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Plasma Total Homocysteine, Folate, and Vitamin B12 Status and Hospitalizations for Cardiovascular Disease*

Bakhouche Houcher¹, Mirande Candito², Pierre Gibelin³¹Department of Biology, Faculty of Sciences, University of Sétif, Sétif 19000, Algeria; ²Biochemistry Laboratory, Inserm U 145, CHU de Nice, Nice, France, ³Cardiology Department, Hôpital Pasteur, Nice, France*Dedicated to Dr. Mirande Candito on the occasion of her 60th birthday

Abstract

Elevated plasma total homocysteine (tHcy) is an independent risk factor for cardiovascular disease (CVD). Also known is that plasma folate and vitamin B12 influence homocysteine metabolism as cosubstrate and cofactor, respectively. This population-based study was conducted to evaluate the plasma concentrations of tHcy, folate, and vitamin B12 in 54 older patients aged ≥ 51 years (40 males; 14 females) of Nice hospital cardiology service. After excluding cases with a serum creatinine >120 mmol/L, we established the test properties of a plasma tHcy concentration <15 $\mu\text{mol/L}$ (Group 1) or ≥ 15 $\mu\text{mol/L}$ (Group 2). In the population aged ≥ 51 years, plasma tHcy was higher in women (18.0 $\mu\text{mol/L}$) than in men (15.5 $\mu\text{mol/L}$; not significant), conversely, serum vitamin B12 was higher in men (376.9 pg/ml) than in women (340.7 pg/ml; not significant). Average plasma tHcy was 11.5 $\mu\text{mol/L}$ in Group 1 and 21.6 $\mu\text{mol/L}$ in Group 2. *Vice versa*, serum vitamin B12 was higher in Group 1 (419.5 pg/ml) than in Group 2 (307.2 pg/ml) ($p < 0.05$). Correlation analysis (Pearson's r) in the total study population (20-84 years) indicated an inverse correlation between serum folate and age ($r = -0.231$, $p < 0.05$). In the subjects, aged ≥ 51 years, there was a significant negative correlation between age and tHcy levels ($r = -0.283$, $p < 0.05$) and serum vitamin B12 concentrations ($r = -0.326$, $p < 0.01$) but not with serum folate. However, in subjects with tHcy <15 $\mu\text{mol/L}$, a significant inverse correlation existed between plasma tHcy and serum folate ($r = -0.455$; $p < 0.05$). In conclusion, these results highlight the relevance of the vitamin status and particularly of folate levels in the modulation of fasting tHcy levels in the patients with clinical hyperhomocysteinemia, defined as plasma tHcy >15 $\mu\text{mol/L}$.

Key words: cardiovascular disease, hospitalizations, aging; homocysteine, folates, vitamin B12

Introduction

Aging is associated with alteration of both vascular structure and function, and with an increased exposure time to cardiovascular risk factors. The geriatric population is increasing worldwide at a rate of 2.8% /year (1). Cardiovascular and cerebrovascular problems, with high morbidity and mortality, are more frequently seen in geriatric populations. Among emerging risk factors for cardiovascular disease (CVD), homocysteine has been postulated as one of the more appealing. Homocysteine is a sulphur containing amino acid formed during the metabolism of methionine, an essential amino acid derived from dietary protein. Homocysteine is metabolised with folate as co-sub-

strate and vitamin B12 as a co-factor and low levels of these vitamins causes hyperhomocysteinemia (HHcy) (2).

Plasma concentrations of total homocysteine (tHcy) are influenced by several factors including age and gender, nutritional factors (vitamin B6, vitamin B12, folic acid, coffee consumption and alcohol intake), genetic determinants (polymorphisms in genes directly or indirectly associated with methionine metabolism) and inflammation. Increased tHcy concentration may be correlated with low plasma levels of vitamin B6, vitamin B12 or folic acid (3). Consequently, the status of these nutrients may be associated with CVD risks.

In 1969, Kilmer McCully linked elevated plasma

Correspondence to: B. Houcher, Department of Biology, Faculty of Sciences, University of Sétif, Sétif 19000, Algeria, Fax: 00 213 36 92 51 22, e-mail: bhoucher@yahoo.fr

tHcy concentrations with vascular disease for the first time (4). He noted a high prevalence of early arteriosclerotic and thromboembolic disease in patients with congenital homocystinuria, a rare genetic disorder associated with greatly elevated blood concentrations of tHcy (5).

Epidemiological studies indicate that hyperhomocysteinemia is a risk factor for atherothrombotic disease, though the molecular mechanism is not clear (2). A study found an association between low serum folate and CVD mortality (6). Wald *et al.* (7) have found that in patients with CVD a dosage of 0.8 mg/d appears to be necessary for maximal lowering of tHcy. However, very recent evidence from patients with heart disease indicates that optimal tHcy lowering may be achievable at much lower folic acid doses (8).

Serum creatinine, a marker with low sensitivity for early decline in renal function, is a strong determinant of plasma tHcy concentration (9,10,11). Almost every study has shown a highly significant positive correlation between the concentrations of creatinine and tHcy. In renal disease, both the creatinine and tHcy concentrations increase, and in end-stage renal disease, plasma tHcy concentrations may become 3-5 times higher than normal (9,12,13).

Thus, much of the epidemiologic association found in prospective and retrospective studies and in cross-sectional studies between modestly elevated plasma tHcy concentrations and cardiovascular risk or severity of atherosclerosis is explained by the renal mechanism (14). However, several studies have shown that folic acid supplementation is effective in lowering blood tHcy levels and may thus also ameliorate the vascular situation in patients with atherosclerotic or coronary disease (15). Recent evidence suggests that this association may be causal (16), although up-to-date contradictory data are available on this issue (17).

The aim of this study was to determine, whether levels of tHcy and vitamin B12 and folate were correlated with the presence of CVD, with clinical evidence of angian or myocardial infarction and to exclude renal deficiency, which is a known cause of HHcy.

Subjects and Methods

Subjects

This research was carried out as a prospective study on 84 patients (65 men and 15 women), hospitalized in the Cardiology Department in CHU Pasteur of Nice, France. The patients suffered from coronary heart disease, with clinical evidence of angian or myocardial infarction. All subjects resided in Nice or the surrounding areas. From these participants, a subgroup of 54 subjects (40 males; 14 females) was selected,

excluding those aged under 51 years. We did not include epileptic patients, since their treatment possibly has antifolate activity; patients treated with vitamins of B group (e.g., folic acid, vitamin B12 and vitamin B6), or those taking fibrate treatment, which can increase tHcy concentrations (18, 19), were excluded, as were patients who presented with creatinine concentrations $>120 \mu\text{mol/L}$, to exclude renal deficiency, which is a known cause of HHcy. The patients divided into: Group 1 (G1) (tHcy $<15 \mu\text{mol/L}$) and Group 2 (G2) (tHcy $\geq 15 \mu\text{mol/L}$).

Biochemical analysis

Blood samples were collected from patients after fasting but on normal diet. To evaluate plasma tHcy concentrations, EDTA samples were immediately put on ice and centrifuged within 1h. Plasma was kept at -20°C and analysed in the following week. tHcy concentration was measured using an enzyme-based immunometric test after chemical reduction of homocystine (Abbott, Rungis, France). Folic acid and vitamin B12 levels were measured in serum, for which blood was clotted at room temperature and centrifuged within 2 h. The serum was kept at -20°C and analysed within 1 week by a microbiological method using *Lactobacillus casei* and *Lactobacillus leishmannii*, respectively. Creatinine concentrations were measured in heparinized plasma in the 2h following sampling by the colorimetric Jaffe method (Boehringer-Roche 917 analyser, Grenoble, France).

Statistical analysis

Concentrations of vitamins and tHcy were characterized by means and standard errors. Comparisons of mean differences were calculated by student's t-test and correlations between biochemical data were determined using Spearman rank correlation coefficients (statistical Software for Windows Version 5.1).

Results

17 patients who presented with severe renal deficiency with creatinine concentrations $>120 \mu\text{mol/L}$, with a mean $159 \pm 30 \mu\text{mol/L}$, which is a known cause of HHcy, are excluded in this study. Table 1 describes gender-specific plasma tHcy, serum folate and vitamin B12 concentrations. At blood sampling, men were only slightly older than women and had somewhat lower tHcy (not significant = n.s.) and folate ($p < 0.05$) and higher vitamin B12 and creatinine concentrations (both n.s.) (Table 1). A substantial proportion of subjects (24%) had tHcy levels $\geq 20 \mu\text{mol/L}$ and 3.7% of them had tHcy $\geq 30 \mu\text{mol/L}$.

The study population was divided in 2 groups

Table 1. Gender influence on biological markers in the study population (total homocysteine = tHcy). n = 54 (Age ≥ 51 years). *p < 0.05

Subjects	Age (years)	Vitamin B12 (pg/ml)	Folates (ng/ml)	tHcy ($\mu\text{mol/L}$)	Creatinine ($\mu\text{mol/L}$)
Men (n = 40)	65.8 $\pm$ 9.2	376.9 $\pm$ 196.2	4.27 $\pm$ 1.86*	15.5 $\pm$ 5.86	88.5 $\pm$ 17.6
Women (n = 14)	65.2 $\pm$ 9.2	340.7 $\pm$ 159.3	5.63 $\pm$ 1.98	18.0 $\pm$ 7.28	80.6 $\pm$ 15.0

according to tHcy concentrations < or $\geq 15 \mu\text{mol/L}$. The two groups differed in the proportion of male subjects, age, vitamins and creatinine levels (all p values ≤ 0.05 , Table 2). The distribution of tHcy concentrations in groups of patients was similar: 53.7% for Group 1 (G1) had low (<15 $\mu\text{mol/L}$) tHcy concentrations, and 46.3% had elevated levels ($\geq 15 \mu\text{mol/L}$) for Group 2 (G2) (Table 2).

tHcy levels were higher in Group 2 (21.55 $\pm$ 5.04 $\mu\text{mol/L}$) as compared with Group 1 (11.51 $\pm$ 2.18 $\mu\text{mol/L}$) patients (p < 0.01). Vitamin B12 levels were higher (419.5 $\pm$ 220.6 pg/ml) in Group 1 vs. 307.2 $\pm$ 114.4 pg/ml in Group 2 (p < 0.05) (Table 2).

Discussion

Several epidemiological studies have shown a correlation between tHcy levels and the presence of CVD and homocysteine has therefore been considered an independent risk factor for CVD. We investigated the relationship between tHcy and the CVD hospitalizations. The results showed that tHcy is a predictor of CVD morbidity in elderly individuals.

Several population-based studies have demonstrated that homocysteine levels are influenced by age and gender (20,21,22), suggesting that the gender difference could be due to larger muscle mass in men, since

Table 2. Comparison of biological markers in the two groups with low (Group 1: tHcy <15 $\mu\text{mol/L}$) or high (Group 2: tHcy $\geq 15 \mu\text{mol/L}$) total homocysteine (tHcy) concentrations (***p < 0.001; **p < 0.01; *p < 0.05)

Subjects	Men (%)	Age (years)	Vitamin B12 (pg/ml)	Folates (ng/ml)	tHcy ($\mu\text{mol/L}$)	Creatinine ($\mu\text{mol/L}$)
Group 1 (n = 29)	53.7	63.55 $\pm$ 8.47	419.5 $\pm$ 220.6*	4.84 $\pm$ 1.88	11.51 $\pm$ 2.18***	81.55 $\pm$ 17.0**
Group 2 (n = 25)	46.3	68.12 $\pm$ 9.36	307.2 $\pm$ 114.4	4.38 $\pm$ 2.08	21.55 $\pm$ 5.04	94.72 $\pm$ 14.47

The percentage of subjects with folate and vitamin B12 levels <3.7 nmol/L and 150 pmol/L in two groups (G1 and G2) were respectively 27.6% vs 44% and 6.9% vs 8.0%. Correlation coefficient in the total study population (20-84 years) indicated an inverse association between serum folate and age in both sexes (r = -0.231, p < 0.05).

In subjects, aged ≥ 51 years, there was a significant negative correlation between age and tHcy levels (r = -0.283, p < 0.05) and serum vitamin B12 concentrations (r = -0.326, p < 0.01) but not with serum folate. In subjects, aged ≥ 51 years with tHcy <15 $\mu\text{mol/L}$, the variation in tHcy levels was explained only by folate (r = -0.455; p < 0.05). Folate and vitamin B12 were not correlated with tHcy level in subjects aged ≥ 51 with tHcy $\geq 15 \mu\text{mol/L}$.

the formation of muscles is associated with the simultaneous formation of homocysteine in connection with creatine/creatinine synthesis (10). Furthermore, it has been demonstrated that estrogen-induced an increased turnover of folate, thus determining the increase of tHcy levels (23). Part of the relationship between tHcy and age in women might be explained by menopause, since tHcy concentration was found to be higher in post-menopausal compared with premenopausal women (24,25,26). A significant difference was found between men and women in terms of tHcy, our results provide further support that this difference is associated with the sex.

Previous investigations conducted in the developed countries suggested that low vitamin B12 concentrations were common only in elderly adults (27). Because the ability to absorb food-bound dietary vita-

min B12 is significantly reduced after 50 years, those investigations were predominantly conducted in the populations older than 60 years (28). It is also known that about 30% of elderly people have an atrophic gastritis which may lower the absorption of vitamin B12 and lead to a suboptimal status of this vitamin over time (29) and age-related renal impairment (30).

tHcy concentrations increased significantly with aging, and hyperhomocysteinemia prevalence has been reported to be higher in the elderly than in other groups (31). The reasons for the higher tHcy concentrations at older age are not well understood, although changes in renal function and impaired renal metabolism of homocysteine are certainly involved (32). Declining folate concentrations may result from inflammation and lymphocyte proliferation and could be responsible for the enhanced formation of tHcy (33). Consequently, the relatively strong association between homocysteine and neopterin concentrations and kynurenine/tryptophan (kyn/trp) implies that immune activation is related to the development of moderate hyperhomocysteinemia in the elderly (34). In accordance, the mean plasma tHcy concentration was elevated in the heart failure group when compared to age- and sex-matched controls (35).

Moderate, intermediate and severe hyperhomocysteinemia are defined as plasma tHcy concentrations in the range 16-30, 31-100, and >100 $\mu\text{mol/L}$ respectively (36). They are usually attributable to folate and/or cobalamin deficiencies (37), and also genetically determined (38).

A growing body of evidence from epidemiological studies suggested that even individuals with moderate levels of tHcy (fasting tHcy >15 $\mu\text{mol/L}$) have an increased risk of CVD (39). We also found that more than one third of the patients had clinical hyperhomocysteinemia, defined as plasma tHcy >12 μM (40). Depending on the laboratory, the definition of elevated tHcy concentrations may differ, but generally a level above >15 μM (41) is considered elevated.

The tHcy blood concentration increases with age and reaches levels of 10-15 $\mu\text{mol/L}$ in healthy adults of middle age. Elderly persons show tHcy concentrations of about 10-25 $\mu\text{mol/L}$ (29). In our patients, 53.7% had a lower tHcy concentration (<15 $\mu\text{mol/L}$), these patients thus would not have been noted as vitamin B12 deficient. This is not surprising because the intake level of vitamin B12 is generally higher than the recommended level in developed countries. Vitamin B12 concentration did not correlate significantly with tHcy in this group of patients. A strong inverse association between tHcy and folate was seen. Serum levels of both vitamin B12 and folate can be influenced by recent vitamin intake and the relative vitamin status of each other, whereas red blood cells folate more accu-

rately reflects hepatic stores (42). The poor correlation between serum vitamin B12 level and tHcy suggests that red cell folate is a better measure of cellular cobalamin deficiency than serum vitamin B12 (43).

Vitamin B12 and folates deficiency may be responsible for an increase in plasma levels of tHcy. In addition, vitamin B12 and folates deficiency has been proposed as an independent risk factor for vascular disease (44). In the present study, no abnormality of the vitamin B12 status was observed in patients with tHcy <15 $\mu\text{mol/L}$ compared with those with tHcy $\geq$ 15 $\mu\text{mol/L}$. Folate is the most important modifiable determinant of the tHcy concentration, it seems attractive to this vitamin as a means to reduce the tHcy concentration (45). The homocysteine levels in our patients were high, for patients with CVD (46.3 and 20% had tHcy concentrations >15 and 20 $\mu\text{mol/L}$, respectively). It was observed in our subjects $\geq$ 51 years, 3.7% of severe hyperhomocysteinemia (>30 $\mu\text{mol/L}$). These results highlight the relevance of the vitamin status and particularly of folate levels in the modulation of fasting tHcy levels.

Moreover, HHcy predicted cardiovascular (CV) hospitalization in the Hordaland Homocysteine Study (persons aged 65-67 years; with tHcy >15 $\mu\text{mol/L}$) and both CV death and all-cause mortality among elderly persons (46). Plasma tHcy was the strongest modifiable determinant of mortality, and the mortality rate amongst individuals with tHcy <9.0 $\mu\text{mol/L}$ was less than 1% per year compared with 6% per year in those with tHcy $\geq$ 15 $\mu\text{mol/L}$ (47). Hyperhomocysteinemia appears to be a predictive factor of mortality in congestive heart failure patients (48), and thus vitamin treatment to reduce Hcy levels (when > 15 $\mu\text{mol/L}$) to near normal can improve the vasodilatory capacity and reduce blood pressure (35). Mild tHcy elevation (>15 $\mu\text{mol/L}$) occurs in $\approx$ 20-30% of patients with atherosclerosis disease (14). Usually, this is easily normalized with oral folate and ongoing trials are assessing the effect of folate treatment on outcomes.

The CVD risk associated with the common C677T MTHFR polymorphism has been extensively studied. The TT genotype was associated with 3.5 $\mu\text{mol/L}$ higher tHcy and a 26% higher CVD risk than the CC genotype. The unexpected finding was made in the hyperhomocysteinemic subgroup (tHcy >15 $\mu\text{mol/L}$): TT subjects had a lower prevalence of CVD risk factors than CC subjects (47). The moderate hyperhomocysteinemia that is dependent on the thermolabile MTHFR mutation is observed for plasma folate concentrations of less than 15.4 nmol/L. Above this concentration, no increase in circulating tHcy is observed (49).

The correlation between hyperhomocysteinemia and CVD is not completely understood. A lot of stud-

ies have demonstrated several potential mechanisms that induced the progression of atherosclerosis and thrombosis, particularly, in elderly subjects. This one is independent of the creatininemia but connected to a deficiency pronounced in folates like with a deficit in B12 vitamin suggesting the interest of a vitamin treatment among these patients. In conclusion, tHcy levels over 15 $\mu\text{mol/L}$, appear higher in patients referred for CVD, is an argument to consider nutritional intervention. Other studies remain necessary to affirm in the clinical plan the benefit of this treatment among old patients.

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References

- Butler RN. Population aging and health. *Br Med J* 1997; 315: 1082-1084.
- Welch GN, Loscalzo J. Mechanisms of disease: homocysteine and atherothrombosis (review article). *N Engl J Med* 1998; 338: 1042-1050.
- Ueland PM, Refsum H. Plasma homocysteine, a risk factor for vascular disease: plasma levels in health, disease and drug therapy. *J Lab Clin Med* 1989; 114: 473-501.
- McCully KS. Vascular pathology of homocysteinemia: implication for the pathogenesis of atherosclerosis. *Am J Pathol* 1969; 56: 111-129.
- McCully KS. Homocysteine and vascular disease. *Nat Med* 1996; 2: 236-239.
- Ford ES, Byers TE, Giles WH. Serum folate and chronic disease risk: findings from a cohort of United States adults. *Int J Epidemiol* 1998; 27: 592-598.
- Wald DS, Bishop L, Wald NJ, *et al.* Randomised trial of folic acid supplementation and serum homocysteine levels. *Arch Intern Med* 2001; 161: 695-700.
- Tighe P, Ward M, McNulty H, *et al.* Plasma homocysteine response to folic acid intervention in ischaemic heart disease patients: implications for food fortification policy. *Proc Nutr Soc* 2004; 63: issue 1 Suppl 1001, 51A.
- Arnadottir M, Hultberg B, Nilsson-Ehle P, *et al.* The effect of reduced glomerular filtration rate on plasma total homocysteine concentration. *Scand J Clin Lab Invest* 1996; 56: 41-46.
- Norlund L, Grubb A, Fex G, *et al.* The increase of plasma homocysteine concentrations with age is partly due to the deterioration of renal function as determined by plasma cystatin C. *Clin Chem Lab Med* 1998; 36: 175-178.
- Wollessen F, Brattstrom L, Refsum H, *et al.* Plasma total homocysteine and cysteine in relation to glomerular filtration rate in diabetes mellitus. *Kidney Int* 1999; 55: 1028-1035.
- Bostom AG, Lathrop L. Hyperhomocysteinemia in end-stage renal disease: prevalence, etiology, and potential relationship to arteriosclerotic outcomes. *Kidney Int* 1997; 52: 10-20.
- Wilcken DE, Gupta VJ, Reddy SG. Accumulation of sulphur-containing amino acids including cysteine-homocysteine in patients on maintenance haemodialysis. *Clin Sci* 1980; 58: 427-430.
- Brattström L, Wilcken DEL. Homocysteine and cardiovascular disease: cause or effect? *Am J Clin Nutr* 2000; 72: 315-323.
- Schroeksnadel K, Fick B, Wirleitner B, *et al.* Moderate hyperhomocysteinemia and immune activation. *Curr Pharm Biotech* 2004; 5: 107-118.
- Hankey GJ, Eikelboom JW. Homocysteine and stroke. *Lancet* 2005; 365: 194-196.
- Kaul S, Zadeh AA, Shah PKS. Homocysteine hypothesis for atherothrombotic cardiovascular disease: not validated. *J Am Coll Cardiol* 2006; 48: 914-923.
- Jonkers IJ, De Man FH, Onkenhout W, *et al.* Implication of fibrate therapy for homocysteine. *Lancet* 1999; 354:1208.
- Young IS, Woodside JV. Fibrates and homocysteine. *Nutrition* 2001; 17: 973-974.
- Nygard O, Vollset SE, Refsum H, *et al.* Total plasma homocysteine and cardiovascular risk profile. The Hordaland Homocysteine Study. *JAMA* 1995; 274: 1526-1533.
- Lussier-Cacan S, Xhignesse M, Piolot A, *et al.* Plasma total homocysteine in healthy subjects - sex-specific relation with biological traits. *Am J Clin Nutr* 1996; 64: 587-593.
- de Bree A, Verschuren WMM, Blom HJ, *et al.* The homocysteine distribution: (mis)judging the burden. *J Clin Epidemiol* 2001; 54: 462-469.
- O'Connor D, Green T, Picciano M. Maternal folate status and lactation. *J Mammary Gland Biol. Neoplasia* 1997; 2: 279-289.
- Andersson A, Brattstrom L, Israelsson B, *et al.* Plasma homocysteine before and after methionine loading with regard to age, gender and menopausal status. *Eur J Clin Invest* 1992; 22: 79-87.
- Wouters MG, Moorrees MT, van der Mooren MJ, *et al.* Plasma homocysteine and menopausal status. *Eur J Clin Invest* 1995; 25: 801-805.
- Morris MS, Jacques PF, Rosenberg IH, *et al.* Serum

- total homocysteine concentration is related to self-reported heart attack or stroke history among men and women in the NHANES III. *J Nutr* 2000; 130: 3073-3076.
- 27 Clarke R, Refsum H, Birks J, *et al.* Screening for vitamin B12 and folate deficiency in older persons. *Am J Clin Nutr* 2003; 77: 1241-1247.
- 28 Hao L, Ma J, Zhu J, *et al.* Vitamin B12 deficiency is prevalent in 35- to 64-year-old Chinese adults. *J Nutr* 2007; 137: 1278-1285.
- 29 Pietrzik K, Brönstrup A. Homocysteine and cardiovascular diseases. *Asia Pacific J Clin Nutr* 1996; 5: 157-160.
- 30 Refsum H, Ueland PM, Nygard O, *et al.* Homocysteine and cardiovascular disease. *Annu Rev Med* 1998; 49: 31-62.
- 31 Selhub J, Jacques PF, Wilson PWF, *et al.* Vitamins status and intake as primary determinants of homocysteine in the elderly. *JAMA* 1993; 270: 2693-2698.
- 32 Giusti B, Gori AM, Marcucci R, *et al.* Homocysteine as an emerging risk factor for cardiovascular disease in the elderly. *Aging health* 2006; 2: 983-997.
- 33 SchroECKSnadel K, Fick B, Wirleitner B, *et al.* Homocysteine accumulates in supernatants of stimulated human peripheral blood mononuclear cells. *Clin Exp Immunol* 2003; 134: 53-56.
- 34 Frick B, SchroECKSnadel K, Neurauter G, *et al.* Increasing production of homocysteine and neopterin and degradation of tryptophan with older age. *Clin Biochem* 2004; 37: 684-687.
- 35 Andersson SE, Edvinsson M-L, Edvinsson L. Reduction of homocysteine in elderly with heart failure improved vascular function and blood pressure control but did not affect inflammatory activity. *Basic and Clin Pharmacol Toxicol* 2005; 97: 306-310.
- 36 Kang SS, Wong PWK, Malinow MR. Hyperhomocyst(e)inemia as a risk factor for occlusive vascular disease. *Annu Rev Nutr* 1992; 12: 279-298.
- 37 Brattström L, Israelson B, Lingarde F, *et al.* Higher total plasma homocysteine in vitamin B12 deficiency than in heterozygosity for homocystinuria due to cystathionine beta-synthase deficiency. *Metabolism* 1988; 37: 175-178.
- 38 Christensen B, Frosst P, Lussier-Cacan S, *et al.* Correlation of a common mutation in the methylenetetrahydrofolate reductase gene with plasma homocysteine in patients with premature coronary artery disease. *Arterioscler Thromb Vasc Biol* 1997; 17: 569-573.
- 39 Bates CJ, Mansoor MA, van der Pools J, *et al.* Plasma total homocysteine in a representative sample of 972 British men and women aged 65 and over. *Eur J Clin Nutr* 1997; 51: 691-697.
- 40 De Vries AS, Verbeke F, Schrijvers BF *et al.* Is folate a promising agent in the prevention and treatment of cardiovascular disease in patients with renal failure? *Kidney Int* 2002; 61: 1199-1209.
- 41 Ueland PM, Refsum H, Stabler SP, *et al.* Total homocysteine in plasma or serum: methods and clinical applications. *Clin Chem* 1993; 39: 1764-1779.
- 42 Snow CF. Laboratory diagnosis of vitamin B12 and folate deficiency. *Arch Intern Med* 1999; 159: 1289-1298.
- 43 Curtis D, Sparrow R, Brennan L, Van Der Weyden MB. Elevated serum homocysteine as a predictor for vitamin B12 or folate deficiency. *Eur J Haematol* 1994; 52: 227-232.
- 44 Pancharuniti N, Lewis CA, Sauberlich HE, *et al.* Plasma homocysteine, folate, and vitamin B12 concentrations and risk for early-onset coronary artery disease. *Am J Clin Nutr* 1994; 59: 940-948.
- 45 de Bree A, Verschuren WMM, Kromhout D, *et al.* Homocysteine determinants and the evidence to what extent homocysteine determines the risk of coronary heart disease. *Pharmacol Rev* 2002; 54: 599-618.
- 46 Nurk E, Tell GS, Vollset SE, *et al.* Plasma total homocysteine and hospitalizations for cardiovascular disease: the Hordaland Homocysteine Study. *Arch Intern Med* 2002; 162: 1374-1381.
- 47 Nygard O, Vollset SE, Refsum H, *et al.* Total homocysteine and cardiovascular disease. *J Intern Med* 1999; 246: 425-454.
- 48 Gibelin P, Serre S, Candito M, *et al.* Prognostic value of homocysteinemia in patients with congestive heart failure. *Clin Chem Lab Med* 2006; 44: 813-816.
- 49 Jacques PF, Bostom AG, Williams RR, *et al.* Relation between folate status, a common mutation in methylenetetrahydrofolate reductase, and plasma homocysteine concentrations. *Circulation* 1996; 93: 7-9.