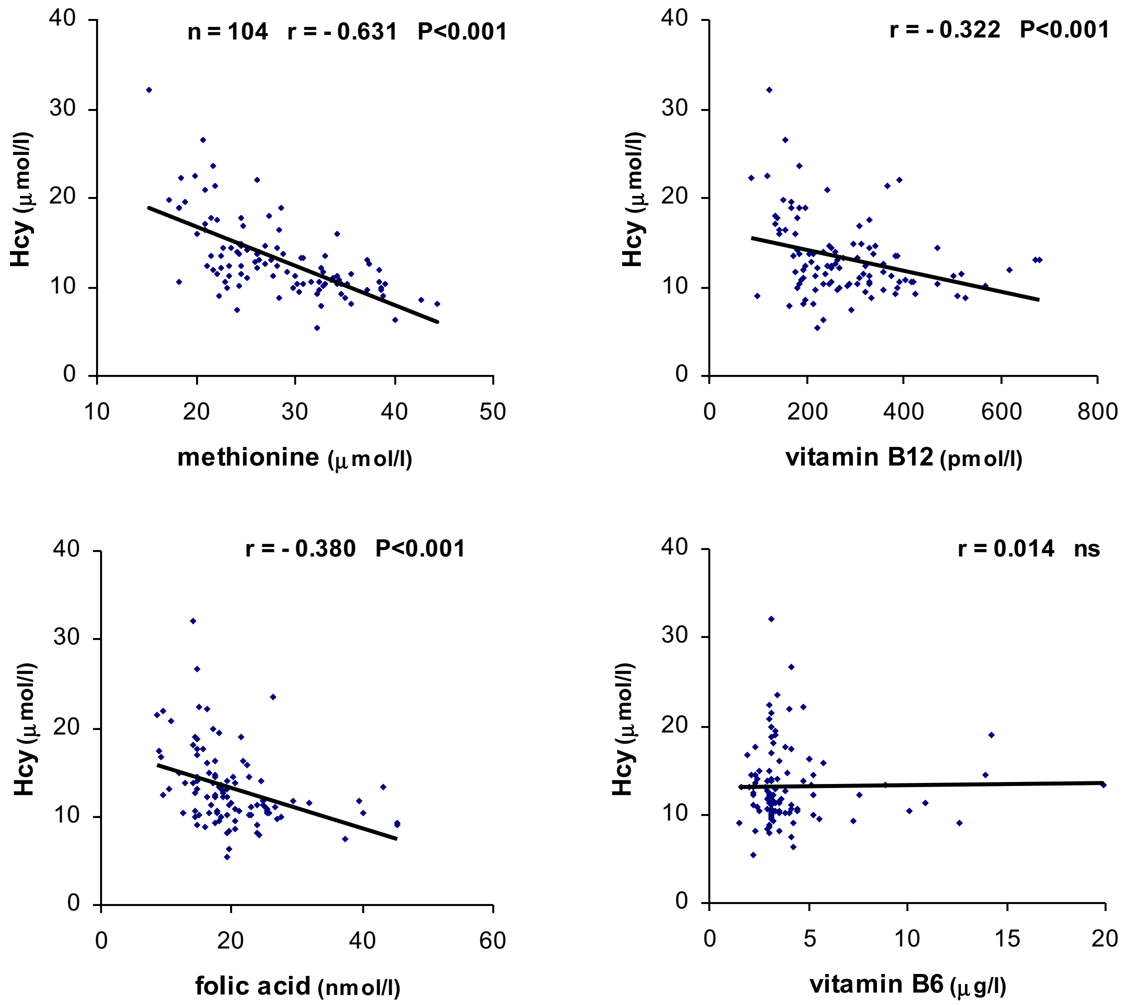

Fig. 1. Relationship between homocysteine concentrations (Hcy) and concentrations of methionine, vitamin B12, folic acid or vitamin B6.

in vegetarians (Tab. 1) with no subjects deficient vs 8 % of subjects deficient in non-vegetarian group. The subjects from general population may have a problem in meeting the RDA for folate, since they consume fewer legumes, whole grain products, seed, fruit and vegetables or, do not consume some these items at all (18). The average folate intake represented 108 % of RDA (216 μg) vs 206 % of RDA (412 μg) in alternative nutrition (13).

Methionine blood concentration in group of overweight and obese non-vegetarians (BMI >25) was not detected. Base on the following statistically significant relationships and values, we suppose a higher methionine intake and a higher plasma level: 1) direct linear correlation between body mass index and cholesterol concentrations in all 128 studied subjects (Fig. 2); 2) a significantly higher cholesterol concentration in overweight and obese vs normal weight subjects (Tab. 1); 3) a significant positive linear correlation between vitamin C and folic acid concentration in all 128 subjects (Fig. 2), (vitamin C – determinant of prevailingly fruit consumption, folic acid – determinant of mainly vegetable consumption); 4) a significantly reduced vitamin C and vitamin B6 concentration in overweight and obese vs normal weight subjects with deficiencies in 54 % vs 38 % for vitamin C, 67 % vs 40 % for vitamin B6 and 29 % vs 8 % for folic acid (Tab. 1); 5) the highest vitamin B12 plasma level in the group with body mass index >25 in comparison to normal weight subjects of both groups (Tab. 1). These correlations and plasma levels for group of overweight and obese subjects as well as the higher occurrence of vitamin deficiencies predominantly from plant sources and no deficiency of vitamin contained only in animal sources suggest the overnutrition with prevalence of animal food consumption. Animal proteins are richer in methionine compared to plant proteins (e.g. if methionine content in hen egg is 100 %, then in cow milk it is 85 % and in chicken 81 %, but in soya flour it is 41 %, in oat flakes 49 %, lentil seed 21 %,

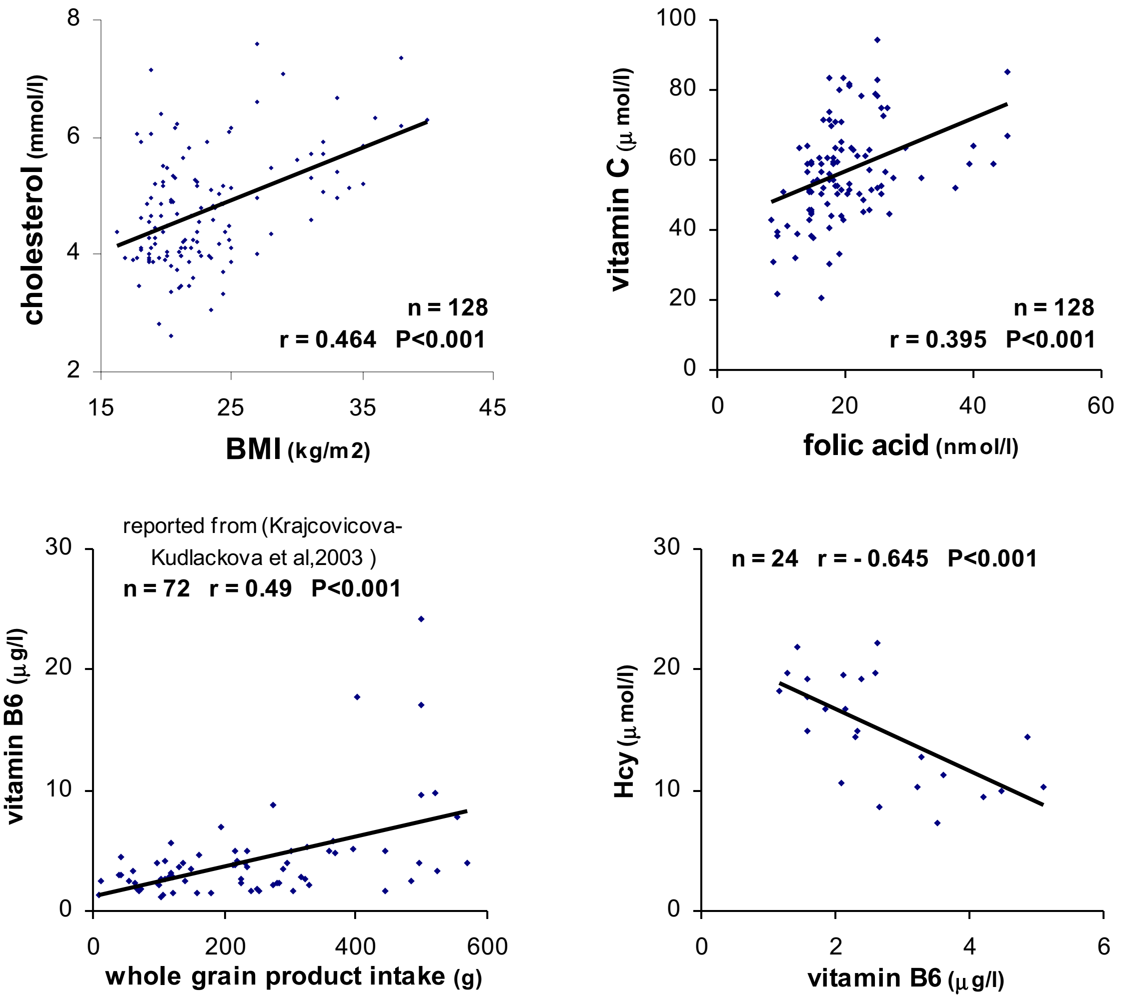

Fig. 2. Correlations between: cholesterol concentration and body mass index (BMI), vitamin C and folic acid concentration, vitamin B6 concentration and intake of whole grain products, Hcy and vitamin B6 concentration.

walnut 31 % (14)). In case of a higher methionine intake, a transsulfuration Hcy metabolic pathway dependent on vitamin B6 dominates. In the group of the overnutrition, the serum vitamin B6 concentration was significantly reduced in comparison to non-obese subjects (Tab. 1) with 67 % of subjects with deficiency vs 40 % of subjects with deficiency in non-obese non-vegetarian group and 10 % in vegetarian group. In our previous study, we reported a significant positive linear correlation between whole grain product consumption and serum vitamin B6 concentration (19) (Fig. 2). Rich in vitamin B6 are also pulses, oil seeds and several vegetable sorts (19). These food commodities of plant origin are non-dominant in overweight and obese non-vegetarians. The Hcy concentration is significantly higher in overnutrition subjects in comparison to normal weight non-vegetarian subjects (Tab. 1) and 46 % of persons had Hcy values higher than 15 μmol/l. Table 2 clearly shows that a mild hyperhomocysteinemia in subjects with BMI >25 is a consequence of vitamin B6 deficiency. We noted a significant inverse correlation between Hcy and vitamin B6 concentrations in 24 overweight and obese subjects (Fig. 2).

The mechanisms by which increased Hcy impairs vascular function are not completely understood (4). Laboratory investigations have revealed several potential mechanisms: impairment of endothelial function, increased monocyte adhesion to the vessel wall, thrombotic tendency mediated by activation of coagulation factors and platelet dysfunction, activation of the inflammatory pathway, stimulatory effects on smooth-muscle proliferation, oxidation of low density lipids, increased lipid uptake and retention (4, 20, 21). These findings provide a coherent and biologically plausible basis for a direct role of Hcy in the promotion of atherosclerotic and thrombotic vascular disease. Numerous epidemiological studies reported the association between hyperhomocysteinemia and vascular risk but the results of these studies are not unanimously validated. The homocysteine stud-

Tab. 2. Concentrations of homocysteine and its determinants in subjects with hyperhomocysteinemia.

	Normal weight vegetarians	Normal weight non-vegetarians	Overweight and obese non-vegetarian
n	18	5	11
homocysteine (μmol/l)	19.9±1.0	19.7±1.1	19.2±0.5
range	15.9–32.1	16.7–22.0	16.7–22.2
vitamin B12 (pmol/l)	154±7	328±25	356±29
range	85–196	244–390	226–562
<179	78 %	0 %	0 %
folic acid (nmol/l)	23.2±1.9	9.4±0.4	17.9±1.2
range	14.4–45.4	8.6–10.8	11.2–26.0
<9.5	0 %	80 %	0 %
vitamin B6 (μg/l)	4.31±0.61	3.24±0.40	1.88±0.16
range	2.35–14.22	1.88–4.10	1.16–2.62
<3	6 %	20 %	100 %

The results are expressed as mean±SEM.

ies collaboration (22) pooled evidence from 12 prospective and 18 retrospective studies from 1966 to 1999. A total of 5 073 coronary artery disease events and 1 113 stroke events were observed among 16 786 healthy individuals. The results showed that a 25 % increase in the serum Hcy mconcentration (an approximately 3 μmol/l increase) was associated with a 49 % higher risk of ischemic heart disease in the retrospective studies. In contrast, a weaker association was observed in the prospective studies (20 % risk increase). With respect to stroke, the strength of association was reversed – a non-significant 16 % higher risk in retrospective studies compared with a significant 30 % higher risk in prospective studies.

Therapeutic options for lowering an increased Hcy call for the use of vitamin supplements.

Folate supplementation with 0.5–5 mg/day significantly reduces total Hcy concentration by 25 % in patients with mild to moderate hyperhomocysteinemia (23). In the review article of Ubbink (24), a decrease of total Hcy concentration by 34–52 % was observed in healthy volunteers consuming folic acid in dose 0.6–10 mg/day and a 27 % decrease in patients with an acute myocardial infarction who were supplemented with 2.5 mg of folic acid daily. Supplementation with vitamin B12 produces a small additional effect (5–15 %) except the vitamin B12 deficient vegetarians (23, 24). In these subjects supplemented by vitamin B12 in the total dose 2.2 mg, the total Hcy concentration decreased by 42 % (25). Vitamin B6 treatment alone reduces an increased Hcy levels from a high methionine load concentrations (23). Optimal concentration of vitamin B6 is important for the decrease of Hcy values in case of a higher methionine intake from animal food (>3 g/day) (12)), which leads to the predominance of vitamin B6 dependent transsulfuration metabolic pathway (Tabs 1 and 2, Fig. 2).

Routine screening for the increased Hcy is yet not recommended (2, 3, 26, 27). However, screening may be advisable in individuals who manifest atherothrombotic disease that is out of range of their traditional risk factors or who have a family history of premature atherosclerotic disease. In healthy population, a correct nutritional regimen with sufficient daily intakes of nutritional determinants of Hcy metabolism, which provide their optimal plasma concentration, is crucial for the maintenance of total Hcy plasma concentrations in normal range and for the prevention of hyperhomocysteinemia.

References

1. **Hankey GJ, Eikelboom JW.** Homocysteine and vascular disease. Lancet 1999; 354: 407—413.

2. **Refsum H, Smith AD, Ueland PM, Nexo E, Clarke R, McPartlin J, Johnston C, Engbaek F, Schneede J, McPartlin C, Scott JM.** Facts and recommendations about total homocysteine determinations, an expert opinion. Clin Chem 2004; 50: 3—32.

3. **Malinow MR, Bostom AG, Krauss RM.** Homocysteine, diet and cardiovascular diseases, a statement for healthcare professionals, from the Nutrition Committee, American Heart Association. Circulation 1999; 99: 178—182.

4. **Kaul S, Zadeh AA, Shah PK.** Homocysteine hypothesis for atherothrombotic cardiovascular disease, not validated. J Amer Coll Cardiol 2006; 48: 914—923.

5. **McCully KS.** Vascular pathology of homocysteinemia, implications for the pathogenesis of arteriosclerosis. Amer J Pathol 1969; 56: 111—128.

6. **Wilcken DE, Wilcken B.** The pathogenesis of coronary artery disease. A possible role for methionine metabolism. J Clin Invest 1976; 57: 1079—1082.

7. **Gibelin P, Serre S, Candito M, Houcher B, Berthier F, Baudouy M.** Prognostic value of homocysteinemia in patients with congestive heart failure. Clin Chem Lab Med 2006; 44: 813—816.

8. **Omar S, Ghorbel IB, Feki H, Souissi M, Feki M, Houman H, Kaabachi N.** Hyperhomocysteinemia is associated with deep venous thrombosis of the lower extremities in Tunisian patients. Clin Biochem 2007; 40: 41—45.

9. **Melnyk S, Pogribna M, Pogribny I, Hine RJ, James SJ.** A new HPLC method for the simultaneous determination of oxidized and reduced plasma aminothiols using coulometric electrochemical detection. J Nutr Biochem 1999; 10: 490—497.

10. **Houze P, Gamra S, Madelaine I, Bousquet B, Gourmel B.** Simultaneous determination of total plasma glutathione, homocysteine, cysteinylglycine, and methionine by high performance liquid chromatography with electrochemical detection. J Clin Lab Anal 2001; 15: 144—153.

11. **Cerhata D, Bauerova A, Ginter E.** Ascorbic acid determination in serum by high performance liquid chromatography and its correlation with spectrophotometric determination. Ces Slov Farm 1994; 43: 166—168.

12. **Mudd SH.** Diseases of sulfur metabolism, implications for methionine-homocysteine cycle, and vitamin responsiveness. Ciba Found Symp 1980; 72: 239—258.

13. **Krajcovicova-Kudlackova M, Blazicek P, Kopcova J, Bederova A, Babinska K.** Homocysteine levels in vegetarians versus omnivores. Ann Nutr Metab 2000; 44: 135—138.

14. **Krajcovicova-Kudlackova M, Babinska K, Valachovicova M.** Health benefits and risks of plant proteins. Bratisl lek Listy 2005; 106: 231—234.

15. Herbert V. Staging vitamin B12 status in vegetarians. Am J Clin Nutr 1994; 59: 1213—1222.

16. Selhub J, Jacques PF, Wilson PWF, Rush D, Rosenberg JH. Vitamin status and intake as primary determinants of homocysteinemia in an elderly population. J Amer Med Ass 1993; 270: 2693—2698.

17. Haan MN, Miller JW, Aiello AE, Whitmer RA, Jagust WJ, Mungas DM, Allen LH, Green R. Homocysteine, B vitamins, and the incidence of dementia and cognitive impairment, results from the Sacramento area latino study on aging. Amer J Clin Nutr 2007; 85: 511—517.

18. Krajcovicova-Kudlackova M, Spustova V, Paukova V. Lipid peroxidation and nutrition. Physiol Res 2004; 53: 219—224.

19. Krajcovicova-Kudlackova M, Blazicek P, Ginter E. Vitamin B6 level from the aspect of nutrition. Hygiena 2003; 48: 73—78.

20. Lentz SR. Homocysteine and cardiovascular physiology (441—450). In: Carmel R, Jacobsen DW (Eds). Homocysteine in health and disease. Cambridge, Cambridge University Press, 2001.

21. Hofmann MA, Lalla E, Lu Y, Gleason MR, Wolf BM, Tanji N, Ferran LJ, Kohl B, Rao V, Kisiel W, Stern DM, Schmidt AM. Hyperhomocysteinemia enhances vascular inflammation and accelerates atherosclerosis in a murine model. J Clin Invest 2001; 107: 675—683.

22. Klerk M, Verhoef P, Clarke R, Blom HJ, Kok FJ, Schouten, EG. MTHFR 677C-T polymorphism and risk of coronary heart disease, a meta-analysis. J Amer Med Ass 2002; 288: 2023—2031.

23. Griend R, Biesma DH, Haas FJ, Faber JA, Duran M, Menwissen DJ, Banga JD. The effect of different treatment regimens in reducing fasting and postmethionine-load homocysteine concentrations. J Intern Med 2000; 248: 223—229.

24. Ubbink JB. The role of vitamins in the pathogenesis and treatment of hyperhomocysteinemia. J Inher Metab Dis 1997; 20: 316—325.

25. Krajcovicova-Kudlackova M. Vitamin regulation of homocysteine levels. Klin Biochem Metab 2002; 10: 87—92.

26. Morris CD, Carson S. Routine vitamin supplementation to prevent cardiovascular disease, a summary of the evidence for the U.S. Preventive Services Task Force. Ann Intern Med 2003; 139: 56—70.

27. Vogel JHK, Bolling SF, Costello RB, American College of Cardiology Foundation task Force on Clinical Expert Consensus Documents. Integrating complementary medicine into cardiovascular medicine. J Amer Coll Cardiol 2005; 46: 184—221.

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