Original Research

Low nourishment of B-vitamins is associated with hyperhomocysteinemia and oxidative stress in newly diagnosed cardiac patients

Mostafa I Waly[1,2], Amanat Ali[1], Amira Al-Nassri[3], Mohamed Al-Mukhaini[4], John Valliatte[4] and Yahya Al-Farsi[5]

[1]Food Science and Nutrition Department, College of Agricultural and Marine Sciences, Sultan Qaboos University, Muscat, 123, Sultanate of Oman; [2]Nutrition Department, High Institute of Public Health, Alexandria University, El-Hadrah, 165, Egypt; [3]Nutrition Department, Royal Hospital, Ministry of Health, Muscat, 111, Sultanate of Oman; [4]Cardiology and Cardiac Surgery Departments, Royal Hospital, Ministry of Health, Muscat, 111, Sultanate of Oman; [5]Family Medicine and Public Health Department, College of Medicine and Health Sciences, Sultan Qaboos University, Muscat, 123, Sultanate of Oman
Corresponding author: Mostafa Waly. Email: waly.mostafa@gmail.com

Abstract

We are currently witnessing a dramatic change in lifestyle and food choices that is accompanied with an increase in the rate of morbidity and mortality from cardiovascular diseases (CVD). Although studies have reported an association of CVD with hyperhomocysteinemia-mediated oxidative stress, the biochemical basis is not known. This case–control study was aimed to evaluate the nutritional and biochemical status of B-vitamins in relation to hyperhomocysteinemia and oxidative stress in newly diagnosed cardiac patients. The retrospective dietary intake of the study subjects (cases and controls) was estimated using a semi-quantitative food frequency questionnaire, and fasting blood samples were drawn to assess their serum levels of B-vitamins (folate, vitamins B_6 and B_{12}), homocysteine (HCY), and oxidative stress indices such as glutathione (GSH), total antioxidant capacity (TAC), malondialdehyde (MDA), and nitrites and nitrates (NN). It was observed that the cases had a lower dietary intake of B-vitamins as compared to their matched control subjects as well as to the corresponding recommended dietary allowances. Biochemical analysis of cases, as compared to controls, indicated depletion of GSH, impairment of TAC, and an elevation in the serum levels of HCY, MDA, and NN. These results suggest that lower status (dietary intake and serum levels) of B-vitamins is involved in the etiology of hyperhomocysteinemia and oxidative stress, the typical risk factors for CVD.

Keywords: Folic acid, vitamin B_{12}, vitamin B_6, hyperhomocysteinemia, oxidative stress, cardiac patients

***Experimental Biology and Medicine* 2016; 241: 46–51. DOI: 10.1177/1535370215596860**

Introduction

Cardiovascular diseases (CVDs) are one of the non-communicable diseases (NCDs), associated with westernization of lifestyle and food choices, and can be prevented through healthy diet, and regular physical activity.[1] CVDs are a major global health problem, and it has been postulated that by the year 2020, the CVDs will surpass other NCDs and infectious diseases as the world's leading cause of mortality and morbidity.[2]

The CVDs risk factors include both modifiable and non-modifiable risk factors; B vitamins (folate, vitamin B_6 and vitamin B_{12}) are one of the modifiable dietary risk factors for CVDs, based on their significant metabolic effects on homocysteine (HCY) metabolism as illustrated in Figure 1.[3–6] It has been reported that nutritional deficiencies of folate, vitamins B_6, and B_{12} inhibit the enzymes that mediate the HCY-dependent transmethylation and transsulfuration pathways resulting in hyperhomocysteinemia and are associated with pathophysiological consequences mainly the induction of oxidative stress as evident by the intracellular glutathione (GSH) depletion.[7–11] Hyperhomocysteinemia and oxidative stress, as reported by many studies, are involved in the pathogenesis of CVDs.[12–14] Previous studies conducted in relation to the etiological factors of CVD incidence mainly focused on the non-modifiable risk factors, classical risk factors, including hypertension, lipidemia, and hypercholesterolemia. However, no studies attempted to identify the biochemical and nutritional basis of B-vitamins as modifiable risk factors for CVDs. Therefore, in the present study we evaluated the biochemical and nutritional status of folate,

vitamins B_6, and vitamin B_{12} in relation to HCY, and oxidative stress in newly diagnosed cardiac patients.

Materials and methods

Study subjects

A case–control study design was used to conduct this study at Royal Hospital Muscat, a tertiary hospital and the main referral hospital for cardiac cases, in the Sultanate of Oman. The cardiac patients included in this study were recruited from the recently diagnosed Omani nationals suffering with acute CVDs and admitted to cardiology department. The criteria of CVDs diagnosis were based on the symptoms, enzymatic changes, and electrocardiogram changes. The inclusion criteria was newly diagnosis with CVDs (angina and myocardial infarction), with no previous diagnosis of CVD, atherosclerosis or any other types of hyperlipidemia. The exclusion criteria were the presence of metabolic disorders, cancer, type-2 diabetes, hypothyroidism, or chronic renal diseases. According to the inclusion and exclusion criteria, 25 cases were enrolled during the period of this study, and controls ($n = 25$), healthy subjects, were recruited on voluntary basis and matched for age and gender with the cases. None of the study participants were consuming lipid-lowering drugs. The study was approved by the Medical Research Ethics Committee of Ministry of Health, Oman, MESRC#76.

Study questionnaire

In-person interviews were scheduled for all the study participants. The enrolled cardiac patients and controls were asked to complete the study questionnaire that included questions related to: (1) Socio-demographic data: smoking, alcohol drinking, medical family history, intake of vitamins and nutritional supplements, monthly income, and physical activity; (2) Anthropometric measurements: weight and height to calculate body mass index (BMI kg/m^2) for all study participants; (3) Dietary intake assessment: the retrospective dietary intake of the study participants was estimated using a semi-quantitative food frequency questionnaire (FFQ). All study participants were asked to report the frequency of food intake, how often, and what portion size for each food item they consumed during a period of six months prior to the CVDs attack for cases, and prior to the study questionnaire interview for controls. The eight different food groups included in the FFQ were: breads/cereals, vegetables, fruits, meat/meat substitutes, milk/dairy products, deserts, beverages, and sandwiches. The collected dietary intake data were analyzed using the Food Processor Software Version 10.2 (ESHA Research, Salem, OR) to calculate the means of daily intake of macronutrients (protein, carbohydrate, and fat), folate, vitamin B_6, and vitamin B_{12} as estimated from the frequency of consumption, reported portion size, and nutrients content for all foods reported by each study participant. The FFQ was developed in collaboration with the Nutrition Department, High Institute of Public health, Alexandria University, Egypt.

Biochemical analysis

Fasting blood samples were drawn after overnight fasting into two types of tubes (plain top and purple top tubes). The purple top tube was used for red blood cells folate measurement,[15] and the plain top tube was used for serum separation by centrifugation and storage at $-80^{\circ}C$ for subsequent

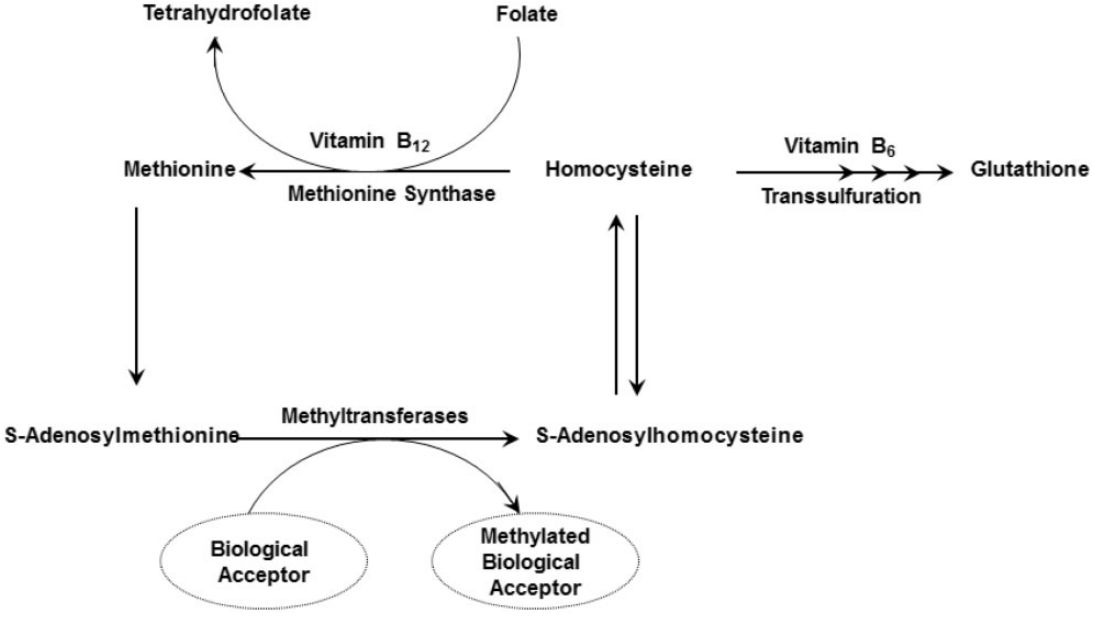

Figure 1 Simplified schematic of the homocysteine-dependent transmethylation and transsulfuration pathways. Homocysteine is methylated into methionine by methionine synthase enzyme, which utilizes vitamin B_{12} as a cofactor and acquires a methyl group from folate which is subsequently converted to tetrahydrofolate. Methionine is further converted to *S*-adenosylmethionine, through the activity of methionine adenosyl transferase enzyme, which is the major methyl donor for all methyltransferases that add methyl groups to various acceptor molecules such as DNA, RNA, phospholipids, and proteins. *S*-adenosylmethionine is then converted to *S*-adenosylhomocysteine which is reversibly converted to homocysteine in a reaction catalyzed by hydrolase enzyme. Based on the methionine synthase enzyme activity and the availability of folate and vitamin B_{12}, homocysteine is remethylated back to methionine, or transulfurated into glutathione biosynthesis pathway that requires vitamin B_6

analyses. Folate, vitamins B_6, and B_{12} were measured in the sera of the study participants using an automated random-access immunoassay system (Siemens Medical Solutions Diagnostics, ADVIA Centaur Chemistry Analyser, Bohemia, NY). The serum HCY levels were determined by the Immulite 2000 Homocysteine Analyser.[16]

Serum oxidative stress markers were measured according to the manufactures' instructions (BioVision, Inc., CA) in each kit: GSH by assay kit K251, the total antioxidant capacity (TAC) by assay kit K274, malondialdehyde (MDA) by assay kit K739, and nitrites and nitrates (NN) by assay kit K262.

Statistical analysis

The collected data were reviewed for completeness and accuracy. Data were presented as mean ± standard deviation (SD), and analyzed by the appropriate statistical procedures using ''The Statistical Package GraphPad Prism version 5''. Chi-squared (χ^2) test was used to analyze the categorical variables. The unpaired Student's *t*-test and simple correlation coefficients (*r*) were quantified for assessing the correlations between HCY and different variables. $P < 0.05$ is considered statistically significant.

Results

General characteristics

Twenty-five cases, cardiac patients (12 males and 13 females), were recruited on voluntary basis in accordance to the inclusion and exclusion criteria for this study. For each case, one control (healthy subject) was matched for age and gender. It was observed that 64% of the enrolled cases were suffering from myocardial infarction, 24% from stable angina, and 12% from unstable angina. There was no significant differences between mean age (years) for the cases and controls, 54.72 ± 10.3 and 53.81 ± 6.8, respectively, $t = 0.368$, $P > 0.05$. The percentage distribution of the cases by their smoking status was majority of the cases (72%) were non-smokers and only 28% were smokers, meanwhile none of the enrolled controls were smokers.

Physical activity and anthropometric measurements

The cases were distributed by their physical activity level into two categories: 76% were in the category of light activity whereas 24% had moderate activity. The same pattern was observed for controls, where 68% had light activity and 32% had moderate activity. None of the enrolled cases or controls was performing any routine physical exercise. The difference between the cases and controls with regard to their physical activities was non-significant, $\chi^2 = 0.511$, $P = 0.774$. The mean body weight (kg) for the enrolled cases and controls was 68.8 ± 14.8 and 69.2 ± 13.1, respectively. The mean height (cm) was 156.2 ± 9.2 and 155.9 ± 8.4, respectively. The average BMI (kg/m^2) for cases and controls was 28.3 ± 4.8 and 28.8 ± 1.55, respectively, with no significant difference, $t = 0.496$, $P > 0.05$, showing prevalence of overweight among cases and controls.

Daily macronutrients intake

The daily macronutrients intake of the cases and controls is presented in Table 1. It was observed that the cases consumed significantly higher amounts of protein as compared to controls ($t = 4.22$, $P < 0.05$), however the cases consumed significantly lower daily intake of fat, carbohydrate, and total energy as compared to controls ($t = 6.81$, $t = 3.49$ and $t = 4.04$, $P < 0.05$, respectively).

Daily intake and serum levels of B-vitamins

The data on dietary intake and serum levels of folate, vitamin B_6, and vitamin B_{12} are summarized in Table 2. It was noted that the cases had lower levels of dietary intake of these micronutrients as compared to both their matched controls as well as with respect to the dietary reference intakes. The same pattern was observed for these micronutrients in the serum levels of cases as compared to controls and serum reference values. The differences were statistically significant, $P < 0.05$.

Table 1 Distribution of the studied sample according to daily macronutrients and total energy intake

Nutrient	Cases	Controls
Protein (g/day)	72.8 ± 19.7*	55.1 ± 7.01
Total fat (g/day)	58.4 ± 17.4#	95.5 ± 20.85
Carbohydrates (g/day)	228.6 ± 70.2#	280 ± 21.92
Total energy intake (kcal/day)	1743.2 ± 442.3#	2199.9 ± 353.55

Values are expressed as mean ± SD. The asterisk denotes that the data in cases are significantly higher than controls, whereas the hash mark denotes that data are significantly lower than controls group, $P < 0.05$.

Table 2 Dietary and serum measurements of folate, vitamin B_6, and vitamin B_{12}

	Serum levels (mean ± SD)		Daily dietary intake (μg/day) (mean ± SD)	
Nutrient	Cases	Controls	Cases	Controls
Folate	2.1 ± 0.4	6.4 ± 0.9*	291.48 ± 9.5	489.65 ± 6.3*
Vitamin B_{12}	209.2 ± 14.7	369.8 ± 32.1*	1.74 ± 0.41	4.56 ± 0.6*
Vitamin B_6	28.9 ± 5.3	56.7 ± 6.2*	1043.2 ± 133.2	1736.4 ± 211.4*

Values are expressed as mean ± SD. The asterisk denotes that data are significantly higher than cases, $P < 0.05$. Serum folate reference value, 3–20 μg/L, serum vitamin B_{12} reference value, 250–1250 pg/mL, serum vitamin B_6 reference value, > 40 nmol/L. Dietary intake reference values for folate, vitamin B_{12}, and vitamin B_6 (400, 2.4 and 1300 μg/day, respectively).

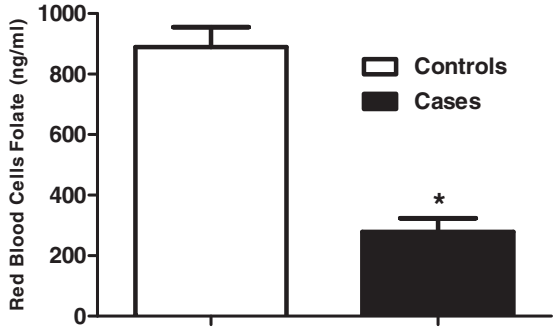

Figure 2 Red blood cells' folate measurement in cases and controls. *Significantly lower than controls. Red blood cells' folate reference values are 450–1400 ng/mL

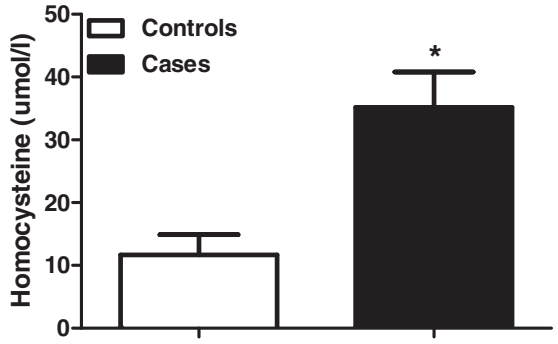

Figure 3 Serum homocysteine levels in cases and controls. *Significantly higher than controls. Serum homocysteine reference values are 10–15 μmol/L

Red blood cells' folate

Figure 2 shows the mean red blood cells folate content of cases and controls. The cases had significantly lower folate levels as compared to controls. These results suggest that cases had a chronic low dietary intake of folate. The red blood cells' folate content of cases was also lower than the corresponding folate reference value, 450–1400 ng/mL.

Hyperhomocysteinemia

Serum HCY levels of the cases were significantly higher ($t = 18.22$, $P < 0.05$) than controls and higher than the normal HCY serum reference value, 10–15 μmol/L; indicating the existence of hyperhomocysteinemia among cases (Figure 3). The serum HCY was negatively correlated with serum folic acid, vitamins B_6, and B_{12} levels ($r = -0.465$, $r = -0.321$, $r = -0.398$, $P < 0.05$, respectively). However, there was no correlation between the serum levels of HCY, BMI, or age. The correlation between serum HCY and red blood cells folate level was weak and non-significant ($r = -0.213$, $P > 0.05$).

Oxidative stress

Significantly lower levels of serum GSH and TAC (8.21 ± 0.89 μmol/L and 28.92 ± 1.1 mmol/L, respectively) were observed among cases as compared to controls (28.92 ± 1.1 μmol/L and 122.3 ± 12.89 mmol/L, respectively), $P < 0.05$. The oxidative stress indices (MDA and NN) were significantly higher in cases as compared to controls, $P < 0.05$, Table 3.

Table 3 Measurements of various oxidative stress parameters in cases and controls

Parameter	Cases	Controls
GSH (μmol/L)	$8.21 \pm 0.89^{*}$	28.92 ± 1.1
TAC (mmol/L)	$69.6 \pm 11.2^{*}$	122.3 ± 12.89
MDA (μmol/L)	$3.19 \pm 0.2^{\#}$	1.43 ± 0.14
NN (μmol/L)	$80.23 \pm 16.8^{\#}$	22.11 ± 4.1

GSH: glutathione; TAC: total antioxidant capacity; MDA: malondialdehyde, nitrites and nitrates, NN. Values are expressed as mean ± SD. The asterisk denotes that data in cases are significantly lower than controls group and the hash mark denotes that data in cases are significantly higher than controls group, $P < 0.05$.

Discussion

A steady increase in the prevalence of CVDs has been linked to adopting a sedentary lifestyle, with the classical risk factors such as hypertension, smoking, hyperlipidemia, and type-2 diabetes. There was an association between the elevated serum HCY level and an increased risk of CVDs incidence.[13] The serum HCY concentration of >10 μmol/L can increase the risk of CVD in a linear dose–response relationship with no specific threshold levels, and it was suggested that a reduction of elevated HCY concentrations (<10 μmol/L) may theoretically prevent up to 25% risk of cardiovascular events.[17] High fasting serum HCY and low folate and vitamin B_{12} have also been suggested as clinical biomarkers for the early diagnosis of different human diseases among various population groups.[18]

Folate deficiency is considered as the most common cause of hyperhomocysteinemia, and there is an increasing demand for the diagnosis and treatment of elevated serum HCY level in high-risk individuals in general and patients with manifest vascular disease in particular.[19] Our study has addressed the preventable risk factors associated with CVDs, using biochemical and nutritional approaches. Inadequate dietary intake of folate, vitamins B_6, and B_{12} has been associated with hyperhomocysteinemia based on their biochemical role as cofactors for enzymes mediated trans-methylation and transsulfuration pathways of HCY. The results of the present study highlighted this relationship among newly diagnosed cardiac patients, where it was observed that the dietary intake of folate in cases was lower than the dietary reference intakes for folate among adults and this was a determinate factor for lower levels of serum folate and red blood cells folate contents, short-term and long-term indicators of folate nutritional status, respectively. The same observation applied for vitamin B_6 and vitamin B_{12}.

Folate and vitamin B_{12} are required for methylation of HCY to methionine, and it was suggested that reduced plasma levels of folate, vitamin B_6, and vitamin B_{12} are associated with elevated level of HCY, hyperhomocysteinemia. Furthermore, our study revealed that hyperhomocysteinemia causes depletion of GSH and impairment of TAC

indicating a cellular oxidative stress condition, as evident by an increase in reactive oxygen species products, MDA and NN. These results are in agreement with data from previous studies that an imbalance between ROS production and antioxidant levels exists in patients with CVDs and hyperhomocysteinemia.[20,21]

It was recommended that food grain fortification with folate and cobalamin supplement as an effective approach for CVD prevention.[22] Vitamin B_6 is required as a cofactor for transsulfuration of HCY into its metabolites, cystathionine, cysteine, and GSH. Lower levels of vitamin B_6 induce the accumulation of HCY and subsequently the development of hyperhomocysteinemia.[22] The mean red blood cells' folate content in cases was significantly lower in cases as compared to controls. The measurement of folate in red blood cells is preferred since it reflects long-term folate status in the body as compared to plasma or serum folate that may be influenced by recent dietary intakes.[23] The correlation between serum HCY and red blood cells folate content was however weak and non-significant. These findings are consistent with the body of evidence in research that elevated HCY level is an etiological factor for CVD.

Adopting a healthy lifestyle pattern reduces CVD risk factors such as dyslipidemia and obesity.[17] The CVD patients enrolled in this study had a sedentary lifestyle as demonstrated by their low level of routine occupational physical activity (76%) as well as lack of physical activity in their leisure time (68%). The incidence of CVD is common among those who adopt a sedentary lifestyle and is considered to be a global health problem among different population groups and nations.

Lack of physical activity predisposes obesity, insulin resistance, high level of low density lipoprotein, and triglycerides, which are considered as the common risk factors for CVD.[24,25] Regular physical activity is considered as an effective intervention for reducing the risk of CVD.[24] The American Heart Association has listed obesity as a major risk factor for CVD, and our data revealed that the majority of CVD cases were overweight, this is quiet alarming since the rate of mortality and sudden death has been reported to be higher among subjects with BMI $>27\,kg/m^2$.[24]

The cases consumed significantly higher amounts of protein in particular from animal sources as compared to controls. The intake of animal protein was also higher than the daily dietary recommended allowance (60 g/day). The high protein intake has been associated with CVDs risk, in particular if it is from animal sources. The cases had a lower intake of total fat, mainly from saturated fats, which is contraindicating the notion that there is a causal relationship between saturated fat consumption and the risk of developing CVD. The daily intake of carbohydrate as well as total energy in cases was also lower than the controls. Previous studies reported a decrease in usual food intake of people identified at high risk of CVD prior to diagnosis, as evident by low intake of fruits, vegetables, bread, grains, and fat.[25,26] Therefore nutrition knowledge of healthy-eating recommendations in relation to CVD risk is recommended to initiate and maintain lifestyle changes among high risk groups.[27] High dietary fat intake is generally involved in the etiology of high rate of adiposity and fat storage for prolonged periods leading to obesity and is considered to be atherogenic in terms of its effect on serum lipids profile and effect on thrombosis and endothelial functions.[28] It has been suggested that adults consuming energy-dense foods have been shown to have higher risk of CVD.[29] HCY is a cytotoxic metabolite that promotes oxidation of low density lipoproteins, and induces inflammation in vascular smooth muscles and impairs the endothelial function with a potential of pathogenesis of atherosclerosis, a major risk factor for CVD.[30,31]

In conclusion, our findings suggest that hyperhomocysteinemia and oxidative stress were common among the enrolled newly diagnosed cardiac patients; they also had a low dietary intake of folate, vitamins B_6, and B_{12} as compared to controls. The measured serum HCY of cases was negatively correlated with their serum folate and vitamins B_6 and B_{12} levels. No correlation was observed between serum HCY, BMI, and age. The mean red blood cells folate content in cases was significantly lower in cases as compared to controls. The correlation between serum HCY and red blood cells folate content was weak and non-significant. Our study highlights the importance of adopting early nutritional and healthy community-based intervention programs for the primary prevention of CVDs among high risk groups in particular with the adolescent population.

Author contributions: All authors contributed equally to this study.

ACKNOWLEGMENTS

This research was supported by the internal grant fund (IG/AGR/FOOD/14/02) offered by Sultan Qaboos University through the college of Agricultural and Marine Sciences.

DECLARATION OF CONFLICTING INTEREST

The authors declare that there is no conflict of interests regarding the publication of this paper.

REFERENCES

1. Bauer UE, Briss PA, Goodman RA, Bowman BA. Prevention of chronic disease in the 21st century: elimination of the leading preventable causes of premature death and disability in the USA. *Lancet* 2014;**384**:45–52
2. Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, Cushman M, de Ferranti S, Després JP, Fullerton HJ, Howard VJ, Huffman MD, Judd SE, Kissela BM, Lackland DT, Lichtman JH, Lisabeth LD, Liu S, Mackey RH, Matchar DB, McGuire DK, Mohler ER, Moy CS, Muntner P, Mussolino ME, Nasir K, Neumar RW, Nichol G, Palaniappan L, Pandey DK, Reeves MJ, Rodriguez CJ, Sorlie PD, Stein J, Towfighi A, Turan TN, Virani SS, Willey JZ, Woo D, Yeh RW, Turner MB. American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Executive summary: heart disease and stroke statistics-2015 update: a report from the American heart association. *Circulation* 2015;**131**:434–41
3. Abraham R, Joseph M, Calton R, Dhanoa J. Raised serum homocysteine levels in patients of coronary artery disease and the effect of vitamin B12 and folate on its concentration. *Ind J Clin Biochem* 2006;**21**:95–100
4. Aronow WS. Homocysteine, the association with atherosclerotic vascular disease in older persons. *Geriatrics* 2003;**58**:22–8

5. Angel F, Joan C, Jose L, Xavier G. Vitamin B12 deficiency, hyperhomocytenemia and thrombosis: a case and control study. *Int J Hematol* 2011;**93**:458–64
6. Dhonukshe R, deVies JHM, deGroot LC. Dietary intake and status of folate and vitamin B12 and their association with homocysteine and cardiovascular disease in European populations. *Eur J Clin Nutr* 2009;**63**:18–30
7. Selhub J. Homocysteine metabolism. *Ann Rev Nutr* 1999;**19**:217–46
8. Finkelstein JD. Pathways and regulation of homocysteine metabolism in mammals. *Semin Thromb Hemost* 2000;**26**:219–25
9. Debreceni B, Debreceni L. The role of homocysteine-lowering B-vitamins in the primary prevention of cardiovascular disease. *Cardiovasc Ther* 2014;**32**:130–8
10. Martijn G, Floor V, Robert J. Hyperhomocysteinaemia and vitamin B12 deficiency: The long-term effect in cardiovascular disease. *Cardiology* 2007;**107**:57–62
11. Wilson CP, McNulty H, Scott JM, Strain JJ. Postgraduate symposium The MTHFR C677T polymorphism, B-vitamins and blood pressure. *Proc Nutr Soc* 2010;**69**:156–65
12. DeChiara B, Sedda V, Parolini M, Campolo J, De Maria R, Caruso R, Pizzi G. Plasma total cysteine and cardiovascular risk burden: action and interaction. *Scient World J* 2012;**30**:36–54
13. Steed MM, Tyagi SC. Mechanisms of cardiovascular remodeling in hyperhomocysteinemia. *Antioxid Redox Signal* 2011;**15**:1927–43
14. Müller KB, Galdieri LC, Pereira VG, Martins AM, Almeida VD. Evaluation of oxidative stress markers and cardiovascular risk factors in Fabry disease patients. *Genet Mol Biol* 2012;**23**:418–23
15. Piyathilake CJ, Robinson CB, Cornwell P. A practical approach to red blood cell folate analysis. *Analyt Chem Ins* 2007;**7**:107–10
16. Quillard M, Berthe MC, Sauger F, Lavoinne A. Automated measurement of plasma homocysteine on DPC Immulite 2000: comparison with measurement carried out on Abbott IMX apparatus. *Ann Biol Clin* 2003;**61**:699–704
17. Lippi G, Plebani M. Hyperhomocysteinemia in health and disease: where we are now, and where do we go from here. *Clin Chem Lab Med* 2012;**50**:2075–80
18. Al-Farsi YM, Waly MI, Deth RC, Al-Sharbati MM, Al-Shafaee M, Al-Farsi O, Al-Khaduri MM, Gupta I, Ali A, Al-Khalili M, Al-Adawi S, Hodgson NW, Ouhtit A. Low folate and vitamin B12 nourishment is common in Omani children with newly diagnosed autism. *Nutrition* 2013;**29**:537–41
19. Hobbs CA, Cleves MA, Macleod SL, Erickson SW, Tang X, Li J, Li M, Nick T, Malik S. National Birth Defects Prevention Study. Conotruncal heart defects and common variants in maternal and fetal genes in folate, homocysteine, and transsulfuration pathways. *Birth Defects Res A Clin Mol Teratol* 2014;**100**:116–26
20. Bogdanski P, Miller-Kasprzak E, Pupek-Musialik D, Jablecka A, Lacinski M, Jagodzinski PP, Jakubowski H. Plasma total homocysteine is a determinant of carotid intima-media thickness and circulating endothelial progenitor cells in patients with newly diagnosed hypertension. *Clin Chem Lab Med* 2012;**50**:1107–13
21. Petramala L, Acca M, Francucci CM, D'Erasmo E. Hyperhomocysteinemia: a biochemical link between bone and cardiovascular system diseases. *J Endocrin Invest* 2009;**32**:10–4
22. Cacciapuoti F. Hyperhomocysteinemia: a novel risk factor or a powerful marker for cardiovascular diseases? pathogenetic and therapeutical uncertainties. *J Thromb Thromb* 2011;**32**:82–8
23. Chew SC, Khor GL, Loh SP. Association between dietary folate intake and blood status of folate and homocysteine in Malaysian adults. *J Nutr Sci Vitaminol* 2011;**57**:150–5
24. Goff DC Jr, Gerstein HC, Ginsberg HN, Cushman WC, Margolis KL, Byington RP. Prevention of cardiovascular disease in persons with type 2 diabetes mellitus: current knowledge and rationale for the action to control cardiovascular risk in diabetes (ACCORD) trial. *Am J Cardiol* 2007;**99**:4–20
25. McCourt HJ, Draffin CR, Woodside JV, Cardwell CR, Young IS, Hunter SJ, Murray LJ, Boreham CA, Gallagher AM, Neville CE, McKinley MC Young Hearts Study Group. Dietary patterns and cardiovascular risk factors in adolescents and young adults: the Northern Ireland Young Hearts Project. *Br J Nutr* 2014;**112**:1685–98
26. Olinto MT, Gigante DP, Horta B, Silveira V, Oliveira I, Willett W. Major dietary patterns and cardiovascular risk factors among young Brazilian adults. *Eur J Nutr* 2012;**51**:281–91
27. Eilat-Adar S, Mete M, Fretts A, Fabsitz RR, Handeland V, Lee ET, Loria C, Xu J, Yeh J, Howard BV. Dietary patterns and their association with cardiovascular risk factors in a population undergoing lifestyle changes: the Strong Heart Study. *Nutr Metab Cardiovasc Dis* 2013;**23**:528–3
28. Jzelenberg W, Hellemans IM, van Tulder MW, Heymans MW, Rauwerda JA, van Rossum AC, Seidel JC. The effect of a comprehensive lifestyle intervention on cardiovascular risk factors in pharmacologically treated patients with stable cardiovascular disease compared to usual care: A randomized controlled trial. *BMC Cardiovasc Disord* 2012;**12**:71–7
29. Pearson TA, Mensah GA, Alexander RW, Anderson JL, Criqui ROM, Fadl YY. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: a statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. *Circulation* 2003;**107**:499–511
30. Ali A, Waly MI, Al-Farsi YM, Essa MM, Al-Sharbati MM, Deth RC. Hyperhomocystenemia among Omani autistic children: a case-control study. *Acta Biochim Polonica* 2011;**58**:547–51
31. Petramala L, Acca M, Francucci CM, Erasmo ED. Hyperhomocysteinemia: a biochemical link between bone and cardiovascular system diseases. *J Endocrinol Invest* 2009;**32**:10–4

(Received March 4, 2015, Accepted June 26, 2015)